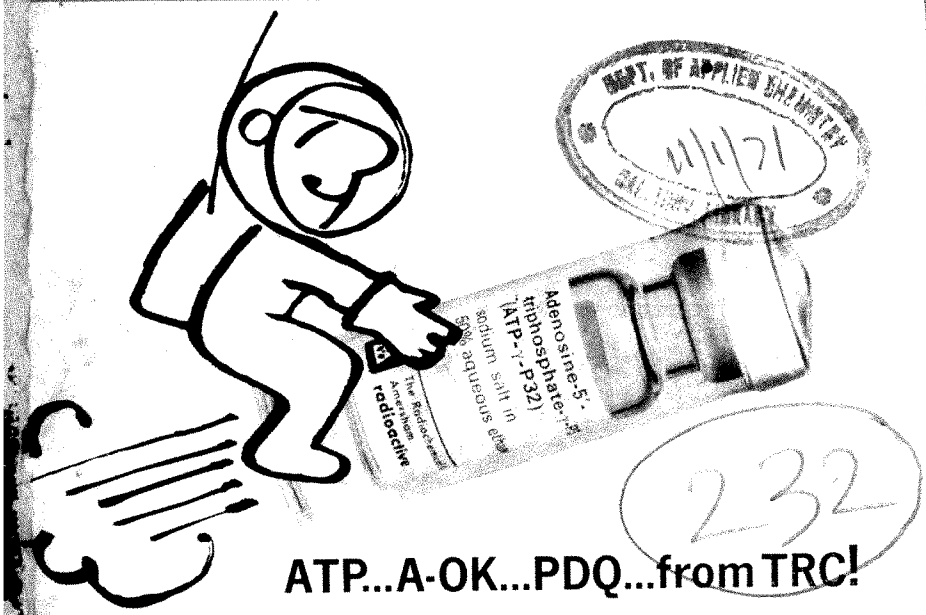
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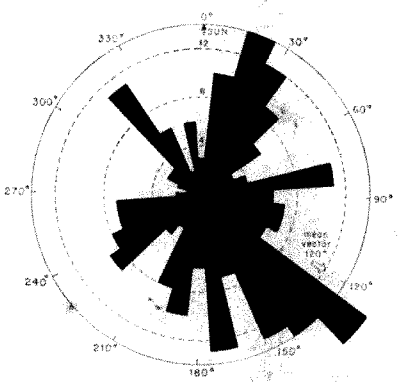
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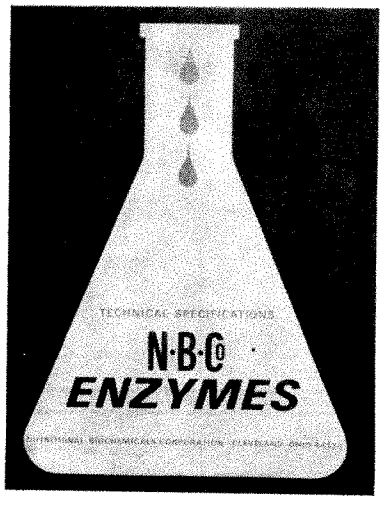
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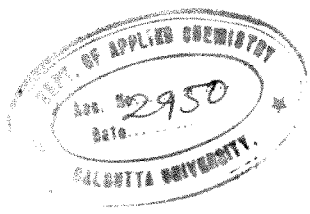
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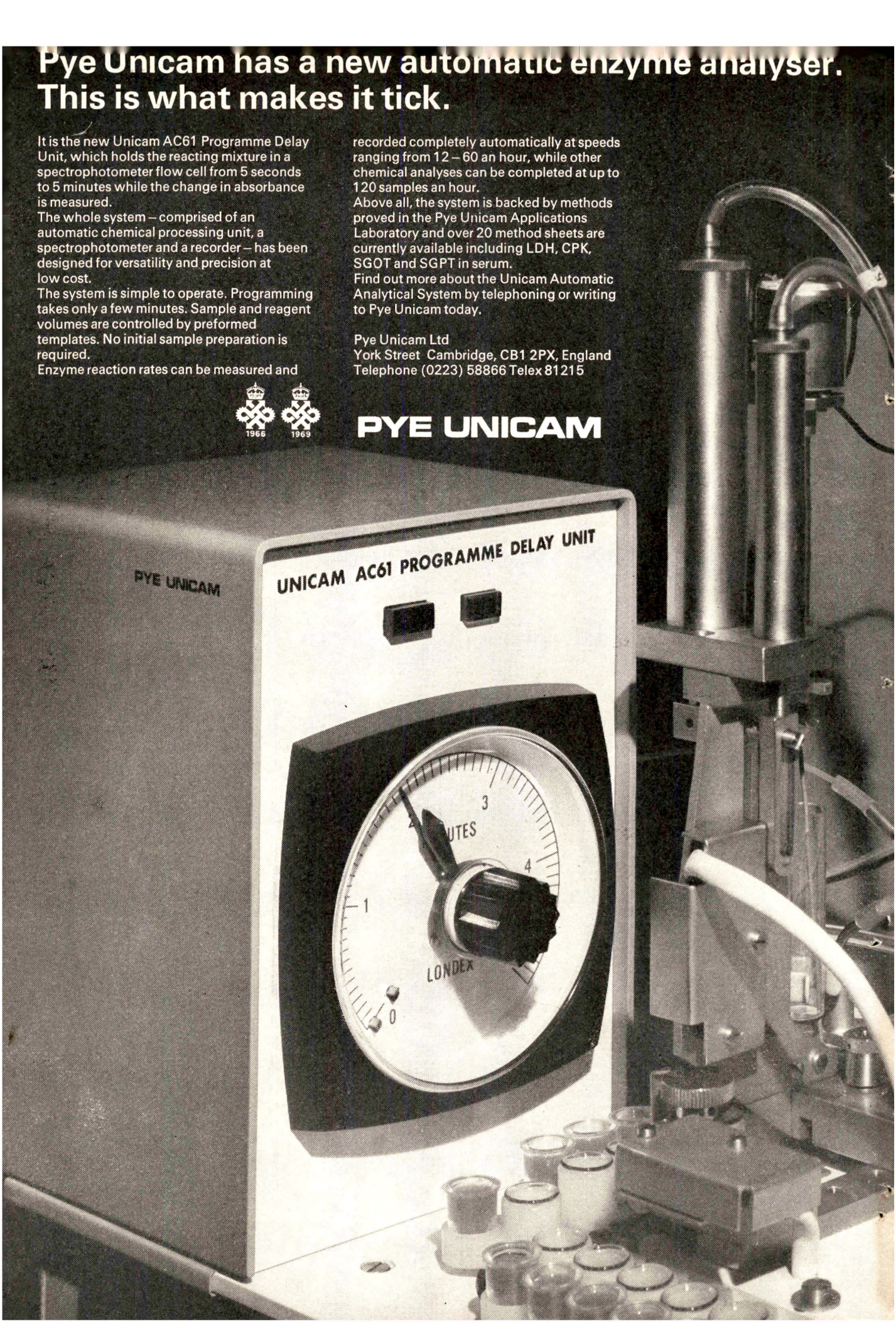
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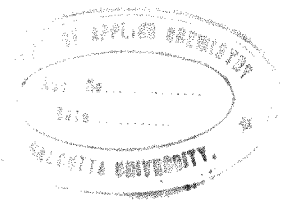
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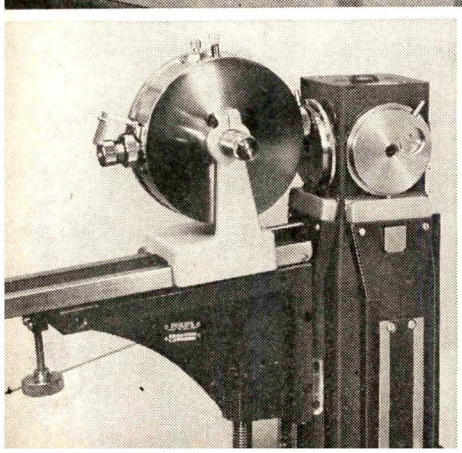
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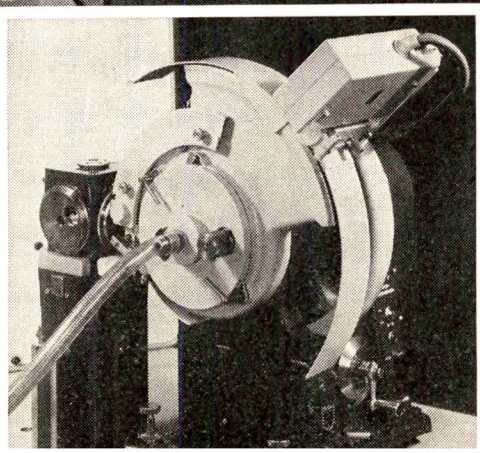
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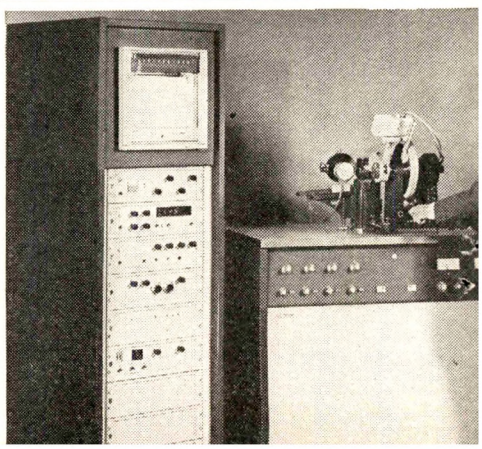
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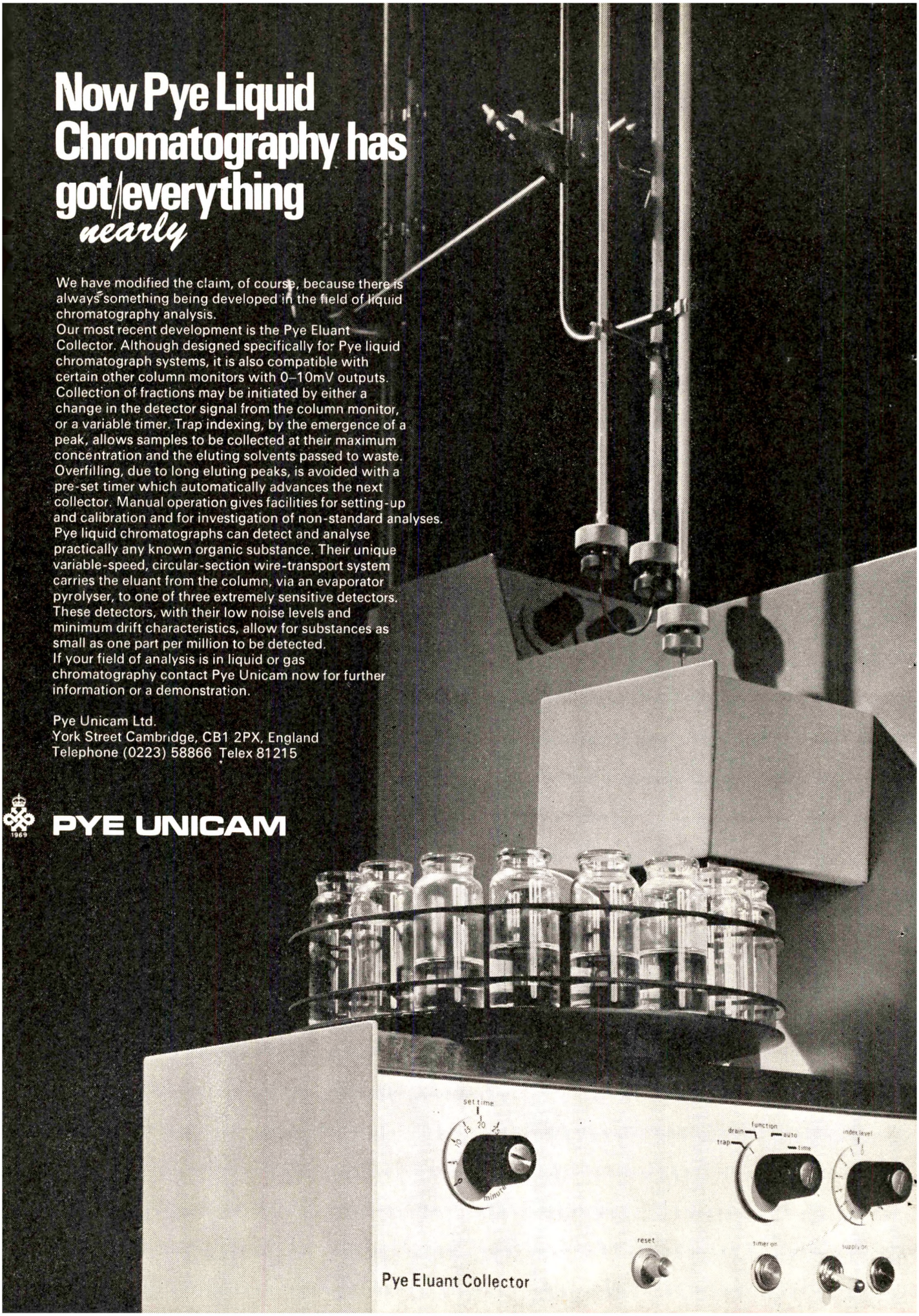
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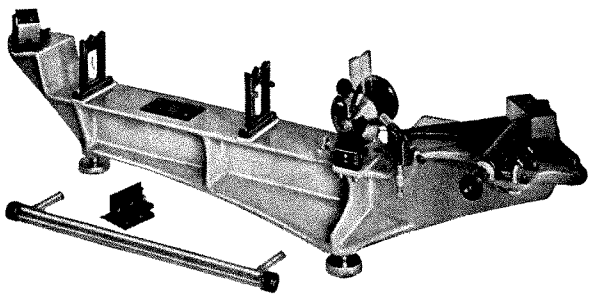


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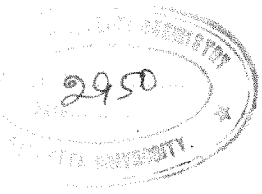
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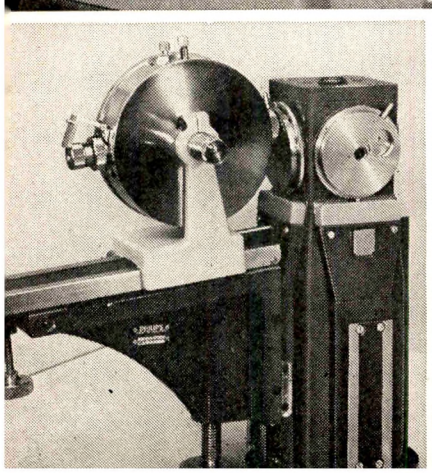
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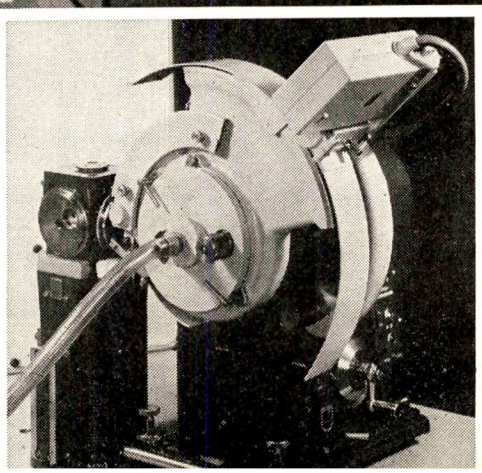
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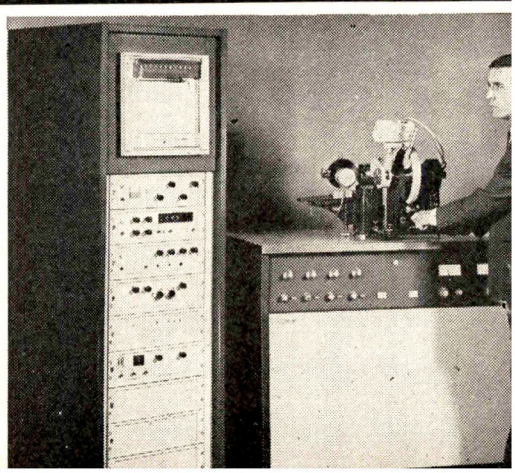
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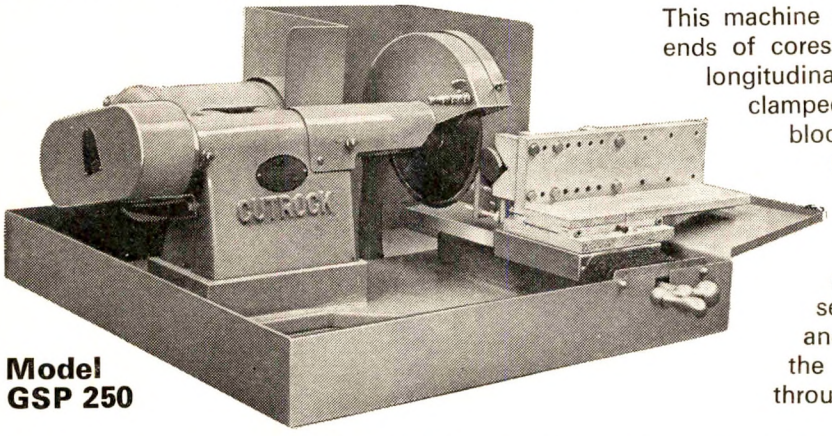
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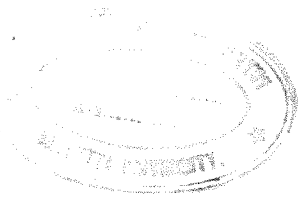
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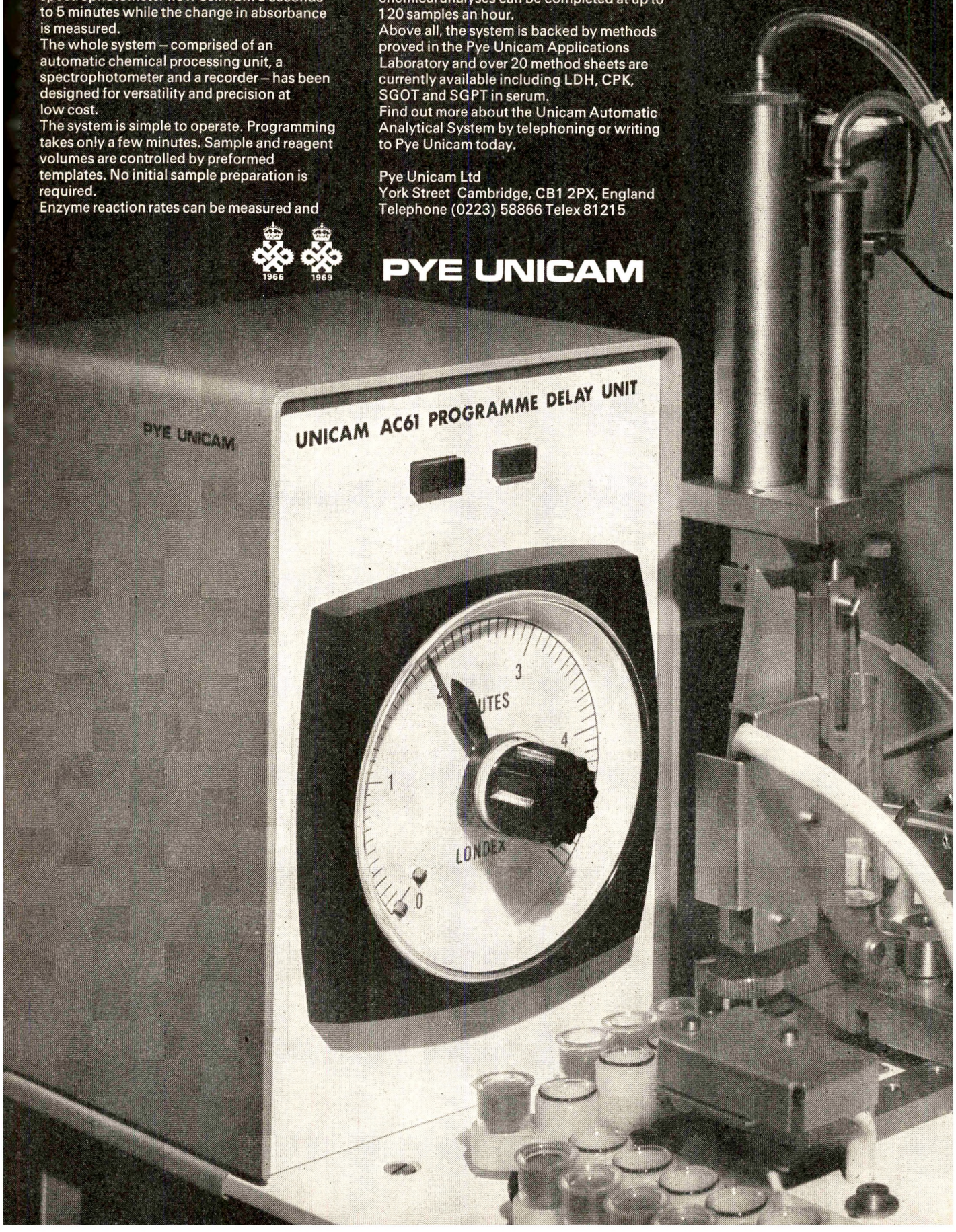
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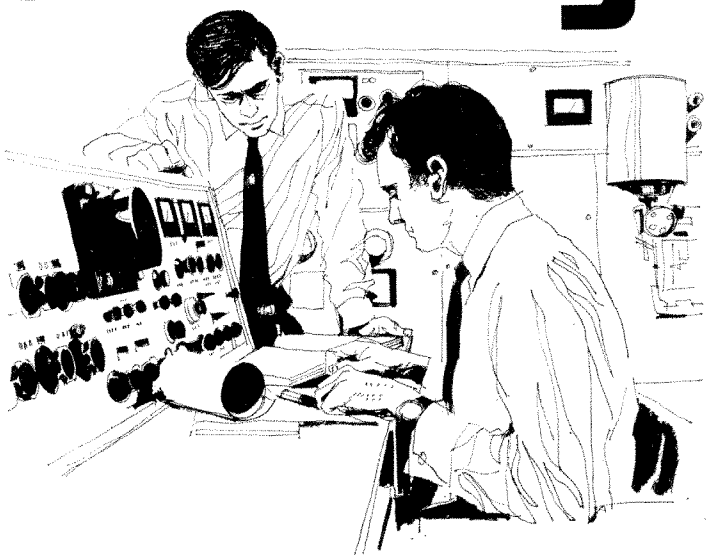
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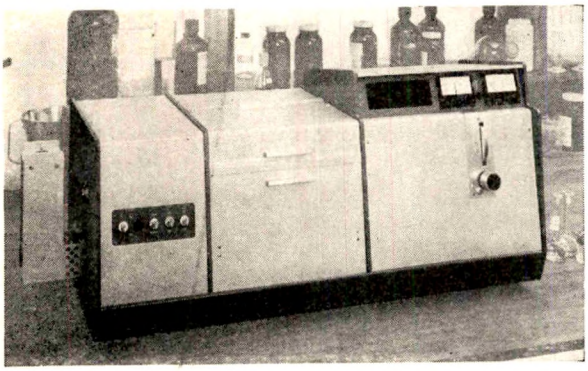
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NATURE Volume 228
OCTOBER 3, 1970

Green Paper to Burn

At the Conservative Party conference next week, the new British Government must finally declare itself on science and technology. Some hesitation in bringing forward new policies may be understandable and even seemly, but the point is now approaching when the government will lose the initiative and even its reputation if it allows uncertainties about future organization to persist. Several related issues need to be decided. There is the organization of government laboratories, called into question by Mr Wedgwood Benn's Green Paper and by the protests which it occasioned from the directors of laboratories concerned and from organizations such as the Confederation of British Industry, the present Minister of Technology's old stamping ground. There is the question of how the research councils should be related to each other and financed, and a host of important if tactical questions such as British membership of the project to build a 300 GeV proton accelerator beneath the CERN umbrella—Mrs Margaret Thatcher's trip to Geneva last week with Sir Brian Flowers as usher is probably a sign of which way the wind is blowing. Finally, there is a whole complex of questions about the organization of universities and, for example, their relationship with the polytechnics. Until something is said, the new government cannot boast of having a policy for science, technology and higher education.

On the government laboratories, the first need is somehow to resolve the uncertainties which have been generated by Mr Wedgwood Benn's Green Paper and its aftermath. What is the government to do? One obvious need is to complete the reorganization of the Atomic Energy Authority begun in 1968 and now suspended in uncertainty. Although the decision to saddle private industry with responsibility for the development of fast reactors should delight the Conservatives on ideological grounds, there is a risk, to say the least, that private industry will not be able to handle the development of fast reactors if, through no fault of the companies now engaged in this work, the problem of making economic reactors turns out to be more difficult than the optimists now expect. A modest insurance policy would seem to be worth while, which in turn implies that there should be continued government involvement in nuclear power developments. This is where the new government may have to work over its predecessor's thinking a second time. The arrangements which have been suggested for the organization of the fuel company are sensible enough, but nothing has yet been decided about the future of Aldermaston, which really ought to become a defence research establishment pure and simple. And the problem of Harwell persists—is it really going to become a successful freelance research association and, if so, should it remain within the present hierarchy of research establishments?

These tactical questions provoke more general considerations of the function of government laboratories as such. Obviously there is a need for some housekeeping. Defence research is an obvious claim on public funds. So too are laboratories such as the National Physical Laboratory, which have statutory as well as useful functions to perform. To be sure, it is important that in the management of these establishments, the best use should be made of scarce resources and of the opportunities which present themselves. Although there is no reason why defence research should decline in volume with the decline of expenditure on military equipment—the correlation might even go the other way—there is every reason to hope for considerable savings in the present pattern of defence research in Britain. Protestations to the contrary notwithstanding, it is exceedingly hard to believe that the Royal Aircraft Establishment at Farnborough, the Royal Radar Establishment at Malvern, the Atomic Weapons Research Establishment at Aldermaston, the Signals Research Establishment at Christchurch and a dozen other smaller defence laboratories can be at present fully stretched by the needs of the defence programme. Is this not a case in which the electronically inclined defence establishment could be made into a nucleus of a research organization for carrying out the long-term development of telecommunications which the new Post Office Corporation will be wishing, a decade from now, that it had carried out? One proper and important function of the complex of government laboratories is, after all, precisely to look after the development of fields of technology in which there is some realistic hope of long term benefit.

But what kind of organization will best suit the needs of government intervention in civil research and development under a Conservative government? Temperamentally, the new ministers will wish to give industry as much freedom as possible, but there is a limit to the extent to which *laissez faire* principles make sense. Evidently there has to be some means of regulating the application of industrial standards. Evidently there has to be some device for making sure that the very large industrial companies are not the only organizations able to afford industrial research. Yet experience has shown that the pattern of the cooperative research association is often unhelpful. Will the Conservatives choose to follow the United States Government in providing indirect subsidies to private industry by means of contracts for the supply of publicly required machinery or will they follow the traditional Labour method of helping causes con-

sidered to be good? Both instruments of intervention have their defects. Indirect subsidies by means of contracts are easier to provide in some fields (such as aircraft technology) than in others. And even well-meaning governments can make bad decisions about desirable goals in technology. Even though the new Conservative Government may be spending a comparatively small part of the GNP on the support of industrial research and development, it is important that it should find an equitable administrative machine for doing this. Quite possibly, the best instrument would be an extension of the research council technique towards the industrial field or, in other words, a straightforward application of what used to be spoken of as the Industrial Research and Development Authority in the days of the Trend Report, in the early sixties. Of the decisions which are required in the channelling of government support towards industrial research and development, the creation of suitable machinery is the most urgent need.

It goes without saying that the most ineradicable of any British Government's functions in the support of science and technology must be in the support of higher education and here it must sadly be acknowledged that Mrs Margaret Thatcher has done very little to give her voters and taxpayers confidence. Too willingly, she has been prepared to support the Labour Government in its mistakes. On July 23 this year, Mrs Thatcher went gratuitously as far as to say that "we endorse the role of the polytechnics as outlined in the White Paper of May 1966". By doing so, she has made it more difficult to see how the new government can bring about an easy relationship between the universities and the poor cousins by whom they are surrounded. Yet the present system in which the polytechnics and the universities are supposed to coexist in parallel should be on simple political considerations an offence against everything for which the Conservatives stand. First of all, the separation of these two instruments of higher education is meant to be a device for distinguishing between the academic sheep and the technical goats. Is this really consonant with the notion that the modern world is everybody's oyster? Second, the polytechnics are a burden on the rates. Does Mrs Thatcher wish to become known as the person who shed the burden of higher education from central government to the local authorities? And finally, of course, the polytechnics seem destined to be impoverished of people for teaching and of money for facilities. Is this consistent with the idea that higher education is the pursuit of excellence? In short, if Mrs Thatcher listens to what her colleagues say about the importance of education, she will either abolish the polytechnics altogether or —if she has sense as well as a capacity to hear—will merge them with the universities.

The issue is important because it affects the way in which young people will be educated in the remainder of this century. It is important for practical reasons because it affects the way in which the research councils must function in the years immediately ahead. To begin with, there are anomalies in the way in which at present the research councils enjoy an autonomy within a carefully restricted frame of reference. The Science Research Council, for example, is free to make research grants only within the United Kingdom and only so long as its activities seem not to conflict with those of other research councils or government departments. Although the research councils have an important influence on the pattern of university research, their influence is by no means decisive. Evidently, to tell from the experience of the past few months, the research councils are not their own masters when it comes to deciding whether to spend money on schemes such as the new proton accelerator at CERN. This, as it happens, is a field in which a rigorous application of the Conservative ideology might lead Mrs Thatcher and her colleagues to a position easily acceptable within and outside the government. For would it not make sense to arrange that research councils should be free to spend money where they choose? If the Science Research Council, for example, has set its heart on contributing towards the CERN machine, if it considers that such an expenditure would be in the British interest and if it believes that such a contribution could be made without losing the allegiance of physicists in British universities, is there not a case for letting the money be spent? To be sure, a prudent Science Research Council, edging its way towards such an expenditure of money would undoubtedly take care that the reasons for the expenditure were plainly understood, but that in itself would be a gain and not a loss.

Unhappily, no government can be relied upon to find its way towards these truths by its own devices and it must be admitted even by the present government's enemies that the quality of the advice available to British governments is at present poor. The amount of it is quite inadequate. The Council for Scientific Policy, which could well by now have seized the initiative for deciding issues of importance, such as the way in which the universities and the polytechnics should be related, has spent two placid years in the contemplation of its own future. Quite possibly, the new chairman, Dr F. S. Dainton, will turn out to be a heroic source of counsel, but he will need time to find his most economical stride. And it seems plain that the Central Advisory Council on Science and Technology under Sir Solly Zuckerman is living through a similar period of uncertainty. What will its function be in 1971? Will it still be in business then? Coupled with uncertainties about the future of the Ministry of Technology, these administrative difficulties are bound to sap morale. Good government requires that more permanent arrangements should be made. The trouble is that under the pattern of government which has grown up in Britain, no single minister can hope to have the power of disposition within his own control. Will Mr John Davies, Mrs Margaret Thatcher and even their more senior colleagues have the wit to see the urgency of the task they have so far neglected? Next week should tell.

New Planning for Planners

THE Council for the Protection of Rural England, not conspicuous for its adventurousness, has made in the past few days a sensible protest about the immunity of public authorities in Britain from the restrictions to which ordinary mortals are subjected by planning regulations and authorities. As part of its contribution to European Conservation Year, the council set out some months ago to study the problems created by the pursuit of familiar civilized objectives—agriculture, power generation, the construction of roads, the recovery of minerals and the conservation of water. The upshot seems to have been the setting up of working parties which have now come out with independent but impressively harmonious complaints that much of the damage done to the environment by these familiar activities could be prevented if public authorities themselves were more ready to practise what they preach. Unhappily, there are several circumstances in which the planning legislation which, on the whole, has worked well in Britain, exempts public authorities from detailed control. Some years ago, the attempt by the South-East Gas Board to build a gas holder in the centre of the rural town of Abingdon drew attention to the nature of this problem (and such a torrent of protest that the gas board backed down). The problem now is to ensure that water undertakings are not always free to flood deserted valleys without first winning the consent of local planning authorities, that electricity utilities live by the same rules as other people and that local authorities also have a say about the manner in which mineral resources should be exploited. In the long run, a strengthening of the planning legislation will be sufficient.

The issue may well be brought to a head next week at the public inquiry due to be held about the development plan for Greater London conceived five years ago and published early in 1969. In its way, the plan is a landmark in the process of making planning more effective—it begins with an explicit statement by the Greater London Council about the need somehow to ensure that the population of London remains more or less what it is at present, which implies that deliberate steps must be taken to restrain the centrifugal tendencies of this among other urban populations. But the plan has also become a local source of contention for the way in which it involves the building of a network of urban motorways within the inner city. Is it right that houses should be knocked down to make room for motorways? Who, in any case, can be sure that the city council is the best judge of what the future will require? May not the ideal pattern for a city of the next century be radically different from the uniform distribution of dwelling houses and offices over the face of south-east England?

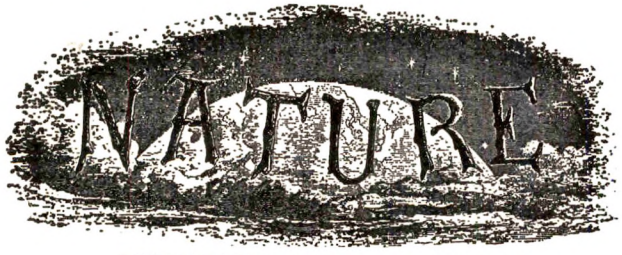
University Unrest Persists

THE Scranton Commission (see page 8) has produced a worthy and even a sober report. It could well have been a more disturbing document if there had been time for the members of the commission to digest the news of the explosion in the mathematics building at the University of Wisconsin on August 24 (see *Nature* **227**, 1085; 1970), which has cast a gloom on the American academic world. At the beginning of the new academic year, the question is not so much whether decent government can bring students and faculty together but whether universities as institutions can hope to survive in the face of all the uncertainties which at present surround them. The fearful loss of good men as university presidents, and the haunting difficulty of replacing them, is one obvious symptom of the present crisis. Beyond that, however, lies the question whether the student body will recover from the upsets of the past few years. The Scranton Commission is both liberal and humane in its appraisal of the immediate problem of how to make American campuses more peaceful. It may have underestimated the extent to which the changes now under way may bring radical upheaval of the teaching pattern in the years ahead.

To be more careful about the mixing of government and university business is, of course, a good objective. So too is the commission's recommendation that public officials—no doubt Mr Agnew is the name that comes most easily to mind—should consider more carefully the effect of their public pronouncements on the tension in the universities. It will be a blow for Mr Nixon that the commission should have pointed out that an ending of the war in South-East Asia would be one of the best ways of ensuring peace in the universities as well, but that has been self-evident for a long time even if, by now, the war is more a catalyst than a cause. The idea that university programmes for the training of military officers should be abandoned will also be a blow for the American Government but by no means a serious one. There are other ways of making soldiers than this. The notion that National Guardsmen should be better trained is sensible enough, but so familiar that it can hardly be counted an important component in the commission's report. The belief that the Administration itself, and the President in particular, can "exercise reconciling moral leadership" and thus help to keep the universities in order is not the strongest part of the Scranton Commission's report. The objective should rather be to find some way of making sure that the university system does not shake itself to pieces whenever the students and the President are at loggerheads.

The weakest part of the Scranton Commission's report is that which deals with the university. It is all very well to ask that university faculties should acknowledge the need for qualitative changes to accommodate the much larger numbers of students now clamouring for university places—the essence of the difficulty with which faculties are confronted is that of reconciling flexibility and firmness. In the past few years, a great many universities have leant over backwards to give students what they ask for—meaningless courses on subjects such as Black studies, for example—and have in the process lost their self-respect. There is a point at which a university faculty should insist on its competence to teach only what it knows, and thus deny the Spockian view that a university should by definition be able to provide all young people with whatever they may want. The essence of the present conflict between the universities and their customers—the students—is that the interests of both sides are in genuine conflict. Whatever the effects of the Scranton Commission's report—and they are almost certain to be beneficial—there is no avoiding the tendency towards universities in which faculties and student bodies are increasingly isolated from each other. Whether in these circumstances universities will be as competent as in recent decades to provide students with a vocational training of great value to industry, commerce or government is less certain. In short, the changes in the function of the American university which are now in prospect may go much deeper than the Scranton Commission imagines.

100 Years Ago



SCIENTIFIC ADMINISTRATION

NO reflecting Englishman can contemplate the great events of the present time without desiring to extract from them such warnings and instruction as may be serviceable to his country in case she should be drawn into war. Accordingly the press teems with discussions on every branch of the military art. We leave these to others. In what respects the constitution, the discipline, the training, and the arming of one army are superior to those of the other, it is scarcely the function of this journal to point out. Taking the broad fact that the Prussian army has, up to the present point, proved itself superior on the whole to that of France, and indeed to any army that has ever existed—a fact that no unprejudiced person will deny—let us ascertain, if we can, whether there may not be recognised some one broad cause to account for so broad a fact.

From Nature, **2**, 449, *October* 6, 1870.

OLD WORLD

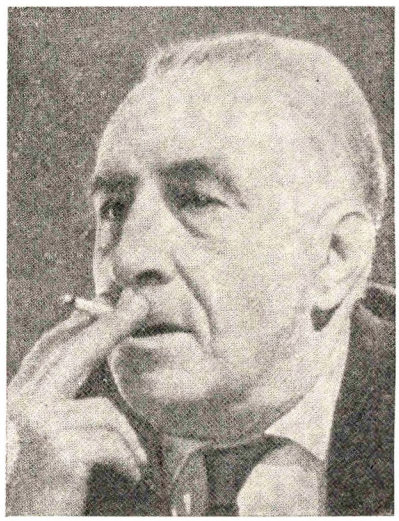
SOVIET UNION

Arrests and Reinstatements

by our Soviet Correspondent

THE wave of protests by scientists and intellectuals against the limitation of academic freedom by the Soviet state continues. The latest to be arrested is the mathematical physicist, R. I. Pimenov, who, according to underground sources, was recently arrested in Leningrad and is awaiting prosecution under the criminal code of the Russian SFSR on a charge of disseminating "falsehoods derogatory to the Soviet State and social system".

Pimenov, who is 40 years of age, is well-known for his monograph on *Spaces of the Kinematic Type*, which has appeared in print both in the Soviet Union and in the USA. The facts behind the charges against him are not easy to ascertain, but certain rumours have linked his case with that of geneticist Zhores Medvedev, forcibly committed to a Kaluga mental hospital earlier this year for circulating privately produced (*samizdat*) essays criticizing state interference in science and the censorship of private mail.



Nikolai V. Timofeev-Resovskii.

Happier news, however, has been received of another member of what may be termed the "Medvedev circle". In August 1969, Medvedev's friend and colleague, Nikolai V. Timofeev-Resovskii, at the Obninsk Institute of Radiology was forced into premature retirement on a minimal pension. Timofeev-Resovskii has always been a somewhat "doubtful" character to the authorities, in spite of an excellent record in the Soviet cause in the revolution and civil war, since, during the '20's and '30's he was working in Germany and was trapped there by the war. In spite of the fact that he played an active part in anti-Nazi resistance work, and his own son fell a victim to the SS, Timofeev-Resovskii was accused of being a collaborator and a German spy and was imprisoned for several years. Following his release, he had worked for some time as head of the section of general genetics and radiobiology in Obninsk. Unfortunately, in 1968, certain well-wishers, without his knowledge, proposed his election to the Academy of

Sciences. This drew considerable publicity and not only was his election quashed, but a campaign against him was started at the Party level. He was accused of being an "emigré" and a "Morganist", resulting in his premature retirement on a near starvation-rate pension.

Happily, Timofeev-Resovskii, winner of three major international prizes for genetics (The Darwin Plaque, (GDR), the Mendel Medal (Czechoslovakia) and the Kimber Prize (USA)), seems to have received a certain amount of rehabilitation in recent months. The current issue of *Priroda* (No. 8, 1970) publishing an article of his, "The Biosphere and Man", which is an expansion of a lecture delivered in February 1970, refers to him as now working at the Institute of Medico-biological Problems of the Ministry of Health of the USSR. If this news is correct, it can only be a favourable sign, similar in significance to Medvedev's own release from the mental hospital, after the wave of protests on his behalf by other campaigners for intellectual freedom.

ASTRONOMY

Collaboration in South Africa

A REMARK in the annual report of the Science Research Council that discussions between the SRC and the South African Council for Scientific and Industrial Research are making good progress seems to have been something of an understatement. The SRC announced last week that agreement has been reached "in principle" to merge the facilities at the Royal Observatory at the Cape and at the Republic Observatory in Johannesberg into a single station situated near Sutherland, some 250 miles north-west of Cape Town.

Although some facilities will be maintained at the Cape Observatory, the new station, which will be called the South African Astronomical Observatory, will be the chief observatory in South Africa. In the short term, the SRC and the CSIR will have little to gain from the venture, but in the long term it offers considerable scope for expansion—the two separate observatories, situated in the centres of large cities, are unsuitable for development.

The new observatory is expected to come into operation early in 1972, when it is hoped that three telescopes from the Cape Observatory and two from the Republic Observatory will have been moved to the site. It will function as an institute of the CSIR, but the SRC will retain some say in its policies through an advisory committee, consisting of representatives of the SRC and the CSIR. Sir Richard Wooley, the Astronomer Royal, and director of the Royal Observatory at Herstmonceux, Sussex, will retire at the end of 1971 to become the first director of the new observatory. The SRC at present spends about £125,000 a year on the Cape Observatory but expenditure on the new observatory could be as much as £200,000 a year.

Another statement in the SRC's annual report, that approval has been given to "investigations preliminary to consideration of a proposal to plan an accurately figured dish capable of operating in the millimetre band", has been couched in vague terms for good reason, it seems. Sir Brian Flowers, chairman of the SRC, pointed out last week that millimetre wavelength astronomy is one area of research which must regrettably be passed over because of lack of funds. The difficulty is that such a dish must be accurate to within about one-sixteenth of the wavelength under investigation and it therefore seems that the SRC has already made up its mind that construction of a fifty-foot dish to that sort of accuracy would be too expensive at present.

New Centre for NERC

MARINE pollution is to be the primary interest of a new institute which has been established by the Natural Environment Research Council (NERC). The Institute for Marine Environmental Research has been set up not only as a centre for new research, but also to bring together various projects already in progress in universities and other organizations, under the auspices of NERC. The new institute will eventually be housed at Plymouth, in a complex of research groups which is planned to include extensions to the existing Laboratory of the Marine Biological Association of the United Kingdom. The director of the institute will be Mr R. S. Glover, who will retain the directorship of the Oceanographic Laboratory of the Scottish Marine Biological Association in Edinburgh, which has been incorporated into the new institute.

The prime objective of the institute's research programme will be to provide a deeper understanding of the biological basis of the productivity of the oceans and the way in which they are influenced by natural changes and by human activity, which of course includes pollution. This will be done chiefly by monitoring long term changes in marine communities in relation to their physical environment. These studies are intended to complement those already undertaken by other organizations.

Within the new institute it is planned to extend the plankton surveys which were started in Hull in 1930 by Sir Alister Hardy, and which are now carried out from the Oceanographic Laboratory in Edinburgh. They are to be extended, and linked with environmental surveys developed in cooperation with universities and other organizations, to concentrate on inshore areas and estuaries which are susceptible to pollution. While the new buildings are being planned, the expenditure on monitoring at the Edinburgh laboratory will be increased from £150,000 to £250,000 a year.

PHYSICS IN FRANCE

Policy for Solids

A DEMAND that the French Science Research Council and physics departments throughout France should remedy the inadequacy of crystal growing facilities and of new materials has come from a French Government committee investigating the state of research on the physics of solids and liquids (*Prospective: Physique des Milieux Condensês, Le Progrès Scientifique*, No. 139;

Documentation Française, 4 fr.). The committee, headed by Professor J. Friedel of Orsay, has also some sharp criticism of teaching methods in French universities.

Why, the report proclaims, do physics departments persist in teaching undergraduates virtually nothing about simple molecules? And why are physicists so reluctant to try to fabricate new materials with interesting properties in the way that chemists do? Why, also, the total lack of summer schools in France on condensed media? On the research side, although the committee has clearly set out to forge something resembling a national science policy for condensed media the impression remains that the drive towards investigating new materials—amorphous solids and liquids, for instance—and the growing predominance of a handful of powerful techniques have established a momentum of their own which the committee has no wish to more than trim.

The committee suggests that a national laboratory be set up to tackle the vital problems of sample preparation including the growth of highly pure crystals, thin films and materials of special structure. This would be comprised of high calibre physicists and engineers of the CNRS and would complement the simpler facilities which should be provided in every major physics department. With regard to surface physics, the committee warns of the danger of rushing to apply new phenomena in devices before fundamental research on a wider range of materials has been tried.

The terms of reference of the committee, set up by the Délégation Générale à la Recherche Scientifique et Technique in conjunction with the French Science Research Council, the CNRS, were inspired by the report of a government scientific committee in 1968 which prescribed the need for tighter coordination and firmer prediction of scientific policy. One area which the committee believes French physicists are well poised to exploit is non-linear optics, which has blossomed forth recently as a technique for studying relaxation processes. It offers significant improvement over traditional shock tube methods and ultrasonics and has great potential for exploring phonon coupling, very fast chemical reactions and phase transitions. The development of the technique has been hampered by the limitations on laser wavelengths, and the committee underlines the urgency of research into low wavelength lasers and laser systems for producing extra short pulses.

Two new spectroscopes using synchrotron radiation and photoemitted electrons are singled out for special mention, primarily for studying electron states in solids. In its attitude to research on liquids, the committee echoes much of what was said in a recent report of the British Science Research Council on amorphous materials (see *Nature*, **227**, 108; 1970), which placed strong emphasis on neutron and X-ray studies and on computer simulation (molecular dynamics). Professor Friedel's committee looks forward, however, to the completion of the joint French–German high flux beam reactor in July 1971 at the Institut Laue–Langevin in Grenoble, and highlights some of the experiments on macromolecules, critical phenomena and magnetic scattering—to name but a few—which will be competing for the 10^{15} slow neutrons cm^{-2} s^{-1} from the reactor. Twenty experimental areas are being prepared for measurements in solid state physics and another fifteen will be installed later on. The reactor is also being equipped with a hot source to increase by a factor of 10 the flux of neutrons at 0·5 eV. The committee issues a reminder that the novelty of a high flux neutron beam should not be allowed to dull the complementary part played by other techniques in the study of condensed media.

Work on high magnetic fields seems to be flourishing in France. The next two years will see the installation of superconducting magnets for fields up to 150,000 Oersteds and the extension of pulse field techniques to reach 500,000 Oersteds for more than 0·1 s. A wide range of applications is envisaged including detailed studies of magnetic transitions and the effects on magnets of high pressures up to 7·10 torr. The scope for studying different forms of phase transitions is reiterated frequently throughout the report, but the committee fights shy of suggesting any possible redeployment of facilities to this end.

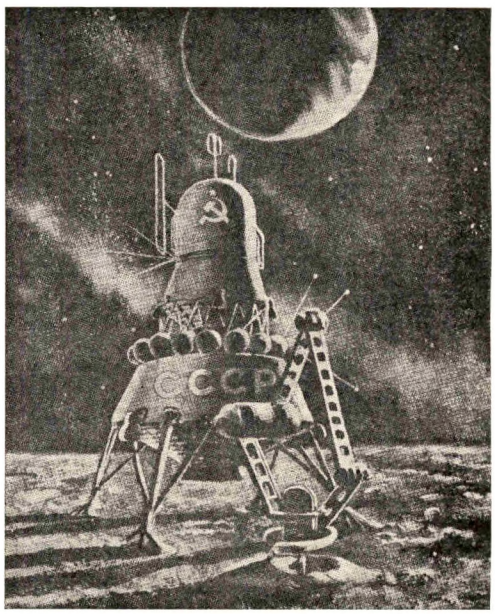
SPACE RESEARCH

After Luna 16

from our Soviet Correspondent

THE return to Earth of Luna 16 with its cargo of Moon rocks is an undoubted triumph for Soviet space exploration, but it also raises once again the thorny question of manned versus unmanned projects in Soviet space research.

Although it seems certain that the planning of a manned mission to the Moon once took priority in the Soviet space programme, the loss of the captain designate (Gagarin) and chief training officer (Belyaev) caused some considerable setbacks and consequent rethinking. According to Academician B. N. Petrov (*Pravda*, September 24, 1970), the Moon programme is at present conceived of in two stages: (1) "preliminary (reconnaisance) investigations with the aid of automatic spacecraft; (2) subsequent multilateral



Artist's impression of Lunar 16 on the Moon. Drawn by A. Sokolov.

systematic research and investigation by means of both automatic and piloted craft for the wider use of the achievements obtained for the aims of science and the national economy".

At present, Soviet space flight is still in the early reconnaisance stage. Petrov pointed out that manned Moon-flights and interplanetary missions would not only involve considerable additional expense, but they also present a risk to human life which is unjustifiably great at the present time. Petrov gives no absolute costs, but he does claim that a manned mission would cost more than twenty times as much.

For the time being, therefore, Soviet long-range space flight seems likely to remain automatically based. After the acquisition and safe return to Earth of the lunar samples by Luna 16, what other robot successes may be expected in the future ? The academicians who have commented on the current exploit are, as usual, not eager to commit themselves. B. V. Raushenbakh, a corresponding member of the Academy of Sciences of the Soviet Union and an expert in automatic control, suggests in a *Pravda* interview, however, that one of the advantages of an automatic space-craft is that it can operate in conditions which would be highly hazardous for a human crew, notably in radiation belts. This might be taken to mean that a future project of equal scientific if less publicity value will be a prolonged investigation of the Earth's radiation belts. A long-term mapping of the activity of the radiation belts would, of course, be a reasonable preliminary to the construction of a manned earth orbital space station, as a base for manned Moon missions.

MOLECULAR BIOLOGY

No Home for EMBO Laboratory

A SITE in Munich for the International Laboratory of the European Molecular Biology Organization has just been offered by West Germany to a working group of the European Conference on Molecular Biology (the association of fourteen European states which provides the funds to support EMBO) which met earlier this week at the Hague to consider what steps the ECMB should now take in establishing the proposed EMBO laboratory.

The second plenary session of the ECMB itself will take place at the end of next month to consider the proposal of the working group that four further working parties should be set up to consider in detail the budget of the EMBO laboratory, the way in which it should be organized, the legal framework within which it should operate, and offers of four sites where it could be built.

Although tentative sites for the laboratory were offered by France, Austria and Italy some time ago, and EMBO itself proposed that the most convenient site would be Geneva (where CERN is already located), the offer of the site at Munich is the first firm proposal and it seems quite likely that the ECMB will decide to accept it. One of the attractions of this offer is that temporary accommodation could be provided in new buildings which are presently under construction at the site; this would enable the laboratory to start work before its own buildings could be completed.

If the ECMB agrees to the proposal of its working group, the four working parties could start work at the end of November and report back to an extraordinary session of the ECMB in April. This would mean that work could start next year on building the laboratory and the original aim of having it in operation by 1973 and fully functional by 1975 may still be met.

EMPLOYMENT

Biologists leave the Laboratory

BIOLOGY graduates are likely to have to broaden their horizons when seeking employment in the future. There were signs at a meeting of members of the Institute of Biology in London on September 25 that biology and allied disciplines will not be able to absorb all the biologists who want to stay in research. Too many biologists, it seems, have been reluctant to drop the study of plants and animals and pursue careers in banking, operational research, the civil service and so on.

Professor L. Broadbent (University of Bath) said that although there are more jobs than graduates in some branches of biology, the percentage of biologists unemployed six months after graduation has been increasing recently, according to figures from the University Grants Commission. Although unemployment is not a big problem for biochemists and microbiologists, 6·4 per cent of general biologists, botanists, zoologists and some other specialists were unemployed in 1968, compared with 3·4 per cent for all scientists.

The general pattern of employment among biologists, he said, was 36 per cent in school teaching, 7 per cent in further education, 22 per cent in universities, 22 per cent in state aided research and 13 per cent in industry. One of the best professions for a young biologist to enter is horticulture said Professor Broadbent; there are some very good jobs for well trained people. But biochemistry still takes the most biologists—18–26 per cent of them work in this discipline.

More biologists are taking the plunge into industry and commerce because, said Professor P. N. Campbell (University of Leeds), it is becoming increasingly difficult for those with a degree less than a good II(i) to find research posts. He was pleased to note that some really good students are now taking up school teaching. Professor Campbell also thought that the increase in medical education now in progress would absorb a good many biologists.

Professor G. Belyavin (University College Hospital, London) predicted that biologists will find a warmer welcome in medical research as the need for fundamental investigators increases. He did not think that prejudice against non-medically qualified people in medical laboratories would ever quite disappear, but the barriers were coming down. Diagnostic techniques are being developed by biochemists, microbiologists, cytologists and so on, and Professor Belyavin saw the demand for their services increasing in the future.

The problem raised for the educationalists is, of course, what to teach biologists to equip them for the roles they will have to play in the future. One step towards the general education of biologists is the undergraduate course in human biology, which is about to begin in Oxford. One of its founders, Professor J. W. S. Pringle, describing the broad scope of biology, anthropology and sociology that it contains, said that, when asked what jobs his graduates will do, he replies that he hopes they will become prime ministers.

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NEW WORLD

Scranton Commission Prescribes

by our Washington Correspondent

THE President's Commission on Campus Unrest has made a visibly sincere effort to understand what students are bothered by and to prescribe against the causes of violent dissent at the universities. In a report of some 350 pages made public this week the commission criticizes the role of students, faculty and administration as well as the part played by the Federal government and the police in response to university unrest. A major theme of the report is that universities and faculty members should make substantial accommodations towards student demands for better teaching and more responsive administration; at the same time the commission calls for universities to adopt a firmer and more clearly defined stance towards disruptions.

The commission, chaired by the former governor of Pennsylvania, Mr William W. Scranton, was appointed by President Nixon in June in the wake of the nationwide riots that followed the invasion of Cambodia and the shooting of the four students at Kent State University. In the past few weeks there have been several attempts to shoot the commission's findings down before they were even launched, based on the expectation that they would blame the President's Cambodian policy for the rioting. Although the commission could not ignore the part played by the invasion, its analysis of the problem has gone far deeper than the immediate causes of the unrest. But the attempt, such as it is, to spare Mr Nixon's political profile may not be much help. With an eye on the approaching Congressional elections most politicians see more sense in excoriating student unrest than in trying to understand it.

The commission prefaces the list of its recommendations with the observation that "the roots of student activism lie in unresolved conflicts in our national life, but the many defects of the universities have also fuelled campus unrest". Its recommendations, many of them couched in general rather than specific terms, are addressed to the President, to the government, to those charged with enforcement of the law, and to universities and students (see page 9). Both the President and government officials are urged to avoid divisive rhetoric. The President is reminded of the importance of ending the Vietnam war. The government should increase its support of education, particularly its financial aid to Black colleges and universities. Officers sent on to the campus should be trained how to control disorders without resorting to excessive measures. Universities should strengthen their disciplinary procedure and acknowledge with new courses and organization the changes imposed by the expansion of higher education and the emergence of the "new youth culture". Universities and faculty members should reduce their outside commitments, since these have reached a volume that seems to compromise the devotion to research and teaching. Finally, the students should learn to appreciate the necessity of tolerance and allow hearing to views with which they disagree.

In its chapter on university reform the commission says that in the past few decades there has been a serious erosion in the universities' sense of mission and a failure to give priority to their central purposes. The failure, the commission says, has arisen in four major ways. First, academic freedom has been threatened by increasing political pressure both from within the university and outside it. Second, teaching and research have been compromised by devoting vital resources to peripheral activities. Third, too few new and academically respectable programmes have been developed because the pace of educational change has been too fast at some universities and too slow at others. Finally, the traditional assumptions about how a university should operate have been invalidated by the erosion of the "minimal but essential sense of community once shared by most faculty, students and administrators".

Turning to the political pressures on the university, the commission notes that universities may have to act politically when the interests of higher education are at stake. But such institutional positions compromise the academic ideal of neutrality and should be taken only as a last resort and when there is clear evidence concerning the direct effects of government policy on higher education. Political involvement of individual members of universities is, of course, quite another matter.

The commission observes that services performed by universities for other organizations have become a major source of income that on some campuses now provides the bulk of the operating budget. The commission recommends that these outside service commitments be reduced; in particular, although much of the research undertaken for the Department of Defense has academic merit, universities should refuse to undertake any classified research and should "terminate existing classified projects unless it is clear that the undesirable results of undertaking such a project are outweighed by compelling advantages". The commission notes ironically that student protests against defence research have increased as the quantity of research has decreased. Nevertheless, the requirement appended to the Military Authorization Act of 1970 that all defense research projects bear a direct and apparent relationship to a specific military function has placed university scientists in the embarrassing position where, having certified for the benefit of Congress that their research has a direct military application, they must then contend with student accusations that they are "working for the war machine". Noting that the universities cannot avoid sharing in the national defence research effort, the commission says it "does not advocate termination of either defence-funded or government-funded research in general, but we urge that such research be planned so as to interfere as little as possible with the normal function of the schools in which it takes place".

Not only the university as an institution but its individual members should reconsider their services to outsiders. The "entrepreneurial professor" who is so heavily engaged in outside research that he has become

almost inaccessible to his students is compromised in their eyes because of his "attachment to rewards more material than the discovery of truth". The commission recommends that universities should establish general guidelines governing the acceptance of outside research both at an institutional level and by individual faculty members. The guidelines should restrict any outside service activities that drain energies away from research and teaching, but they should be sensitive both to the individual rights of faculty members and to the differences between teachers in various disciplines. Committees of faculty members and administrators should develop and enforce the guidelines. The general criterion should be not that "every full professor is entitled to his sideshow" but whether the sideshow will contribute to teaching or to knowledge.

The commission finds some justice in student complaints at the quality of teaching, generally blamed on excessive outside faculty commitments, university reward systems biased in favour of research and publication and faculty indifference to improving the teaching system. As one way of improving the quality of teaching, the commission urges that the practice of tenure be reconsidered. "Tenure has strong justifications because of its role in protecting academic freedom of senior faculty members. But it can also protect practices that detract from the institution's primary functions, that are unjust to students, and that grant faculty members a freedom from accountability that would be unacceptable for any other profession."

Another object of the commission's criticism is that practice of relying heavily on graduate students to do much of the teaching. Graduate students are "necessarily inexperienced, often distracted by the demands of their own degree programme . . . and in some cases deeply disillusioned". The commission says it would strongly recommend abolishing graduate teaching assistantships altogether were it not for the fact that the universities cannot at present afford to do so.

Major Recommendations

"We recommend that the President seek to convince public officials and protesters alike that diversive and insulting rhetoric is dangerous. In the current political campaign and throughout the years ahead, the President should insist that no one play irresponsible politics with the issue of 'campus unrest'.

"We recommend that the President take the lead in explaining to the American people the underlying causes of campus unrest and the urgency of our present situation. We recommend that he articulate and emphasize those values all Americans hold in common.

"To this end, nothing is more important than an end to the war in Indochina. Disaffected students see the war as a symbol of moral crisis in the nation which, in their eyes, deprives even law of its legitimacy. Their dramatic reaction to the Cambodian invasion was a measure of the intensity of their moral recoil.

"We recommend that the President take steps to assure that he be continuously informed of the views of students and Blacks, important constituencies in this nation.

"We strongly urge public officials at all levels of government to recognize that their public statements can either heal or divide. Harsh and bitter rhetoric can set citizen against citizen, exacerbate tension and encourage violence.

"We recommend that the federal government review all its current policies affecting students and universities to assure that neither the policies nor administration of them threatens the independence or quality of American higher education. At the same time government should increase its financial support of higher education.

"We recommend greatly increased financial aid for Black colleges and universities. All agencies of government that support such institutions should massively increase their grants to enable these colleges to overcome past shortcomings.

"Bombing and arson pose an increasing threat to lives and property on campus. We urge prompt enactment of strict controls over the sale, transfer, and possession of explosive materials.

"Too frequently, local police forces have been undermanned, improperly equipped, poorly trained, and unprepared for campus disturbances. We therefore urge police forces, especially those in smaller communities, to improve their capacity to respond to civil disorders.

"The university should promulgate a code making clear the limits of permissible conduct and announce in advance what measures it is willing to employ in response to impermissible conduct. It should strengthen its disciplinary process. It should assess the capabilities of its security force and determine what role, if any, that force should play in responding to disorder.

"When criminal violence occurs on the campus, university officials should promptly call for assistance of law enforcement agencies.

"When faced with disruptive but non-violent conduct, the university should be prepared to respond initially with internal measures. It must clearly understand the options available to it and be prepared to move from one to another if it is reasonably obvious that an earlier tactic has failed.

"Faculty members who engage in or lead disruptive conduct have no place in the university community.

"The university, and particularly the faculty, must recognize that the expansion of higher education and the emergence of the new youth centre have changed the makeup and concerns of today's student population. The university should adapt itself to these new conditions. We urge that the university makes its teaching programmes, degree structure, and transfer and leave policies more flexible and more varied in order to enhance the quality and voluntariness of university study.

"We call upon all members of the university to reaffirm that the proper functions of the university are teaching and learning, research and scholarship. An academic community best serves itself, the country, and every principle to which it is devoted by concentrating on these tasks.

"One of the most valid criticisms of many universities is that their faculties have become so involved in outside research that their commitment to teaching seems compromised. We urge universities and faculty members to reduce their outside service commitments. We recognize that alternative sources of university funding will have to be developed to take the place of the money attached to these outside commitments.

"Large universities should take steps to decentralize or reorganize to make possible a more human scale.

"University governance systems should be reformed to increase participation of students and faculty in the formulation of university policies that affect them. But universities cannot be run on a one man, one vote basis with participation of all members on all issues.

"Students must protect the right of all speakers to be heard even when they disagree with the point of view expressed. Heckling speakers is not only bad manners but is inimical to all the values that a university stands for.

"Students must face the fact that giving moral support to those who are planning violent action is morally despicable."

APOLLO CUTBACK

Has NASA Finished with the Moon?

by our Astronomy Correspondent

WITH Luna 16 back on Soviet soil, carrying an unspecified amount of lunar material, it is conceivable that the Soviet Union could be taking the initiative in the exploration of the Moon within a few years. Luna 16 has come at an awkward time for NASA which has just turned its back on the Moon; planetary scientists in the west are still licking their wounds after the loss of a further two Apollo missions, cancelled apparently so as to keep within the budget for the current financial year and to have something in hand for possible post-Apollo programmes. Only four more flights to the Moon are now planned, Apollos 14, 16, 17 and 18. NASA's decision flies in the face of both the Space Science Board of the National Academy of Sciences and NASA's own Lunar and Planetary Missions Board. On August 13 the two boards were asked by the then administrator of NASA, Dr Thomas O. Paine, to consider the scientific consequences of either continuing with the Apollo programme as then planned or of cancelling two of the missions. "Any reduction in the number of missions will seriously threaten the ability of the total Apollo programme to answer first-order scientific questions", was the boards' answer in their joint report signed by Professor Charles H. Townes of the University of California (Berkeley), chairman of the Space Science Board, and Dr John W. Findlay, assistant director of the National Radio Astronomy Observatory and chairman of the Lunar and Planetary Missions Board. "We are on the very beginning of a learning curve, and it is clear that the loss of one mission will have much greater than a proportional effect on the instrumented experiments and, more critically, on the design and execution of the geology experiments involving the astronauts". Possibly with an eye to the prevailing lack of public enchantment with space science, the report ends by saying that "the consequences of such failure [to answer first order questions about the Moon] for the future of the agency and, we believe, for large-scale science in this country, are incalculable".

By rejecting cancellation, the two boards agree with everybody else that the elementary questions about the origin of the Moon will not be solved until samples have been collected from a variety of sites. Although the capability of the new series of Soviet Moon probes which seems to have begun with the unsuccessful Luna 15 nearly fifteen months ago is an unknown quantity, they, rather than NASA, could soon be providing the material from the desirable diversity of sites. How successful the Luna series will be at this job depends chiefly on whether the ascent stage brings back egg-cupfuls of dust or intact pieces of rock, which for the moment seem to have more to say about the history of the Moon, and whether it gropes for samples like a blind man or is fitted with television so as to pick and choose. Moscow has let slip that a drill penetrated fourteen inches below the surface, so Luna 16 would seem to have some sophistication.

While Luna 16 is laying the foundations for a possible swing towards lunar science in the Soviet Union, in the West people are trying to come to terms with the latest cancellations in the Apollo programme. At best Houston will now receive samples from only six sites, including the two mare sites already visited. After June 1972 no more Apollo flights to the Moon are planned. Although NASA officials must be glad that the cancellations which have been in the air for some time, to the detriment of NASA's planning, have at last been agreed on, the onus is now on the scientists to pick the most profitable sites. Fra Mauro has already been chosen for Apollo 14 in January, of course. The final three missions will be in July 1971, January 1972 and June 1972, and the landing sites in the running are the Marius Hills, Copernicus, Davy, Hadley Rille and Descartes. It is thus conceivable that there will be no samples from a rille, or from the central peaks of Copernicus which are believed to have originated deep within the Moon.

Although the Space Science Board and the Lunar and Planetary Missions Board grudgingly admit in their report that the loss of Apollo 15 from the programme would be bearable, they refuse to countenance the loss of Apollo 19. Apollo 15 was to be the last mission before a factor of two jump in the capabilities of the Moon flights, to be brought about by improvements in the space suits and a doubling of the scientific payload that can be landed on the surface, but chiefly by the inclusion of a lunar vehicle which will be carried from Apollo 16 on. But the loss of Apollo 15 is regarded as serious because it means one less site to be sampled, one less scientific package left on the Moon, and one less opportunity for gaining experience before the scientific exploration begins in earnest with Apollo 16. But in their report to Dr Paine the boards saw the cancellation of Apollo 19 as little short of disastrous because it will reduce by at least 20 per cent the total time spent on lunar surface activity during all the Apollo flights, and the total length of lunar traverses will be reduced by at least 25 per cent. It will now be up to NASA's scientists to make the most of the four flights left by choosing the best of the experiments that would have been carried on the original six flights, and by trying to accomplish next year and in 1972 the experiments that would have taken until the last two Apollo flights that were scheduled for 1974 to prepare.

By cancelling Apollos 15 and 19, renumbering the remaining missions from 15 to 18, Dr Paine says the Apollo budget for 1971 will be reduced by $42·1 million, which will go a long way to making up the deficit of $64 million between what NASA asked for and what they will almost certainly receive. Further appreciable savings will come by deferring the construction of new laboratories and by savings on the tracking and data acquisition side related to the cutback in the Apollo programme. But the cancellations also mean that NASA will have two Saturn 5 boosters in hand which could be mothballed for possibly as long as ten years. Dr Paine sees them as a national resource which can be used in the Skylab project, for tests of the Nerva nuclear rocket, or for manned or unmanned launches to a geostationary orbit. He also says that the cancellations will make things easier in 1972, 1973 and 1974 when money will be freed for future programmes such as the space shuttle and the space station. The same time interval should see several important unmanned projects reaching important stages, the high energy astronomical observatory and the grand tour flights, for example.

Once the Skylab programme is over in mid-1973,

there are no further plans for manned spaceflight until the first tests of the space shuttle, at present little more than a gleam in the eye. The optimists say that could be in 1976 or 1977. For the planetary scientists in the West, the cancellations have come just when appetites have been stimulated by the first two collections of samples. According to Dr Findlay "all scientists would regard the chief loss is the danger, the probability in fact, that we will not get our first order scientific answers about the Moon from the Apollo programme".

It is implicit in this that the elimination of Apollos 15 and 19 will give NASA more elbow room when the Skylab programme is under way in November 1972. It seems a poor swap, however—people are hard-pressed to give a scientific justification for the Skylab programme that will compare with the value of a visit to a lunar rille, for example. NASA's decision hurts because it has brought home to people that science has never been high on the agenda for manned space flight. Following the ructions during Apollo 11 when the lunar scientists complained of being too far from the action, science has had a hey-day in the Apollo programme. The traverses that the Apollo 13 astronauts would have followed were carefully thought out, and people have had a merry time thinking up experiments that could be carried on later flights. Now after little more than a year the real state of play has been brought home. "There is almost nothing in manned space flight I would justify on the basis of science. Science is an adjunct to manned flight", the acting administrator of NASA, George Low, is reported as saying. To be fair, however, NASA's continual searching for new fields to conquer which appears to be behind the Skylab project and the shuttle is as much as anything to appease a Congress that is unenamoured of space activities.

DEFENCE

Arms Race Soldiers On

by our Washington Correspondent

AMERICAN spy satellites orbiting over the Soviet Union have spotted 18 new sites at which construction of SS-9 missile silos has begun. Since the giant missile is usually deployed in groups of six, this raises from 220 to more than 300 the number of SS-9s being deployed by the Soviet Union, Dr John S. Foster, director of Defense Research and Engineering, announced last week. The new silos, first detected in July, represent a renewal of the Soviet SS-9 programme which, whether coincidentally or otherwise, has been kept on ice since the Strategic Arms Limitation Talks (SALT) began in November last year.

The SS-9 missile is a large launching vehicle carrying either a single warhead of 25 megatons or three warheads each of five megatons. Its deployment is alarming to the United States since it is regarded as capable of destroying Minuteman missiles in their hardened silos and as such is a first strike weapon. (The smaller but more accurate multiple warheads which the United States will begin deploying in its Minuteman III missiles are, like the SS-9, silo-busting weapons). Dr Foster's announcement about the Soviet resumption of the SS-9 programme was made, as it happened, a day before representatives of the Senate and the House of Representatives met to discuss the two chambers' different versions of the defence procurement authorization bill which includes the funding for the anti-ballistic missile (ABM) or Safeguard System. Despite Dr Foster's announcement the Senate's position on Safeguard, which allows work on ABM systems to begin at only two more sites in addition to the present two, prevailed over the House's version which would have allowed the Administration's request to expand the ABM system to six more sites, making eight in all. This proposal would have provided a blanket defence of the United States against a "light" missile attack, such as the Chinese may soon be in a position to mount. In the Senate the Chinese defence aspects of Safeguard were killed off at the committee stage before the bill had reached the floor of the House.

In the Senate debate (see *Nature*, **227**, 770; 1970) the spokesmen for the Administration made much of the argument that expansion of the Safeguard system to two additional sites would serve as a bargaining counter in the SALT talks. The Soviet redeployment of the SS-9 is also seen as an attempt to gain more leverage at the SALT talks. Last week Ambassador Gerard Smith, the chief US negotiator, was briefing the House subcommittee on national security policy on the progress of the SALT talks and the prospects of trading away the respective bargaining counters. Meanwhile, the Air Force is requesting approval for a $1,000 million programme to harden its 1,054 Minuteman sites, presumably as an insurance policy against the ABM system either not working or being traded off.

ENVIRONMENT

Senate wins Clean Car Race

by our Washington Correspondent

AMERICANS may be able to breathe again if a bill passed by a 73 to nil vote in the Senate last week becomes law. In a list of measures far more stringent than those called for by President Nixon, the Senate has set out to bring the major sources of air pollution under control by 1975. The most controversial demand is that, beginning in 1975, all new cars must meet the exhaust emission standards which the Administration had proposed should be met only by 1980.

The Senate bill also requires certain industries to install in all new factories and extensions to old ones the most advanced technology available for reducing air pollutants. An important general measure is the provision for national air quality standards setting levels protective of health for the major known air pollutants. These are particulates, sulphur dioxides, hydrocarbons, carbon monoxide, photochemical oxidants and nitrogen oxides, which together account for more than 98 per cent by weight of all air pollution.

Passage of the bill is a triumph for Senator Edmund S. Muskie, chairman of the subcommittee on air and water pollution, but the extent of his final victory will depend on what compromise is reached with the House of Representatives which earlier this year passed as mild a bill as President Nixon had asked for. The present legislation is the third attempt to make air polluters desist since the Clean Air Act of 1963, but it departs from the philosophy of its predecessors by setting standards and letting industry develop the technology to meet them. The previous attitude has

been to see what industry claims is attainable with the technology at hand and making that the basis of legislation, whilst allowing pollution that it is not economically feasible to control to continue unabated.

The policy of putting the horse before the cart has drawn the predictable reaction from the car industry. "General Motors does not at this time know how to get production vehicles down to the emission levels that your bill would require for 1975 models", the president of General Motors, Mr E. N. Cole, wrote in a letter to Senator Muskie; "accomplishment of these goals, as far as we now know, simply is not technologically possible within the time frame required". The refrain was echoed by Mr Lee Iacocca, president of Ford's North American automotive operations, who said the requirement to reduce vehicle emissions by 90 per cent "could prevent production of cars after January 1, 1975".

Table 1. AUTOMOBILE EMISSIONS PERMITTED BY PROPOSED FEDERAL STANDARDS AND BY THE SENATE BILL (IN GRAMS PER MILE)

	Hydrocarbons, new test	Carbon monoxide, new test	Nitric oxides, old test	Particulate matter, old test
Uncontrolled	14·6	116·3	4·0	0·4
1970 standard	2·9	37·0	—	—
Proposed 1975 standard	0·5	11·0	0·9	0·1
Proposed 1980 standard	0·25	4·7	0·4	0·03
Bill language (90 per cent reduction of 1970 or uncontrolled)	0·29	3·7	0·4	0·04

The princes of Detroit might have won more conviction for the prophecies of their own demise had it not been for the annual Clean Air Car Race, run between the Massachusetts and California Institutes of Technology, in which student engineers are scored for the cleanliness as well as performance of their vehicles. Results of this year's contest, announced only a few days before the Senate debate, showed that the Federal emission standards proposed for 1980 (and by Senator Muskie's bill for 1975; see Table 1) were well within the grasp of the college teams, even though no single vehicle met the levels set for all three classes of emission (hydrocarbons, carbon monoxide and oxides of nitrogen). The winner of the race, a modified Ford Capri, entered by Wayne State University, emitted 0·19 grams per mile of hydrocarbons and 1·48 of carbon monoxide, both of which are better than the standards set by the Senate bill, and its emission of oxides of nitrogen was very close to standard.

Where Wayne State University had led, could not Ford and General Motors follow? Their wellwishers on the floor of the Senate explained that there was all the difference in the world between the Wayne State entry and a vehicle suitable for mass production. For one thing, the student car had four converters using a prohibitively expensive platinum catalyst; for another, the manufacturers are being required by the bill to guarantee their vehicles' cleanliness for 50,000 miles. The Senate on this occasion refused to be blinded with technology. Mr Muskie pointed out that the deadline in his committee's bill allows the car manufacturers two years to do the necessary research and development and another two years in which to apply that technology to the mass production of automobiles. If the technology to get to the Moon could be developed in ten years, Mr Muskie implied, the requirement to reduce car exhaust emissions by 90 per cent in four years was asking for rather less.

Table 2. EMISSION OF AIR POLLUTANTS IN THE UNITED STATES, 1968 (MILLIONS OF TONS)

Source	Carbon monoxide	Particulates	Sulphur oxides	Hydrocarbons	Nitrogen oxides	Total
Transportation	63·8	1·2	0·8	16·6	8·1	90·5
Fuel combustion in stationary sources	1·9	8·9	24·4	0·7	10·0	45·9
Industrial processes	9·7	7·5	7·3	4·6	0·2	29·3
Solid waste disposal	7·8	1·1	0·1	1·6	0·6	11·2
Miscellaneous	16·9	9·6	0·6	8·5	1·7	37·3
Total	100·1	28·3	33·2	32·0	20·6	214·2

(From the report of the Council on Environmental Quality.)

The most telling argument against the manufacturers is that cars and other vehicles are responsible for nearly half the total air pollution, accounting for some 42 per cent of the estimated 214 million tons of pollutants released into the air in the United States in 1968 (see Table 2). "Detroit," Senator Muskie remarked, "has told the nation that Americans cannot live without the automobile. This legislation would tell Detroit that if that is the case, they must make an automobile with which Americans can live."

BIOLOGY

Coping with Acanthaster

by our Washington Correspondent

FUNDS totalling $4.5 million have been voted by Congress for a five year programme to control the crown-of-thorns starfish, *Acanthaster planci*, whose ravages of Pacific coral reefs have reached epidemic proportions in the last few years. The programme, which is to be administered by the Department of the Interior and the Smithsonian Institution, calls for research into the ecology of the starfish, together with the development of better methods of control.

The immediate beneficiaries of the programme will be the Pacific territories of the United States such as Guam and Hawaii. Since 1967 more than 23 miles of the coral reefs round Guam have been almost completely destroyed by the starfish. In Hawaii a serious infestation has been found off the coast of Molokai island. Elsewhere in the Pacific the Great Barrier Reef is the best known casualty, having been denuded of 100 square miles between 1964 and 1966. (A recent survey of the Reef, however, suggests that regeneration of the coral may not be so protracted a process as is supposed (see *Nature*, **227**, 5; 1970).)

The bill authorizing the *Acanthaster* research programme passed the Senate in April. In last week's debate in the House, Representative Mink of Hawaii suggested that the funds should be used to set up a Coral Reef Institute which, she thought, should be situated with the University of Hawaii. To begin with, the Institute would devote its energies to the *Acanthaster* problem but, with that solved, it would turn to other questions of coral ecology such as the effects of algae and fish farms being established in the Philippines.

The Smithsonian Institution will use its share of the funds to study the general biology of the crown-of-thorns starfish whilst the Department of the Interior will study control methods. So far the most effective method of halting the plague is for divers to inject starfish individually with formalin or ammonium hydroxide.

NEWS AND VIEWS

Is Your Jamboree Necessary?

NOBODY returning to his laboratory in late September after the round of conferences, commissions, round-tables and workshops has much time to sit down and assess the worth of all this summer activity, which, in any case, continues through the other seasons of the year. Many fatigued travellers may have begun to doubt the value of their conference or colloquium, yet new committees are already well advanced with preparations for 1971 and there are even preliminary announcements for 1972. Is it midsummer madness or does the scientific community gain from the scientific and social fellowship? And which aspect is the more important?

Meetings—at any time of the year—clearly fulfil an essential function in a system in which the written word is at once indispensable and yet ineffectual. The story is better told by word of mouth in some darkened baroque hall in central Europe or over a beer on the beach at Cold Spring Harbor than in an unrelieved text which omits the snags and veils the humanity of research. Even props such as slides can detract from the message and it was said at the recent biochemical meeting in Interlaken (*Nature*, **227**, 1195; 1970) that one speaker who lost his slides performed almost more effectively with some which were hand-drawn at the last moment. But is a big meeting the right place for personal contact? Is there a defence for the jamboree? It is, of course, part of the history of science that much is lost when a larger discipline splinters into specialities, but communication is more efficient at meetings of less than 500 than at those in excess of 5,000. There is the extra difficulty of managing a large audience and finding an adequate auditorium, so the problem is to decide whether to bring together the 5,000 and then split them into ten sessions or to sunder the disciplines into ten specialities and let them go their several ways. Given a superb organization such as that at the federation meeting at Atlantic City, the first method can work efficiently. But if the chairman lags or the transit bus is late, the ranks may just as well be split. The onus is also on the organizers, but as at the International Astronomical Union meeting (*Nature*, **227**, 991; 1970), participants should not be lulled into thinking that the proceedings will always be smoothly conducted.

One saving grace of the jamboree is, of course, the chance it gives for cross-fertilization of ideas. Another is that younger investigators do at least have a sight of the veterans. So often, however, the veterans have themselves just come from a more intimate and exciting gathering. Their latest discoveries are presented somewhat casually for this larger throng, and those on the periphery get a false impression of the state of the art and the co-veterans complain that the meeting had nothing new to offer. Again it is for the conveners to apply the more rigorous criteria necessary for the selection of speakers and contributions. No one would wish them to turn down a brilliant speaker because he is saying no more than he had said many times before, and it would be unjust to turn away a small jewel of discovery just because it is known that the speaker is inaudible and inarticulate. But surely there is some way of avoiding "poster sessions" and papers "read by title only".

From the editorial point of view, there is the question of the timing of publication of the reported findings. In a bygone age, investigators more often subjected their reports to public scrutiny before submitting them to a journal, but now it is common simultaneously to compose an abstract for the conference secretary and a manuscript for a journal. The consequence of this in the case of the recent International Congress of Biochemistry was that a dozen or more contributions corresponded to articles just about to appear in *Nature*, and many others were in the press elsewhere. The procedure is not formally incorrect, but most editors feel safer with work submitted after a meeting and revised in the light of comment, and most organizers anxious to ensure a high level of novelty at their meeting will be happier if more contributions have an air of freshness about them.

There are signs that the value of big meetings is being seriously debated, but so far there is little indication of their universal abandonment. In Interlaken, there were some misgivings about the future of the International Congress of Biochemistry, but it seems that the triennial meetings will continue, with Sweden as the host in 1973. The International Association of Microbiology has decided to discontinue—or to fragment—its mammoth conferences but, on the other hand, an International Society of Immunology has been formed which is looking forward to its first meeting in Washington in 1971. It is up to each society to decide in such matters, preferably after ballot or debate, but if individuals have no inclination to voice an opinion on the best means of communicating with colleagues it is likely that the initiative will be taken by those who ultimately have the greatest say and also the greatest self-interest in the business—the grant-giving authorities.

TUMOUR VIRUSES

Dissecting Virions

from our Cell Biology Correspondent

MOLECULAR biologists attracted to the RNA tumour viruses by the recent discoveries of Temin and Mizutani and Baltimore only to find that this particular field is already overcrowded might consider tackling the more mundane job of analysing the composition of the RNA tumour virus virion. For surprisingly little is known about the collection of RNAs, proteins, lipids and carbohydrates which comprise these viruses.

All the RNA tumour viruses contain an RNA molecule which sediments at about 65S and a collection of much smaller RNAs which sediment at about 4S in sucrose gradients. The 65S RNA is presumed to contain the genetic information of the virus, but it is not known whether the 65S RNA is exclusively viral or contains a mixture of viral and cellular RNA. The structure of the 65S RNA has not been determined unambiguously either. Several lines of evidence suggest that it is made up of three or perhaps four smaller molecules held together by weak bonds, but the possibility that the 65S RNA is a single polynucleotide chain capable of extreme changes in configuration has not been eliminated conclusively.

The 4S RNA in the virions of the RNA tumour viruses has been more fully characterized, and at least some of this RNA seems to be transfer RNA. Two years ago Travnicek reported that unpurified 4S RNA from avian myeloblastosis virus (AMV) particles accepts amino-acids. Now Erikson and Erikson (*J. Mol. Biol.*, **52**, 387; 1970) report that the 4S RNA of this virus includes valine, proline, methionine, glycine, aspartic acid and arginine transfer RNAs. The amino-acid accepting activity of the viral 4S RNA for five other amino-acids was either very much less than that of cellular 4S RNA or undetectable. Apparently each AMV particle contains about 200 tRNA molecules per molecule of 65S RNA. Either during virus maturation the transfer RNAs are selectively incorporated into the virions, or all the tRNAs of the cell are incorporated, but some are selectively inactivated while in the virion or during extraction from it.

The proteins in these viruses occur in the viral envelope and associated with the RNA in the internal nucleoid; those proteins in the nucleoid are believed to give rise to the group specific antigenicity which differentiates the viruses in one group, for example the avian or murine viruses, from those in all other groups. The proteins in the envelope, on the other hand, seem to give rise to the subgroup antigenicity which distinguishes different viruses within a group, and has a role in restricting the host range of a particular virus. Duesberg, Martin and Vogt (*Virology*, **41**, 631; 1970) have recently isolated two glycoproteins containing glucosamine, galactose and fucose from several strains of Rous sarcoma virus and Rauscher murine leukaemia virus. These two proteins which constitute about 10–20 per cent of the total protein in the virions have molecular weights of about 90,000 to 105,000 and about 37,000. The glycoproteins from different strains of Rous sarcoma virus have different electrophoretic mobilities and inhibit the action of neutralizing subgroup specific antisera. It seems therefore that these glycoproteins are part of or identical with the subgroup antigens. Duesberg *et al.* have also detected three proteins common to the Rous sarcoma viruses they examined which are not glycosylated and are probably responsible for the group specific antigenicity. Which, if any, of these proteins correspond to the enzymes that Temin and his followers are discovering remains to be seen.

PROTEINS

Endless Enzymes

from our Molecular Biology Correspondent

THE concentration-dependent association of enzymes into long filamentous formations, which are catalytically active, has been observed in rare cases, the best defined of which is bovine glutamate dehydrogenase. The enzyme in its native environment, in liver mitochondria, is thought to exist at considerable concentrations, and the associated state must therefore be presumed to be physiologically relevant. The addition of the co-factors, NADH and GTP, favours the depolymerization of the long chains to the smallest active form, which consists of six polypeptide chains, each of molecular weight 50,000. This hexamer is triangular in section, being made up of two superimposed rings of three subunits.

The nature of both the disaggregated and associated forms in solution, and of the equilibrium between them, has been studied by Reisler and Eisenberg, whose work is a salutary reminder of the power of hydrodynamic methods, fastidiously applied by masters of the craft. The first report (*Biopolymers*, **9**, 877; 1970) is concerned in the main with the hexamer and its viscometric behaviour. The determination of the intrinsic viscosity of such a molecule by extrapolation to zero concentration is greatly complicated by the association equilibrium, which causes the disymmetry of the particle to change with concentration. At very low protein concentrations, moreover, measurements, at best tiresome enough, are notoriously vitiated by adsorption of a layer of protein to the wall of the capillary. Reisler and Eisenberg have devised a new and effective correction for this effect, based on the introduction of a factor for the effective diminution of the capillary bore. By this means they are able to extend their measurements to prodigiously low concentrations, and so show that the intrinsic viscosity in the associating system is indistinguishable from that in the presence of the co-factors. These therefore do not function by causing a major shape change. The hexamer has been simulated by machined models, in which each subunit is an ellipsoid of the right shape. The frictional properties of this model in viscous fluids give a calculated intrinsic viscosity in precise agreement with that of the protein, and any geometrical deviations, such as a different monomer shape or a displacement of monomers along the three-fold axis, lead to grossly different values. This is good evidence for the validity of the presumed structure. Reisler and Eisenberg have also made the strange observation that saturation of the solution with toluene greatly promotes the association—a finding altogether contrary to the usual and expected effects of hydrophobic additives.

Reisler, Pouyet and Eisenberg (*Biochemistry*, **9**, 3095; 1970) have also examined the sedimentation properties of the enzyme, and working at modest concentrations, where they can show that non-ideality,

the hydrodynamicist's greatest affliction, can be neglected, find that the sedimentation equilibrium distribution can be represented in terms of a single indefinite association. That is to say, the elongation of the protein rods occurs by addition of successive hexamers according to a single equilibrium constant. Velocity runs lead once more to the same extrapolated sedimentation coefficient as is found in the disaggregated system with cofactors present, and its magnitude agrees with predictions from settling rates of the macroscopic hexamer models.

Olsen *et al.* (*J. Mol. Biol.*, **52**, 239; 1970) have discovered another apparently infinitely associating protein. The enzyme, glutaminase, from kidney, is activated or inhibited by common anions. In Tris buffer the activity and molecular weight alike are lowest; they are higher in phosphate and very high in a phosphate–borate medium. The electron microscope reveals small globular aggregates in Tris, and in phosphate–borate long filaments with a regular helical form, which is shown by shadowing experiments to be left-handed. The formation of helices from the globular state is rapid, and accompanies the onset of high activity. The occurrence of highly aggregated, filamentous, and active forms of enzymes may prove to be a more widespread phenomenon than has been suspected.

CHROMOSOMES

More about Structure and Function

from our Cytogenetics Correspondent

More information about the organization of chromosomes emerged from the third Oxford chromosome conference which was held from September 22–25. Dr M. L. Pardue (Edinburgh) described how ribosomal RNA from *Xenopus laevis* can be hybridized to chromosomes of *Xenopus* and Dipterans to locate the complementary nucleolar DNA sequence. She also described how hybridization can be used to locate the regions of chromosomes bearing "satellite" DNA; in mouse this turns out to be around the centromeres in all the chromosomes except the *Y*. Dr B. M. Richards (London) discussed X-ray diffraction patterns of nucleohistone, which are compatible with a supercoiled structure for interphase chromatin. He outlined a model whereby histone F_{2a1} (which has a known amino-acid sequence) could interact with DNA in such a way as to generate supercoiling. Other DNA–protein interactions were dealt with by Dr T. Holt (Nijmegen). Puffs can be induced by heat treatment at certain regions of *Drosophila hydei* salivary gland chromosomes; at the same time acid proteins are taken up at these regions. The kinetics of protein accumulation can be followed by microspectrophotometry and, together with parallel studies on RNA synthesis at the puff sites, lead to the conclusion that these proteins participate in gene activation. The activity of the *X*-chromosome in the mule has been assessed by Dr M. M. Cohen (Buffalo). In fibroblast cultures from four animals it seemed that in 60–90 per cent of the cells the *X* chromosome of the donkey parent was inactivated as judged by autoradiography, and the degree of inactivation correlated well with the amount of the *X*-linked gene product glucose-6-phosphate dehydrogenase in the cell contributed by each parent.

Reports of mitotic (as opposed to meiotic) crossing over are occasionally encountered in the literature on the genetics of higher organisms. Dr K. Patau (Madison) presented evidence for its occurrence in man. In lymphocyte cultures quadriradial configurations are found, some of which can be considered to be due to mitotic chiasmata. Lymphocytes from Bloom's syndrome show a particularly high frequency of such chiasmate configurations. These chiasmata are non-random in their location in the complement. Patau concludes that the chiasmata arise from pairing of homologues in the somatic cell rather than from translocation.

The way in which studies of meiotic chromosome can reveal chromosome evolution in progress was discussed by Drs M. J. D. White (Melbourne), H. Lewis (Los Angeles) and J. Wahrman (Jerusalem). Wahrman described chromosomes of races of *Acomys* found in Sinai ($2n=36$) and Israel ($2n=38$); the inference is that centric fusion between the telocentric chromosomes of the ancestral population was more complete in the Sinai race. That centric fusion is the origin of the present day chromosomes is strongly inferred from a cross between a race of *Acomys* from Cyprus ($2n=38$) and the Israeli animals. At meiosis in the hybrids a long chain multivalent is formed indicating that centric fusion proceeded in different ways in the founders of these two populations.

The role of *B* chromosomes in the structure of a population of *Myrmeleotettix* has been investigated by Dr G. M. Hewitt (Norwich). Although his investigations ranged from the micrometeorology of the grasshopper's environment to an analysis of the DNA composition of the *B* chromosomes, their role is still unclear. Dr G. M. Evans (Aberystwyth) showed that *B* chromosomes can influence cell division in plants. Evans also revealed a difference in the relation of the duration of the mitotic cycle and DNA content in monocots and dicots. Dicotyledons have longer cycles than monocotyledons even when species with identical DNA content are compared.

Analyses of chromosome movements in meiosis and mitosis were presented by Drs R. Deitz (Tübingen) and A. Bajer (Eugene). The latter showed a film of mitosis in *Haemanthus* endosperm and illustrated how chromosome movement is influenced by a short pulse of ultraviolet light focused onto selected areas of the spindle. From the way in which movement is interrupted, together with changes in microtubule structure, Bajer concludes that the centromere fibrils slide along the continuous fibrils during anaphase.

CELLS

Interactions in Populations

from a Correspondent

The study of cell–cell interactions among mammalian somatic cell populations is growing. Some investigations are at present descriptive, providing evidence for associations between cells without any real understanding of their functional significance. Some workers have invented a form of intercellular cooperation to account for their results but cannot suggest the mechanism involved. At the symposium on cell–cell interactions, part of the meeting of the British Society for Cell Biology held in Birmingham on September 24, examples of both genre were presented. Dr C. N. D.

Cruikshank (University of Birmingham) said that his observations on melanocyte–keratinocyte interaction *in vitro* show that such cells can form an intimate association and that pigmented material can pass from one to the other. Mrs P. Mann (University of Birmingham) described a similar interaction between mast cells and eosinophils; the former in response to an appropriate stimulus, *in vitro*, are capable of transferring granular material to eosinophils.

After outlining the conditions which determine cell fusion, Dr J. F. Watkins (University of Oxford) described the ways in which the formation of heterokaryons and the resulting hybrids can usefully be applied to understanding topics as diverse as the control of mitosis, differentiation, malignancy and the permanence of transplantation antigens. He thought it would be many years before the genetic analysis of mammalian somatic cells by hybridization paralleled the refinements possible with microorganisms.

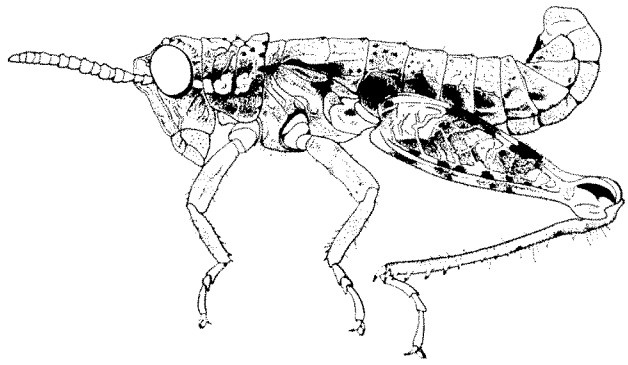
A more direct approach to cell–cell interaction was presented by Dr D. D. Potter (Harvard University). For several years he and his colleagues have studied the contacts which various kinds of cells can make both *in vivo* and *in vitro*. Experimentally in this latter state it had proved possible to find low resistance junctions between cells at which the cell membrane was greatly modified to permit the passage of inorganic ions, and probably also simpler organic nutrients such as sugars and amino-acids. Such apposition is probably equatable with the tight junction as visualized in the electron microscope. Neither the driving force for the passage of material nor the biochemical nature of the alteration at the junction is known. The methods used for this work included the impalation of cells on micro-electrodes by which their cell potential could be both measured and altered.

Cooperation is a fashionable word in immunological circles, and Dr A. J. S. Davies (Chester Beatty Institute, London) spoke of evidence for the existence of two sorts of lymphocyte (T and B) in mice between which an interaction could facilitate antibody production. He felt that it was not necessary to postulate such interaction in cell-mediated immune responses, but it was likely to be a significant feature of certain kinds of antibody production. Dr A. J. Munro (University of Cambridge) stressed some of the technical difficulties encountered in building appropriate models of the immune response *in vitro*, but indicated that cooperation could be demonstrated which was ostensibly similar to that found *in vivo*. Dr G. Roelants (London) and Dr J. H. Humphrey (National Institute for Medical Research, London) presented the negative and positive aspects of their work on macrophage–lymphocyte interactions. Roelants found that the formation of RNA–antigen complexes by macrophages lacked that specificity which would be required for such materials to play a significant part in immunization. On the other hand, Humphrey showed that in a transfer system, macrophages containing antigen were immunogenic, and that the physiological condition of the macrophages could affect the magnitude of the immune response observed.

Summing up, Dr J. G. Hall (Chester Beatty Research Institute, London) discussed the cytotoxic macrophage and the interaction between cells necessary for it to become "armed" with cytophilic antibody. Hall believed that this form of collaboration between cells was of great significance in the various anti-bacterial and anti-viral immune responses which an organism made in order to survive. Nevertheless, Hall thought that interaction between lymphocytes and endothelial cells in the post-capillary venules, resulting in an alteration of the migration pattern of the lymphocytes, was very important and should be the subject of intense scrutiny.

ENTOMOLOGY

Nymphal Grasshoppers



Five new species, representing four new genera of grasshopper, have been found in South Africa. Describing them in the latest issue of the *Journal of Natural History* (**4**, 493; 1970), H. Dick Brown, of the Plant Protection Research Institute in Pretoria, remarks that they are unusual in lacking wings; this gives them a nymphal appearance. The animal shown here has been named *Namatettix denticornis*.

CHROMOSOMES

Chemistry of Excess

from our Cytogenetics Correspondent

THE extra chromosomes which some plants possess as well as their regular set have been found to influence the chemistry of the nucleus. These effects, described by Kirk and Jones (*Chromosoma*, **31**, 241; 1970), might ultimately effect the physical characteristics of the plants.

Several plants and animals possess chromosomes that are supernumerary to, but not homologous with the regular complement. These are the so called *B* chromosomes. Their origin and function can only be inferred; they contain no mappable genes and they are often deleterious to the individual possessing them, but they are maintained in the population. *B* chromosomes are well known in rye (*Secale*), in which they were studied extensively by Müntzing in the 1950s. Some of their properties have been reinvestigated by Jones and colleagues with respect to their effect on the chemistry of the nucleus.

Kirk and Jones isolated nuclei from root tips of rye plants with between nought and six *B* chromosomes, and measured DNA, RNA, total protein and histone protein within each class of nucleus by microdensitometry. They found that the presence of increasing numbers of *B* chromosomes caused the total protein and RNA content of nuclei to decline while the amount of histone increased. There was a curious pattern to the increase: when the amount of RNA or total protein was divided by the DNA content, the ratio was always lower in nuclei with an odd number of *B*s (one, three or five) than with an even number (nought,

two, four and six) of *Bs*. Conversely, the ratio of histone to DNA was higher in nuclei with odd numbers of *Bs* than in those with even numbers.

At present there is no indication of the meaning of these changes in terms of nuclear function and their relation to the phenotype of the plants. There are some clues, however. There is a negative correlation between histone and both RNA and total protein content within a nucleus, and so *B* chromosomes seem to cause more nuclear histone to be accumulated, and this may in turn depress or repress the nuclear RNA and protein content. And last year Jones and Rees (*Heredity*, **24**, 265; 1969) showed that plants with odd numbers of *B* chromosomes, besides being less frequent in the population, had a weaker phenotype—lower weight and fewer tillers—than plants with an even number of *Bs*. These results suggest that *B* chromosomes cause changes in nuclear chemistry and that these changes ultimately find expression at the level of the phenotype.

Jones and Rees also showed that the presence of *B* chromosomes influenced chiasma formation and inferred that *Bs* increased the genetic variability of the gametes and consequently the offspring—a conclusion that is borne out by other recent studies (Barlow and Vosa, *Chromosoma*, **30**, 344; 1970; Hanson, *Genetics*, **63**, 601; 1969; Malik and Tripathi, *Z. Biol.*, **116**, 321; 1970) of the influence of *B* chromosomes on meiotic behaviour in three monocotyledonous species. In this way *B* chromosomes can compensate, through benefits to the population, for any deleterious effects on an individual.

ECOLOGY

Energetics in the Tropics

from a Correspondent

ENERGY requirements, conversion and utilization provided the central theme of a symposium held from September 16 to 19 at Makerere University, Kampala, Uganda. The proceedings, under the auspices of the International Biological Programme (IBP), embraced the physical and biochemical aspects of energy fixation, the roles and efficiencies of primary producers, herbivores and carnivores and even the energy efficiency of man.

The units in which energy and performance were expressed caused some difficulty. For some delegates, the contribution from Professor J. L. Monteith (University of Nottingham) provided the first experience of applying joules and watts to biological systems, but, at least in his derivation of a light energy conversion efficiency in green plants of 0·01 per cent (taking as 100 per cent the total measurable radiation above the Earth's atmosphere), the choice of units was amply justified. Dr R. Hill (University of Cambridge) then laid the biochemical foundations for a discussion of alternative pathways for carbon dioxide fixation in certain tropical plants.

The importance of production measurements in understanding community structure and interaction was illustrated by the work of the IBP Lake George team and the Nuffield Unit for Tropical Animal Ecology, both based in Queen Elizabeth National Park, Western Uganda. The aim of the former is to construct a complete energy balance sheet for a tropical freshwater ecosystem. Professor C. D. Pigott (University of Lancaster), using data collected in Queen Elizabeth Park, suggested that a knowledge of the primary production of savanna grassland can be used to estimate fairly accurately the site capacity for large herbivores. But he and other speakers stressed that such estimates could be misleading, if expressed solely in total energy equivalents, without taking into account the nutrient content of food, plants in relation to grazing preferences and conversion efficiencies at the second trophic level.

Food energy conversions in animals were discussed in detail by Professor A. T. Phillipson (University of Cambridge), and other speakers dealt with the ecological implications of water use, temperature stress and the inverse relationship between basic metabolic rate and body size. Many animals have obvious adaptations to their environment: the Thomson's gazelle, for example, seems to be highly efficient in its water conservation. Mr. D. Hopcraft (Athi River, Kenya) showed that, under rangeland management, this small ungulate can, at lower production cost, perform better than cattle in terms of lean meat production and yet, even at very high stocking rates, have a less damaging effect on the vegetation than cattle.

Discussing crop production, Professor R. Holliday (Makerere University, Kampala) and others used tea, sorghum, coffee, maize, cotton and legumes to illustrate the concept of economic yield and discussed techniques for manipulating it. Professor M. Dagg (Ahmadu Bello University, Nigeria) emphasized the limiting effects of the availability of water in crop production in the tropics. Another contribution concerned with productivity in East African forests, demonstrated how natural ecosystems can be managed to give economic yield.

A discussion led by Dr D. S. Miller (Queen Elizabeth College, London) brought recommendations that agricultural efficiency could be improved in two ways: by replacing traditional hand tools by more efficient instruments and by improving on the efficiency of man himself. Advances in the latter direction must await a better understanding of the effects of diet, disease and temperature.

The scope of the symposium was enormous, but ecosystems, and especially the less well documented tropical types, cannot be treated narrowly. Although some estimates of community bioproduction were given, many delegates expressed strong opinions that understanding and/or management of ecosystems was only likely to be achieved by intensive studies at the level of the species, some of which were discussed at the symposium.

SELENOLOGY

Source of the Lunar Field

from our Geomagnetism Correspondent

IF the Moon possesses a global magnetic field today it must be a very small field. A few years ago, magnetometers on Explorer 35, for example, showed that there was no measurable field at the altitude of the satellite. Taking into account the limitations of the apparatus used, this can be interpreted as setting an upper limit of 10^{20} gauss cm^3 on the moment of a possible dipole at the centre of the Moon. In other words, the lunar dipole moment is at least six orders of mag-

nitude lower than the terrrestrial dipole moment. But the Moon must have possessed, or at least been influenced by, a fairly strong magnetic field in the past, for some of the material brought back by Apollo 11 contained "natural" magnetization.

Further evidence that the Moon has a magnetic history has come from direct measurement of the field at the lunar surface (Dyal *et al.*, *Science*, **169**, 762; 1970). In November 1969 a three-axis magnetometer was placed on the eastern edge of Oceanus Procellarum by the crew of Apollo 12. The result of the measurements it made can be stated quite simply. During the first twenty days of its operation, the magnetometer recorded a steady field of 36 ± 5 gammas directed 38° below the horizontal and 64° south of east. Any suggestion that this field was produced spuriously by a magnetized artefact was ruled out by the absence of an observed field gradient in the plane parallel to the lunar surface, and by the lack of any change in the field as the lunar module began its ascent for the return to Earth.

What do these data mean? There is a clue in the strength of the observed field. If the field is interpreted as a global dipole, the corresponding magnetic moment comes out at $1{\cdot}7 \times 10^{21}$ gauss cm^3, which is about twenty times greater than the maximum value allowed by the Explorer 35 result. It follows, therefore, that the 36 gamma field must be due to a localized source near the Apollo 12 landing site. The size, shape and depth of the source could, of course, be anything; but calculations show that if it is dipolar and lies near the surface it can stretch no more than about 200 km from the magnetometer site and must possess a magnetic moment between 10^9 and 10^{18} gauss cm^3. These figures are consistent with an extended source having remanent magnetic properties similar to those found in the Apollo 11 samples.

Dyal *et al.* are right, however, to point out that the existence of such a body does not necessarily indicate that the Moon ever possessed a magnetic field. For one thing, the body in question could be a large magnetized meteorite or a region shock-magnetized by meteoritic impact, although the low concentrations of meteoritic material in the Apollo 11 samples militate against such a suggestion. But even if the observed field is produced by a region of native lunar material thermally magnetized in an ambient field, it does not necessarily follow that the ambient field was intrinsically lunar. It could, for example, have been interplanetary, although this is unlikely because, whatever the magnitude of the interplanetary field in the past, directional variations would probably have averaged it out to about zero over the time it took the lunar material to cool through its Curie point. And it is possible that the ambient field was terrestrial.

It is a feature of some theories for the origin of the Moon that the Earth and Moon were once much closer together. If the magnetized body possesses high coercivity, as the Apollo 11 samples do, the ambient field must have been a few thousand gammas. This is the sort of field currently observed at about two to three Earth radii from the Earth, which is dangerously close to the Roche limit for lunar disintegration, although not beyond the realm of possibility. In any case, there is no guarantee that the Earth's field remained constant. A larger terrestrial field in the past would allow the minimum Earth–Moon separation to be increased.

Finally, the ambient field could have been an internal lunar field—an idea which many people hoped the Apollo samples would confirm or refute. But a lunar dynamo implies a hot converting interior and a sufficient lunar angular velocity, neither of which seems to be consistent with the Moon as it is today nor with any stage in its evolution. As far as magnetism is concerned, Apollo has, it seems, raised at least as many questions as it has answered.

TEMPERATURE

Many Methods of Measurement

from a Correspondent

INDUSTRY and government were well represented at the second conference on temperature measurement, held in Warwick from September 21–23. Since the first conference on this topic in 1964 the International Practical Temperature Scale of 1948 (IPTS48) has been revised, and Mr C. R. Barber (National Physical Laboratory) opened the conference with an account of how the new scale (IPTS68) was formulated. Dr T. J. Quinn (NPL) then described how black-body cavities could be made for use above the gold point on the IPTS68, where radiation methods define the scale. Dr J. P. Compton (NPL) explained how the scale can be realized from its new lower limit of 13·8 K up to 273 K, and discussed methods that can be used below this limit.

Mr M. V. Chattle (NPL) described the use of the platinum resistance thermometer between 630·74° C and 1064·43° C—the range of the IPTS68 in which the platinum–platinum 10 per cent rhodium thermocouple is the interpolating instrument. He explained that, as long as care is taken in thermal cycling of the platinum resistance thermometer, it could be used as an interpolating instrument in this range, being far more reproducible than the thermocouple. Mr J. S. Johnston (Rosemount Engineering Co.) explained that the platinum resistance thermometer could be adapted for use in industry, which requires rugged instruments. He showed constructions of thermometers designed for fast response, stability and small size, for use in various environments.

Many speakers described how temperatures, in the range from 300 K upwards, could be measured from the infrared radiation emitted by materials. Mr R. Barber (Land Pyrometers, Sheffield) said that the silicon cell had many uses in radiation pyrometry because it provides a cheap and rugged but sensitive detector. But as he and other speakers explained, care must be taken to ensure that the pyrometer is measuring the required temperature. In steel rolling, for example, difficulties are caused by the oxide layer, by the lack of knowledge of the emissivity and by an environment of steam and electrical interference.

Several speakers described how the temperatures of transparent materials can be measured by choosing the wavelength band carefully. Glasses, polymers and gases have absorption bands in their transmission spectra and so have high emissivity in these bands as well. By isolating these bands with filters and choosing suitable detectors, Mr J. R. Beattie (Pilkington Research and Development), Mr J. M. Rucklidge (Land), Mr B. J. Stay (ICI), and Dr K. C. Lapworth (NPL) were able to measure the temperatures of these materials.

Two sonic methods were among the newer techniques

described. Dr J. F. W. Bell (University of Aston) has used the variation of the velocity of sound with temperature in a solid as the principle behind his thermometer. Mr E. S. Morgan (Central Electricity Research Laboratories, Leatherhead), on the other hand, used this variation in a gas. Morgan used his instrument to measure the average temperature of the gases in a furnace by measuring the time taken for a pulse of sound to travel across the furnace.

VITREOUS STATE

Atoms and Glasses

from a Correspondent

SOME busy experts on the vitreous state managed to fit a Faraday Society discussion on this topic into their conference schedule between September 22 and 24. About half of the contributions presented at Bristol were devoted to electrons or phonons in vitreous materials, and the rest were largely concerned with nucleation, crystal growth and phase separation in glass forming systems. Introducing the electron and phonon section, Professor Sir Nevill Mott (University of Cambridge) reviewed the state of knowledge of conduction mechanisms in amorphous and liquid semiconductors. The existence of localized electron states in these materials has been accepted for some time, and the chief contention centres on the existence and position of the so-called mobility edge, which may divide the electron energy band into localized and non-localized regions. The effectiveness of electron-electron interactions in modifying the already quite complex theoretical picture has also been the subject of much recent discussion. It was taken up again by Professor M. Pollak (University of California) whose chief conclusion was that the interaction can significantly affect activation energies associated with d.c. conductivity in various ways, as well as increasing the a.c. conductivity above a certain critical frequency.

Mr T. Klima and Mr T. McGill (University of Bristol) raised some comment when they dealt with the role of short range order in producing an energy gap in covalent amorphous semiconductors. But, on the whole, phonons sparked off much more discussion than did electrons. Significant theoretical progress is being made on several fronts, for example, with the model based calculations of Bell and Dean, the crystalline approach of Gaskell and the orientationally disordered molecular crystal methods developed by Whalley and his colleagues.

The second half of the discussion was prefaced by Professor J. Zarzycki (Saint-Gobain, Paris) who reviewed phase separated systems. He touched on the failure of some stoichiometric and chemical composition based theories to account for the temperature dependence of miscibility phenomena. He also outlined the basis of the spinodal analysis and remarked on the difficulty in distinguishing between nucleation and spinodal growth mechanisms by small angle X-ray scattering methods; the discussion returned to this difficulty several times.

The significance of Cahn's spinodal analysis was called into question several times, with some particularly pointed evidence for the prosecution coming from Dr R. K. MacCrone (Rensselaer Institute, New York). It is perhaps natural that theories in this area of amorphous research should be inherently more macroscopic or coarse grained in character than those in the realm of electrons or phonons. But there seems to be a growing prospect that the molecular dynamics, or computer simulation techniques already exploited in connexion with simple liquids, can make possible significant progress during the next decade in probing nucleation and miscibility phenomena at the atomic level.

MICROSCOPY

More Scope for Scanning

from our Materials Science Correspondent

FROM a rare laboratory curiosity, the scanning electron microscope has in five years developed into a tool widely used in materials science, microelectronics, biology and even the assessment of lunar dust. Most routine users exploit the instrument in one mode only —the emissive mode, which is used to give information about surface topography. They will be helped to make more varied use of their instruments by D. R. Clarke's recent survey of image contrast in the SEM (*J. Mat. Sci.*, **5**, 689; 1970).

Beginning with the standard emissive mode of image contrast, Clarke distinguishes clearly between back-scattered and secondary electrons and details the special contributions to contrast made by each of these electron types. An important innovation is MacDonald's use of Auger electrons to form an image: this allows highly sensitive atomic number contrast (which is otherwise rather poor in the SEM), and also allows voltage contrast—used for examining microcircuits in operation—to be sharply separated from other forms of contrast. Topographic contrast is interpreted in some detail and the roles of secondary and back-scattered electrons are distinguished.

After a survey of voltage and electric field contrast, of magnetic domain contrast and of beam-induced conductivity contrast, which is used to reveal defects in semiconductors. Clarke surveys very recent developments in distinguishing different crystal orientations. This aspect of image contrast depends on the formation of channelling patterns (a term borrowed from ion-implantation) and indeed the orientation of a crystal can often be determined, though the modest magnification which can be used in this connexion remains a limitation.

Clarke goes on to detail improvements in the collector and display systems (though not referring to the improvement of atomic number contrast which can be achieved by using twin collectors—a Japanese contribution). Electronic processing developed at the Services Electronic Research Laboratory, Baldock, has made it possible for contoured and "quantized" displays to replace simple brightness modulations in images, and a form of stroboscopic display can be used to slow down and display rapid periodic voltage sequences in a microelectronic device.

STRESS ANALYSIS

Computers to the Fore

from a Correspondent

STRESS analysis is one of many engineering subjects which are becoming dominated by digital computer techniques. In this case the technique is finite element

analysis, which simply divides the component under study into elementary shapes (triangles, rectangles and so on) and solves the equations of equilibrium and compatibility by matrix methods. Any conference intended to cover the whole of stress analysis, such as the Institute of Physics Stress Analysis Group annual conference at the University of Surrey on September 23–25, must include this topic to an increasing degree. This certainly happened, but it seemed rather reluctantly done, and reflected the ostrich attitudes of many experimental stress analysts, for whom the Institute of Physics has provided a useful debating ground for many years (the majority of physicists interested in stress analysis are concerned with experimental techniques).

Dr T. K. Hellen (Central Electricity Generating Board) emphasized the success of computer methods for elastic problems, and was supported by Dr J. McEwen (Weir Group Ltd), who had used these programs to solve the centrifugal stressing of impeller blades, and compared the results with other methods. Dr. K. Kedward (Rolls Royce) demonstrated the successful application to anisotropic carbon fibre reinforced beams, describing some very convincing comparisons with photoelastic coating fringe patterns. In this type of application, with anisotropic and non-linear material behaviour, finite element analysis is most promising, for experimental methods bring considerable difficulty in both modelling and analysing this behaviour.

Dr G. C. Mitchell (NCRE, Dunfermline) described some uses of finite elements in ships' structures, showing that it is still necessary to test actual prototypes (or large scale models, at least) to destruction, for practical assessment of failure. Accurate calculation of stresses is only one step in the safe design of large structures.

Several contributors commented on the difficulty of preparing data, and the problem of the vast computer output, which often frightened designers used to decisions based on limited data. The simplification of output for design purposes is an important task.

There were several descriptions of the application of standard experimental techniques to various design and material testing problems, but there were also some interesting developments of instrumentation for high temperature work. Dr V. M. Hickson (Royal Aircraft Establishment, Farnborough) described an ingenious but rather expensive gas gauge, which used the variation of pressure at an orifice to determine small displacements. Stabilization at working temperatures seemed to be difficult, but this did seem a problem with the capacitance gauge, described by Dr B. E. Noltingk (Central Electricity Research Laboratories, Leatherhead), which depended on the change of capacitance between two curved wires, placed side by side, as they were stretched. Preliminary results from this instrument suggested that it is very reliable up to 600° C, and associated instrumentation, described by Dr P. C. F. Walfendale (Automatic Systems Laboratories) could satisfy all practical requirements. For temperatures greater than 600° C, optical techniques seem still to be the only practical, though far from perfect, methods. Dr A. R. Luxmoore (University of Wales, Swansea) described an application of the moiré effect to creep measurements of stainless steel tubes at 800° C.

There was only one description of the application of holography (an elementary study on plates) from Dr K. G. Miles (University of Nottingham). This was surprising, in view of the impact of holography on interferometric measurement of deformation, but perhaps it was attributable to the wide scope of the contributions.

PALAEOMAGNETISM

Can Sediments be Used?

from our Geomagnetism Correspondent

PALAEOMAGNETISM is apparently based on a preposterous premise. Although it is no surprise that rocks which contain small quantities of magnetic minerals can acquire a magnetization, no one had a right to expect that such magnetization could remain stable for hundreds, or even thousands, of millions of years. The reason for the stability is, of course, that some of the magnetic minerals concerned have high coercive forces; and nowhere is this characteristic better illustrated than in haematite-bearing sediments. Very often the natural remanent magnetization (NRM) of red sediments is hardly affected by demagnetizing fields of 2,000–3,000 oersteds.

As far as sediments are concerned, the validity of the palaeomagnetic method is confirmed by consistency of data, as exemplified by close internal grouping of palaeomagnetic directions and comparability of directions with those derived from other types of rock. Nevertheless, a minor stir was created when Smith (R. W.) and Fuller (*Science*, **156**, 1130; 1967) showed that in single crystals of haematite the magnetic moment produced by the interaction of antiferromagnetism with lattice defects and impurities (the so-called defect moment) was much more stable magnetically than the moment produced by the canting of spin sub-lattices (the intrinsic moment). In other words, it seemed that the structure sensitive moment, which can easily be altered by stress or heating to give spurious palaeomagnetic data, was much more stable magnetically than the spin moment, which is less influenced by extraneous phenomena. If this were so, partial demagnetization of the NRM would first remove the very moment which records the true palaeomagnetic direction.

Palaeomagnetists can now breathe freely again, for Dunlop (*Science*, **169**, 858; 1970) has shown that what applies to single haematite crystals does not apply to fine-grain haematite nor to the form of haematite found in sediments. In a long series of magnetic experiments on synthetic haematite powders and natural red sediments in various states of annealing (which removes the defects and so the defect moment), Dunlop has shown that it is the spin-canted moment which is the harder magnetically—and thus that palaeomagnetic directions obtained from sediments partially demagnetized in the laboratory are valid in theory as well as in practice. It turns out that thermal demagnetization should be particularly suitable for the magnetic cleaning of sediments because not only is the average coercivity of the defect moment lower than that of the intrinsic moment, but the process of heating tends to anneal the sample, thereby reducing the number of defects which produce the structure-sensitive moment in the first place.

A Philosophy for Big-Bang Cosmology

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According to recent developments in cosmology we seem bound to find a model universe like the observed universe, almost independently of how we suppose it started. Such ideas, if valid, provide fresh justification for the procedures of current cosmological theory.

MUCH of the effort of current extragalactic optical and radio astronomy is directed to isolating a particular Friedman–Lemaître cosmological model that will reproduce satisfactorily the principal observed features of the universe in the large. Most cosmologists regard this as a meaningful exercise, although they may disagree somewhat as to the particular interpretation to be placed on it. Nevertheless, the Friedman–Lemaître models have not always been treated so seriously since they were first propounded nearly half a century ago. Friedman published his remarkable paper[1] in 1922 in *Z. Physik*, but it seemed to be completely ignored at the time. Lemaître had heard nothing of it when he published his own equally remarkable work in 1927; he did learn of it in time to insert a reference in his paper[2] of 1931. His work, in its turn, was similarly ignored. The two papers, which were in excellent accordance with each other, were rescued from oblivion for rather incidental reasons. Einstein had felt compelled to introduce the so-called cosmical terms into his field relations in order to derive a cosmological model, the Einstein static universe of 1917; he never liked these terms and, when he found out about Friedman's work, he welcomed it as allowing him to discard them and the Einstein universe as well. Contemporaneously, Eddington saw that Lemaître's work showed the Einstein universe to be unstable so that it could be treated as the initial state of an expanding universe; Eddington welcomed Lemaître's work as a way of saving the Einstein universe and the cosmological terms. Now, however, after all these years, it seems that, for the purpose of comparison with the observations, nothing more serviceable than the Friedman–Lemaître models has emerged; they are being taken more seriously than ever before.

The present article arises from an attempt to understand why this is the position. It arose from the circumstance that I have recently had the privilege of occupying the Chaire Georges Lemaître in the University of Louvain, where Lemaître spent almost all his scientific life, so I was brought to reflect afresh on the work of that remarkable man[3]. On the one hand, I came to recognize more than ever the astonishing insight and appreciation of essentials displayed by him—and, of course, by Friedman as well. On the other hand, these two workers had deliberately used as many simplifying assumptions as possible, and yet no one subsequently seems to have gained anything worth while by seeking to relax these restrictions. Surely, therefore, there must be some justification for their work that is deeper than they could have known when they did it. On collecting together recent ideas presented by several writers, this does indeed seem to be the case. If so, the resulting insight must be of considerable interest, although it is still highly tentative.

Another reason for presenting this outlook now is that de Vaucouleurs[4] has published recently an extensive and penetrating criticism of current big-bang cosmology. Taken at its face value, that theory does betray all the apparent weaknesses so unsparingly exposed by him. Nevertheless, so far as it goes, the history of the universe inferred from big-bang theory is self-consistent in so many unexpected ways that it can scarcely be illusory. If the present discussion is valid, it may be claimed to counter much of the criticism expressed by de Vaucouleurs.

Some General Considerations

We begin by asking how cosmology is possible as a scientific study, for scientific study employs physical laws. But the concept of a physical law is that it enables us to treat a system that is being studied as one particular case among many. The universe, however, is a unique system, and so this aspect of a physical law seems to lose all meaning when we attempt to apply it to the universe. Moreover, a "particular case" is usually specified by initial conditions and these in their turn seem to have little meaning for the universe as a whole.

There is nevertheless one consideration that may make the case of the universe in the large different from all other systems in physics. For, if the universe as a whole is evolving, then, of course, the farther away we observe any part of it, the farther back we see it in time. Thus we actually observe the evolution of the universe. Also, if in some appropriate sense one part of the universe is like another, then it might be argued that the various parts make up the many cases needed in order to give significance to the use of physical laws. And a part seen farther away, and so earlier in time, may supply initial conditions for the study of a nearer part.

Such considerations at any rate make it reasonable to try to apply ordinary scientific methods to the study of the universe in the large. Whether this will succeed in yielding results of interest can be known only by making the attempt. In fact, cosmologists have been trying for more than fifty years.

We are, however, compelled to note one possible general difficulty. Manifestly, the farther away we observe parts of the universe (and so the earlier in time) the less information we can get about them. And we certainly could not observe the big bang itself. As we go back in time, therefore, we get less and less information on which to base any predictions until, when we come to what we might wish to treat as the initial state, we have no information at all.

There seems to be only one way in which we might hope that progress would be possible. This would be if we were to discover that, for some reason, we actually require less information than we suppose in order to make desired predictions. We shall see that nature does indeed seem to operate a principle of compensation in favour of cosmologists and that the pursuit of this topic leads us to much fresh insight.

Recent Theoretical Results

In this section, let us provisionally accept at their face value the various physical theories to which we have occasion to appeal; in particular, we take it for granted that they apply to the actual physical world. Afterwards, it will of course be one of our main purposes to examine the status of physical theory in this context.

We recall certain general results of cosmological studies,

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mostly quite recent. (*a*) Hawking, Penrose[5] and their associates have proved some remarkable theorems in the theory of general relativity. Under certain very general assumptions about the behaviour of matter, they showed that any cosmological model admitted by the theory must possess at least one singularity. Any such singularity we should in general regard as a big bang; if there is only one, we should interpret it as "the" big bang.

(*b*) Misner[6] has shown that, if there is one big bang in a homogeneous universe, then even if the circumstances of this big bang are highly anisotropic nevertheless very soon afterwards the model must become highly isotropic. This is brought about by the phenomenon of neutrino viscosity which Misner discovered in the course of the work. Carswell[7] has shown how difficult it is to construct any model that does not conform to Misner's result.

(*c*) If a model universe is everywhere isotropic, it is necessarily homogeneous. The converse is not true, otherwise there would be no point in Misner's result. Also, Misner's result does not allow him to infer isotropy without the assumption of homogeneity, otherwise homogeneity would follow. But it can be argued on physical grounds that a mechanism that tends to produce isotropy must tend also to produce homogeneity. Misner has discussed this aspect[8].

(*d*) Wagoner, Fowler and Hoyle[9] have studied the very early stages of a hot big-bang universe in regard to the nuclear processes operating at each stage. Their work shows in the first place that there is no meaningful sense in which we can speak of an initial chemical composition. A single parameter depending on the hotness and the baryon density is all that signifies. Furthermore, the processes occurring in these early stages produce the raw material for galaxy-formation later on. The chemical composition of this material is the significant chemical composition of the cosmos. The second remarkable inference is that the main features of this composition are not sensitive to the value of the single parameter we have just mentioned: in particular, there is a range of some six powers of ten in the value of this parameter through which the helium abundance (by mass) changes only from about 25 to 35 per cent.

(*e*) Milne[10] long ago remarked that, if we have a cloud of particles in ordinary space, provided only they are not strictly at relative rest, after a sufficient time any particle will see all the rest moving away from himself with speeds proportional to their distances. (By simply waiting long enough, this description must become valid to any desired accuracy.) Thus the time-direction of inevitable ultimate expansion is towards what we call the future, and the Hubble law for this expansion is itself inevitable. In regard to this property, the relativistic treatment does little more than give it a more elegant geometrical form[12].

We may summarize all these conclusions by the statement: (*A*) The universe must start with a big bang (or bangs) and, almost independently of any special initial conditions, it must have a particular chemical composition, it must exhibit a Hubble expansion, it must be isotropic if it is homogeneous, and we do expect it to be homogeneous.

All these predictions seem to be in good agreement with experience. We must comment that they depend on sophisticated developments in various physical theories—general relativity, elementary particle physics, nuclear physics, and so on; this is not elementary cosmology. In the formulation of statement *A*, the "musts" arise from our provisional attitude to the theories; the "almost independently" summarizes various degrees of insensitivity to initial assumptions.

Compensation in Cosmology

In regard to the study of the universe in the large, I now tentatively propound the principle: (*B*) The less information we can get, the less we need in order to make predictions that are confirmed by observation. This may be startling. It implies that, if we ask a sensible question about the universe, but if we necessarily have very little information on which to base an answer, we can nevertheless expect to get a sensible answer by making the best use of what information is available. In any case, so far as the examples given in the previous section are concerned, the proposition *B* states only about the same as *A* when it asserts that certain results follow almost independently of special initial conditions. So proposition *B* is well borne out by those examples. Actually, we shall see later that the number of possible examples of this sort may be quite small.

We must then hasten to state the converse: (*C*) From observed properties of the present state of the universe, we can infer less and less about earlier and earlier previous states, and almost nothing about what we might wish to call the initial state.

By present state, we mean the state of our cosmic vicinity at our cosmic epoch. Statement *C* is then certainly in accordance with our examples. If we are right in saying that, whatever the initial state, a universe must sooner or later show a Hubble behaviour, then, if we do observe a Hubble behaviour, this can tell us almost nothing about the initial state. If we observe the present chemical composition and ask what was the original chemical composition that led to it, then, from what we have seen, this question turns out to be meaningless. But if we observe that the helium content is between, say, 25 and 35 per cent by mass, we can infer that the value of the parameter I mentioned is between 10^{25} and 10^{31} (in the appropriate units) and this tells us rather little ! And so on.

Uncertainty in Cosmology

Some years ago I called[11] attention to some general considerations regarding the uncertainty of inferences in cosmology. The considerations depended on what on general grounds appear to be inevitable limitations on our obtaining the sort of information required in order to make precise inferences. I had to point out, however, that this feature was not yet built into the current formulations of cosmology. So far as the arguments went, I still think they are valid; but I think the discussion given here is complementary. It indicates that the uncertainty is distributed in a quite unsymmetrical way between past and present. On the strength of statements *B* and *C*, it seems that we make the claim, to which I shall return later. (*D*) We can know with a good deal of certainty how the universe is behaving now, but we are quite uncertain as to how it started.

Cosmology Simplified

Perhaps the most immediately significant consequence of this line of thought is that it offers deep reasons for the usefulness of the Friedman–Lemaître cosmological models. As we have remarked, these were based on the most extreme simplifying assumptions—that the universe admits no more than one singularity, that it is homogeneous and isotropic, and so on. In fact, they were toy universes, and they were soon cast aside and almost forgotten, until

Einstein and Eddington recalled attention to them for the rather specialized reasons we mentioned. Then, to everybody's surprise, Hubble, Tolman and others discovered that these toys bore a marked resemblance to the universe revealed by Hubble's observations. Ever since about 1930, a main preoccupation of cosmologists has been to decide which toy universe is most like the real thing. And all this activity, to say the least, has never produced a nonsense.

We may only now be coming to understand why Friedman and Lemaître had wrought better than perhaps they knew—or could know in their day. For from the foregoing discussion we see that, had cosmologists attempted to use more general models not assuming, say, homogeneity and isotropy, then work such as that of Misner would have tended to show that such models would soon become homogeneous and isotropic, and so on as regards other features. Thus cosmologists would, apparently, after all have been led back to the Friedman–Lemaître models.

Uniqueness and Physical Laws

The actual physical universe is by definition unique. As was implied at the outset, a fundamental objection to the procedure of, say, relativistic cosmology is that it compares the actual universe with a whole set of theoretical models, all on the same footing. We are invited to select one of these models that just happens to match better than the rest. It is difficult to assign significance to such a situation.

We have just seen, however, that had we started with models more general than those of Friedman and Lemaître, general physical considerations would have led us to restrict our attention to the more particular cases of the Friedman–Lemaître models. This seems to be enormously significant in the context with which I am now concerned. For, as we have also remarked, further physical considerations of such a sort must be expected to restrict the choice still further. Thus the embarrassing situation of having to select a model universe from among claimants of equal standing may scarcely arise. Or at any rate, if we have to select a model in this way, we should now expect in due course to find some deep physical reason for preferring it.

It is well to remember that the work we are discussing is intended to give at best a model of no more than the "smoothed-out" universe, so some rather artificial features will remain in the situation. Nevertheless, the tenor of the discussion in which we are engaged encourages us to think that we are doing something significant in carrying out the accepted procedures of the subject.

We have noted several properties that a model universe must possess, almost independently of the initial conditions, according to the various physical theories concerned. It seems to be a short step to saying that, according to theory, the actual universe simply has to have these properties. In other words, we think there must be some justification for ignoring any possibility that would follow only from rather special initial conditions. If so, we can assert: (*E*) Physical theory shows that the universe in the large must have certain properties which we observe it does have.

This is not an empty or trivial proposition. As we have already remarked, the examples quoted depend on highly sophisticated physics. By the same token, I am certainly not claiming to be able to say by pure reason what the universe must be like. For physical theory is not the product of pure reason and nothing else.

If statement *E* is significant, then we must further assert: (*F*) In so far as physical theory may be applied to the behaviour of the universe in the large, it must be required to predict only what is observable.

All this provides a new sort of test of physical theory. At one extreme there are the familiar laboratory tests. These are usually very precise, because they concern predictions of a sort that the theory is deliberately designed to yield. At the other extreme, physical theory is not in general designed to make predictions about the universe in the large. If it does, they will be about the smoothed-out universe; for this and other reasons they will not be subject to precise test. But the fact that theory does apparently make generally valid predictions of this uncovenanted sort gives a new kind of confidence in physical theory.

To put the conclusion in perhaps simpler terms: We do not believe that the universe has a singularity (or singularities) because general relativity says it must have; we do not believe the universe to be isotropic because the theory of neutrino viscosity says it must be, and so on. Rather, we believe these theories to be useful ways of thinking about the universe in the large because they lead to these acceptable inferences.

Laws and the Universe

The ideas that are unfolding here may meet two other objections that have been urged against the whole concept of big-bang cosmology. First, it has always seemed that the laws of physics, on the one hand, and the physical universe, on the other, must be interdependent. Any notion that the physical universe came into existence according to certain physical laws—which it, so to say, found waiting for it to obey—seems to be unacceptable. The conclusion *C*, that the laws of physics as we have them permit almost nothing to be said about the "start" of the universe, is certainly in keeping with this view.

Second, the notion of a changing universe evolving according to unchanging laws seems almost as hard to accept. At the same time, the notion of changing laws is useless. Were there such things, we could not know about them. For, if we did know about them, this would be by virtue of a law of change of the laws; this super-law would be unchanging, so we should have a contradiction.

The trend of this new assessment is to regard the laws as being laws explicitly for our study of the universe. The laws are not there to tell us what the universe was doing *n* billion years ago, but to tell us what we can know about this—including, of course, the meaning to be attached to "*n* billion years ago". This viewpoint is clearly consistent with the fact that the certainty of our knowledge is greatest at our present.

It is tempting to say that we could nevertheless go through corresponding theoretical investigations for a time *n* billion years in the past, and so determine what an observer then would have known with certainty. But were we to do this we should still have to find out how much of that knowledge that observer could have transmitted to us. And this is consistent with the demand that we may "predict" only what we can check by observation. We could not check what a past observer would have observed; we can check only what we can observe of what the universe was like at that epoch, as a result of our ability to look into the past in an evolving universe. This should be the same as what the past observer could transmit to us. In this fashion we get away from the concept that physical laws are something that the universe must obey. They are something that our thinking about the universe must obey.

Next Steps

If it is justified to claim that the new outlook enhances the significance of existing studies, we naturally ask how they ought to be developed.

On the astronomical side, it seems proper to continue to seek a Friedman–Lemaître model to fit the observations as well as possible. If one is found that gives a reasonably good fit, then at least it will provide a convenient representation of empirical knowledge of the universe in the large. Also, we know from existing work that we cannot get a fit without allowing for evolutionary effects, and so we expect incidentally to gain knowledge of the evolution of galaxies and so on, that we could gain in no other way.

On the astrophysical side, we ought to look for other properties, such as the helium abundance, that are insensitive to initial conditions, and that do not depend to a first approximation on the "age of the universe". It is not easy to think of such properties, presumably because there are not many of them. Maybe the maximum mass of a galaxy in a cluster is a case in point, or there may be magnetic properties of the cosmos that come in for consideration. The interest is that, just as the instances already discussed apparently serve to narrow down the choice of models to the Friedman–Lemaître cases, additional instances may reduce the choice still further.

We do, indeed, know one fundamental means of discriminating between models of the expanding universe. This concerns the arrow of time. As is well known, Wheeler and Feynman[13] sought to show that, if we take a symmetric combination of advanced and retarded solutions of the equations of electromagnetism, but also take account of the effects produced by a postulated "absorber" at infinity, then the outcome is the same as if we disregard the absorber and follow the usual procedure of arbitrarily selecting the retarded solution for everything in the finite part of the field. Wheeler and Feynman used a static space–time, and it was difficult to understand how a model that is time-symmetric could admit the dissymmetry denoted by selecting only retarded fields. Hogarth[14] therefore sought to adopt these general concepts to non-static space–times. He treated an expanding universe as constituting its own "absorber", in the sense of Wheeler and Feynman, and the expansion provides a natural dissymmetry between past and future. Hogarth discovered that, among the models of the expanding universe that he studied, some do and some do not lead to the emergence of retarded fields. If a particular expanding model does have the property that local electromagnetic effects are correctly treated by the use of retarded fields, then this property would explain why observers in this model would, in fact, describe it as expanding, not contracting.

Were some such results to hold good for the actual universe, since work like Hogarth's again involves sophisticated applications of the physical theories concerned, we should have another striking instance of the ability of the physics to deal with the universe in the large. Further, it would mean, as we have said, that a time-symmetric treatment of electromagnetism (not assuming radiative reaction), that takes explicit account of the behaviour of the universe in the large, would reproduce the same local effects as we get by taking no account of the universe in the large but employing our usual conventions about retarded potentials (and radiative reaction). We do not yet know why we want things to work out that way. The point is, however, that we cannot choose our conventions and our cosmology independently. Thus we seem to be a step nearer to being able to claim that physics as a whole demands just one particular universe.

So far as such work is successful—Hogarth emphasizes the limitations of his treatment and others propose different treatments—it shows how the behaviour of the universe in the large determines local features such as the direction of the arrow of time. Thus we have a "Mach principle" in operation for electromagnetic properties. Now Lynden-Bell[15], in particular, has formulated a criterion for a cosmological model to be "Machian" in a more comprehensive sense. We cannot claim to be compelled to require a model to be Machian. But if we do, Lynden-Bell's work shows that this must impose further severe restrictions.

So far as physics is concerned, our discussion has been concerned with known physics, but we have to ask what new physics may be required for further progress. The change of scale between laboratory physics and cosmic physics is so enormously greater than the change between, say, atomic and laboratory physics that there appears to be abundant scope for new physics. Nevertheless, astronomical observations present no undoubted demand for anything fundamentally new. Astronomers have naturally considered the possibility of new phenomena in galactic nuclei, quasars and pulsars, but there is no definite indication of a failure of known physics. Also, continual creation, or non-conservation of baryon number in certain interactions, if either were required by the astronomy, would be "new" physics, but again there is no firm demand for them, although such ideas are not finally abandoned. Apparently the greatest difficulty of physics is to explain why matter on the small scale is parcelled into identical electrons, identical protons and so on, and why matter on the large scale is parcelled into galaxies and clusters of galaxies. These are very different problems and it might be thought that neither is a cosmological problem. If baryon number, say, is not conserved, however, then the processes in which it changes must be of fundamental cosmological significance; if baryon number is conserved, then the total number of baryons (defined in some appropriate manner taking account of the possible existence of matter and antimatter) is a fundamental feature of the cosmos and a satisfactory theory ought to predict it. Again, the trend of recent cosmological studies is to infer that condensations of matter into galaxies, and so on, exist now because the universe never was homogeneous in detail. Thus the problem of elementary particles and the problem of galaxies are both problems of cosmological origins; proposed solutions would presumably have to be compatible with the conclusion *D* about starting conditions.

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Homotypic and Heterotypic Interference by Defective Particles of Vesicular Stomatitis Virus

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Defective particles can serve as templates for their replication by helper infectious virus. While both the "long T" and "short T" defective particles in VSV preparations are capable of homotypic interference, only the larger "long T" particle is capable of heterotypic interference.

THE presence of functional non-self-replicating particles is being recognized in an increasing number of mammalian virus systems[1-4]. In vesicular stomatitis virus systems (VSV), defective particles, produced as a natural consequence of infection with the bullet-shaped infectious "B" particles, are responsible for autointerference (which results in decreased yields of infectious viral progeny)[5-7].

Defective particles, designated "short T" particles, are produced by wild type VSV of the Indiana serotype (IND-ST) whereas a larger "long T" particle is the only defective particle produced by a heat resistant strain of VSV (HR-LT) isolated from the above strain[8,9]. Both "short T" and "long T" particles are similar in morphology[8,9] and constituent proteins[10,11] to the larger infectious B particle. Whereas "short T" particles are one-third the length of B particles and contain an RNA genome one-third the weight of the B particle genome, the "long T" particles are approximately one-half the length of B particles and contain an RNA genome one-half the weight of the B particle genome[9,12,13].

Huang and Wagner[5] showed that "short T" particles of the Indiana serotype, while capable of interfering with the production of infectious virus of the Indiana serotype (homotypic interference), are much less effective in interfering with the production of infectious virus of the New Jersey serotype (heterotypic interference). We here compare homotypic and heterotypic interference by both "short T" and "long T" particles of the Indiana serotype and propose a model of the possible genome composition of the defective particles.

Preparation of B and T Particles

Pure B particle stocks of the HR-LT and IND-ST strains of the Indian serotype[9] and B particles of the New Jersey serotype were produced by low multiplicity passage (0·1 p.f.u./cell) of plaque purified virus in mouse L cells in culture. Electron microscopic examination of these stocks reveals less than one T particle in one hundred B particles. At least three high-multiplicity serial passages are required to produce a detectable number of T particles.

"Long T" particles were prepared from lysates of serial undiluted passage stocks of HR-LT virus; "short T" particles were obtained from lysates of undiluted passage stocks of IND-ST strain of VSV. Both "long T" and "short T" particles were separately purified by at least three consecutive sucrose gradient purifications. The final infective titre in T particle preparations was less than 10^4 p.f.u./ml.

Interference by Purified "Long T" and "Short T" Particles

The yield of infectious virus produced by cells infected with B particles together with either "long T" or "short T" particles depends on the multiplicity of defective particles added. At low multiplicities of T particles, where the interference effect is least, there is a near linear relationship between the log of the p.f.u. produced and the dilution of the T particle preparation. This linear portion of the interference curve is used as a measure of the interfering activity[17].

In Fig. 1 the interfering activities of both "long T" and "short T" particles with B particles of three virus strains are compared. The interfering activities of both "long T" and "short T" particles are identical when tested against homotypic B particles. By contrast, whereas "long T" particles are as effective in heterotypic as in homotypic interference, the "short T" particles did not produce heterotypic interference.

Particle Production during Interference

The interference assay determines the effect of interference on infectious B particle production only. Consequently, experiments were done to examine the entire extracellular particle yield by quantitation with measurement of absorbance, after sucrose gradient analysis, for each condition of interference. Infection with "long T" or "short T" particles alone, at the highest concentrations

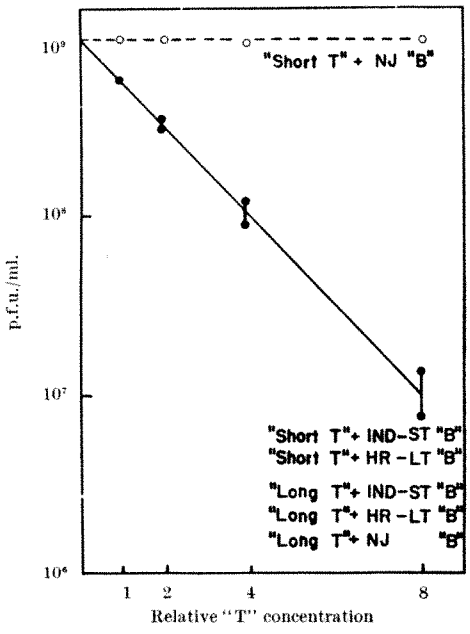


Fig. 1. Interference by "long T" and "short T" particles of the Indiana serotypes. L cell monolayers, 4×10^6 cells in sterile 60 × 15 mm Falcon plastic Petri dishes, were infected with 4×10^6 p.f.u. of B particles of the appropriate strain in 0·1 ml. A similar volume of either growth medium or appropriately diluted "long T" or "short T" particle stock was also added to each plate. After absorption at 37° C for 30 min in an atmosphere of 5 per cent CO_2 and 100 per cent humidity, 5 ml. of minimal essential medium supplemented with 5 per cent foetal calf serum was added and the plates were incubated a further 18 h. At this time the yield of p.f.u./ml. in each cell free lysate was determined by plaque assay[18]. At least three different experiments were done for each particle combination using two distinct "long T" and "short T" preparations. For each T particle preparation the relative numbers of "long T" in the same volume of preparation were determined by direct microscope counting and the dilution scales normalized. In the above graph a relative dilution of 2 corresponds very approximately to one physical T particle per cell. All results are normalized to a control particle yield of 10^9 p.f.u./ml. to allow direct comparisons. The solid line and associated bars show the range of results obtained for the following five combinations: "short T" or "long T" particles with homotypic HR-LT or with IND-ST particles and "long T" with heterotypic New Jersey B particles. The broken line (○ - - - ○) represents the results of "short T" with heterotypic New Jersey B particles.

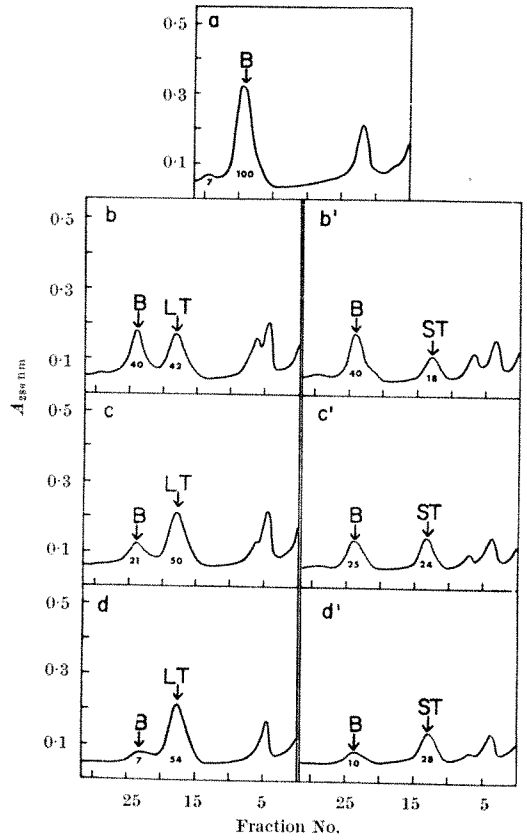


Fig. 2. Effect of "long T" and "short T" particles on the total particle yield of cells infected with IND-ST strain. 10^8 cells were infected with 1 p.f.u. cell of IND-ST B particle stock with or without the addition of appropriately diluted T particles. After absorption for 30 min at 37° C the cells were resuspended (10^6 cells/ml.) and incubated in suspension culture for 24 h. Cells and debris were removed from the lysate by centrifugation at 2,000*g* for 10 min. Virus particles were pelleted from the supernatant by centrifugation at 40,000*g* for 120 min in a Sorvall fixed angle rotor, resuspended in 1 ml. of phosphate buffered saline (PBS) and layered on a linear 5 to 30 per cent sucrose gradient in PBS. After centrifugation at 81,000*g* and 4° C for 35 min in the 'SW27' rotor of a Spinco ultracentrifuge, the gradients were collected and the effluent was continuously monitored for A_{280} using an Isco density gradient fractionator. *a*, Yield from cells infected with B particles alone; *b*, *c*, *d*, yield from cells infected with B particles plus two-fold increasing numbers of "long T" particles respectively; *b'*, *c'*, *d'*, yield from cells infected with B particles and two-fold increasing numbers of "short T" particles. The direction of sedimentation was from right to left. The numbers below the peaks are proportional to the area under the peak and are normalized to a value of 100 for B particles in *a*.

used in this study, yielded neither light scattering nor absorbance bands.

In all gradients illustrated, the position of B particles, "long T" particles or "short T" particles is indicated. Absorbance peaks located at other positions in the gradient will be briefly described. Electron microscopic examination of material in fractions sedimenting just in advance of the B particle revealed only clumps of particles identical to infectious B particles. In some gradients a shoulder of absorbance appears on the trailing edge of the B particle band, but the nature of this material is unknown. Near the top of the gradients there are one or more bands of material which in the electron microscope contain structures similar to the core nucleoprotein of B and T particles. Ribonucleoproteins of both B and T particles accumulate in infected cells[9] and are released to the extracellular medium late in infection.

Fig. 2 shows the particle yield from cells infected with B particles of the IND–ST strain and either "long T" or "short T" particles. With either T particle it can be seen that the greater the input of T particles the less B particles produced, as expected from p.f.u. results. At lower levels of interference the mass loss in B particles is partially compensated by an increase in corresponding T particle production. At high levels of interference, however, the actual mass of particle production is in fact decreased. This is in keeping with observations of viral RNA synthesis as described by Stampfer *et al.*[14].

An important conclusion can be drawn from the results presented in Fig. 2. Although the IND–ST strain of VSV, in normal undiluted passage conditions, gives rise principally to "short T" particles, it can—when superinfected with "long T" particles—lead to their replication. This suggests that the defective particle is acting as a template, presumably at the RNA replication level, for its own synthesis.

Identical results were obtained when the above experiments were repeated using the homotypic HR–LT strain of B particles with either "long T" or "short T" particles. Simultaneous addition of both "long T" and "short T" particles to cells infected with IND–ST B particles resulted in the production of both types of T particles (unpublished data).

When "long T" particles were used in heterotypic interference (Fig. 3*b*, *c*, *d*) there was a progressive reduction in the number of B particles produced as the input number of "long T" particles increased. By contrast with homotypic interference, however, there was only minimal indication of T particle production. If "long T" particles are indeed produced in this infection, their number is not related to the input number of "long T" particles.

"Short T" particles used in heterotypic combination with infectious B particles of the New Jersey serotype have no effect on the yield of B particles (Figs. 3*b'*, *c'*, *d'*) as expected from the lack of interference determined by p.f.u. At the same time it should be noted that no con-

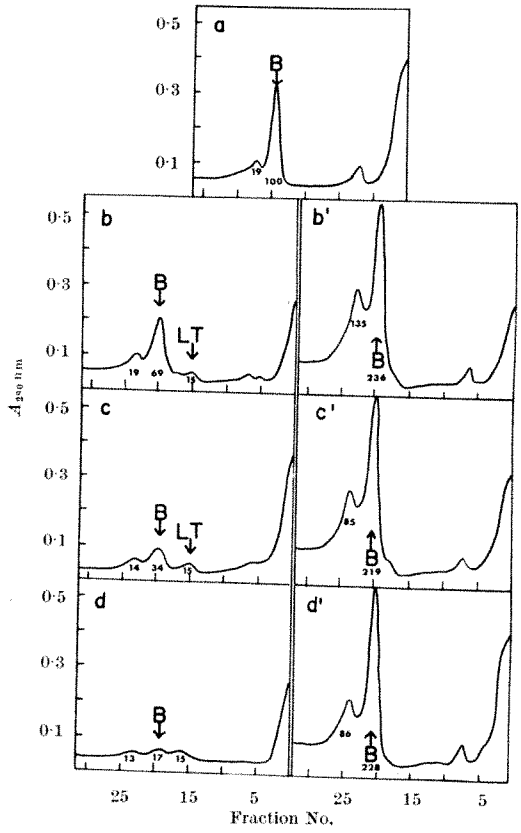


Fig. 3. Effect of "long T" and "short T" particles on the particle yield of heterotypic New Jersey B particle infected cells. The procedures are identical to those described in Fig. 2 except that B particles of the New Jersey serotype were used. All cultures were harvested at 24 h. The virus particles of *b'*, *c'*, *d'* were pelleted by centrifugation at 81,000*g* for 70 min in a Spinco 'SW 27' rotor and somewhat greater particle recoveries were obtained than in *a*, *b*, *c*, *d* which had been pelleted as described in Fig. 2. *a*, Yield from cells infected with B particles alone; *b*, *c*, *d*, yield from cells infected with B particles plus two-fold increasing numbers of "long T" particles; *b'*, *c'*, *d'*, yield from cells infected with B particles plus two-fold increasing numbers of "short T" particles. The direction of sedimentation is from right to left. The numbers below the peaks are proportional to the area under the peaks normalized to a value of 100 for B particles in *a*.

comitant synthesis of "short T" particles occurred in these conditions. Thus the New Jersey virus seems incapable of acting as a helper for synthesis of "short T" particles of the Indiana serotype. This result was not the result of exclusion of "short T" particles from the cell by New Jersey B particles, for pre-infection with "short T" particles gave the same result.

Possible Cistron Assignments for VSV Genomes

The fact that ultraviolet irradiation can eliminate the interfering effect of "short T" particles and "long T" particles (unpublished observations) seems to indicate the need for functional T particle genomes in interference. The question is therefore: What information is carried by the T particle genomes? As a working model we propose that the genomes of B, "long T" and "short T" particles consist of the cistrons as shown in Fig. 4. This model is based principally on a consideration of the molecular weights of the viral structural proteins[10,11] and the molecular weights of the RNA genomes within the three particles[9,13].

Although the polymerase cistron is shown as one unit, the replicase and transcriptase (production of messenger RNA) can be different enzymes or different functions of the same enzyme. Furthermore, partly on the basis of a previous conjecture that each structural protein is coded for by its own monocistronic messenger RNA[9], we may suppose that the genetic information in the T particle is complementary in base sequence to the actual messenger RNA for these proteins. On the basis of these cistron assignments, the lack of self-replication by T particles is explained by the absence of the polymerase cistron. Replication of T particles in the presence of B particles can occur if the viral replicase specified by the B particle genome is capable of replicating the T particle genome.

At least two hypotheses for interference have been previously proposed. One suggests[5] that a product of the T particle genome may act as an inhibitor of an essential "early" protein coded for by the B particle genome. The second hypothesis suggests that the T particle genome may compete with B particle RNA as a template for available polymerase molecules[14]. To distinguish between these possibilities on the basis of the model it will be necessary to determine whether T particles, like B particles[16], carry a virion-associated transcriptase or if transcription from T particle genome is wholly dependent on an enzyme synthesized from the B particle. In particular, it will be important to know whether "long T" particle RNA is replicated in the presence of New Jersey B particles.

Regardless of which mechanism is operative in interference the proposed cistron assignments afford a possible explanation of the difference in heterotypic interference exhibited by the two T particles. Assuming that VSV

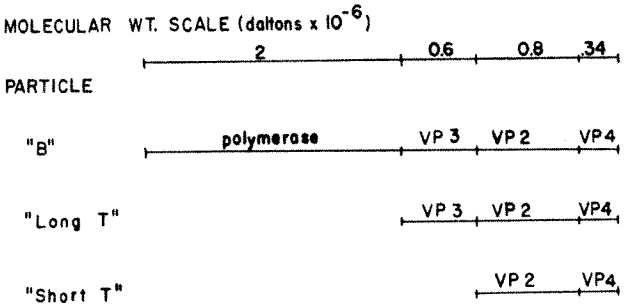


Fig. 4. A proposed model of the cistrons in VSV particles. VP_3, VP_2, and VP_4 refer to the viral structural proteins as previously defined[10]. Apart from the grouping of the cistrons of VP_2 and VP_4 and the grouping of these cistrons with that of VP_3, the linear arrangement of these cistrons along the genome is arbitrary. The expected cistron sizes are derived from the molecular weights of structural proteins as obtained by Wagner *et al.*[11] multiplied by ten. The molecular weights of the genomes are from Nakai and Howatson[13] and Petric and Prevec[9].

polymerase is specific for homologous template RNA, then—because the proteins VP_2 and VP_4 are antigenically distinct in the two VSV serotypes[15]—we suggest that the New Jersey enzyme is incapable of utilizing the RNA contained in the "short T" genome. In this case no interference could occur. The "long T" genome, however, contains the cistron for VP_3, a protein which is antigenically similar in both serotypes[15]. This segment of genome may be sufficiently similar in the two serotypes to allow template recognition and subsequent replication or transcription of all cistrons thus leading to interference.

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Interferon Production by T4 Coliphage

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Evidence is presented that DNA can act as an inducer of interferon.

ALTHOUGH interferon is produced by cells in response to a number of agents, the recognition mechanism of the cell to the inducer, along with subsequent events involved in the production of interferon, is little understood. Viruses, both active and inactivated, are capable of inducing interferon. A phage produced in a mould, *Penicillium stoloniferum*, was found in our laboratories to be the active component of statolon[1,2]. Subsequently, Lampson *et al.*[3] showed that double-stranded RNA extracted from a helenine preparation obtained from *Penicillium funiculosum* was a highly active inducer of interferon. Our studies, and those of others, have shown that such double-stranded RNA is derived from phages (mycophages) carried by these moulds[4,5]. Synthetic double-stranded RNA molecules were also found to be capable of inducing interferon[6].

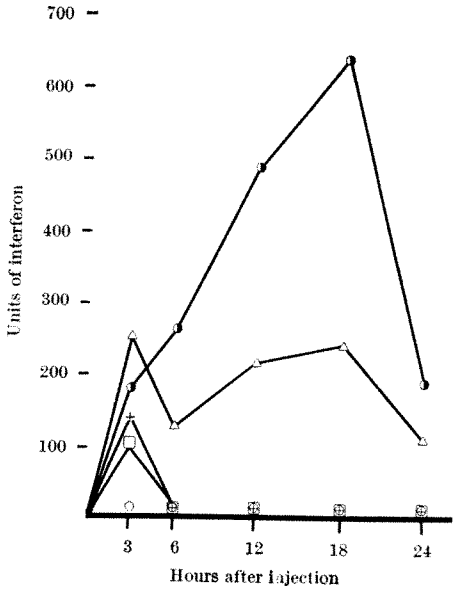


Fig. 1. Production of circulating interferon with T4 and endotoxin. ◑, T4; △, T4+endotoxin; +, endotoxin, heated and sonicated; □, endotoxin; ○, T4, heated and sonicated.

Although DNA viruses can induce interferon and are inhibited by it, DNA, although double stranded, has not proved to be an effective inducer[7]. DNA very likely does not reach the inducer recognition site *in vivo* because of its vulnerability to nucleases or possibly because of structural restrictions. Recently, Friend *et al.*[8] demonstrated that T2 coliphage is taken up and degraded by macrophages. In an attempt to understand more about the mechanism of the stimulation of interferon production, we undertook to determine whether T4 coliphage, a DNA containing virus of nonmammalian origin, and presumably nonreplicating in the mammalian cell could, by virtue of its being a virus, deliver its DNA to the recognition site.

T4 coliphage (T4r) was grown in glycerol medium[9] and titred according to classical methods[10]. Lysates were freed of bacterial debris by filtration or centrifugation, dialysed overnight against three volumes of distilled water, applied to 6 × 70 cm DEAE-cellulose columns, and eluted with a salt gradient (personal communication from D. Fraser). The eluted phage band was pelleted by centrifugation at 15,000 r.p.m. in the 30 rotor on the 'Spinco Model L' centrifuge. The pellet was carefully resuspended and diluted in 0·15 M NaCl, 0·001 M $MgCl_2$ and 0·001 M Tris buffer (*p*H 6·8).

The serum of mice injected intravenously with 2×10^{11} T4 coliphage particles/mouse and bled 18 h after injection was found to be inhibitory to vesicular stomatitis virus (VSV) in mouse L cells. Levels of inhibition in several experiments ranged from 500 to 1,000 units/2·5 ml. serum. Examination of the properties of the inhibitor showed it to be a nonsedimentable, nondialysable, *p*H 2 stable, pepsin-sensitive, species-specific substance, all of which are properties of interferon. The presence of interferon could not be detected in the sera of mice injected 48 h previously; this disappearance with time is generally observed with circulating interferon. Preliminary experiments with T4 DNA showed no production of an inhibitor against VSV in mice injected with DNA and bled 18 h later.

A dose response was determined by injecting intravenously five groups of four mice each with 0·2 ml. of purified T4 coliphage, ranging in dosages from $3{\cdot}60 \times 10^8$ to $1{\cdot}84 \times 10^{11}$ particles/mouse. At 18 h after phage administration, the mice were bled and the sera from each group were pooled and assayed for interferon. The results given in Table 1 show an increased response with increasing quantities of T4 coliphage. No response is seen with T4 until a threshold input of about 3×10^9 particles/mouse

Table 1. DOSAGE RESPONSE OF T4 COLIPHAGE IN MICE

Phage particles per mouse	Units of interferon
$1{\cdot}84 \times 10^{11}$	742
$3{\cdot}60 \times 10^{10}$	159
$1{\cdot}18 \times 10^{10}$	145
$3{\cdot}60 \times 10^{9}$	85
$1{\cdot}18 \times 10^{9}$	< 30
$3{\cdot}60 \times 10^{8}$	< 30

is reached. Lomniczi and Burke (personal communication) have made a similar observation with mutants of Semliki Forest virus. At high multiplicities the RNA of the input virus was the inducer, while at lower multiplicities the inducer was newly synthesized RNA. It seems that interferon synthesis can begin only when the concentration of inducer reaches a threshold concentration whether by initial input or by subsequent increase through synthesis.

Although the T4 phage was of high purity, the possibility of extraneous endotoxin being responsible for interferon production needed to be eliminated. Endotoxin is a relatively stable material—preliminary experiments showed that endotoxin (*Escherichia coli* O111: B4 endotoxin, Difco) withstands heat treatment at 98° C for 15 min plus sonification for 15 min, while T4 is inactivated as an inducer by this treatment. A differential test was therefore available.

T4 at 3×10^{11} particles/mouse and endotoxin at 150 μg/mouse, both untreated and treated with heat and sonication, were each administered intravenously to groups of six mice, and after various time intervals they were bled. The sera of each group of mice were pooled and were then assayed for interferon. A mixture containing equal volumes of the starting materials, T4 and endotoxin, was also tested. Because injection volumes were held constant (0·2 ml.), half quantities of each of the constituents were injected in the latter case.

Endotoxin, both treated or untreated (Fig. 1), produced an early peak interferon followed by a swift decay, as is generally seen with endotoxin[11]. Although some interferon is seen early with T4, it continues to increase with time until a peak level is reached at 18 h. This late peaking is seen with viral inducers of interferon. No detectable interferon is produced with T4 inactivated by heat and sonication, indicating that no measurable endotoxin contaminates the phage preparations. The curve of the mixture of T4 and endotoxin also shows the characteristic contribution made by each component and that the contribution is additive at 3 h. Closer definition of the kinetics of interferon production by T4 during the early period was desirable, and this was included in the next experiment.

Because ghosts of the T-even coliphages can be prepared, we had available a method for testing the contribution of the particulate coat of T4 toward the production of interferon. T4 ghosts were prepared according to the procedure of Herriott and Barlow[12]. Ninety-four per cent disruption was accomplished with three osmotic treatments. T4 at 3×10^{11} particles/mouse, and an equivalent amount of T4 ghosts, less 6 per cent, prepared from the same sample of phage were injected. Because some intact phage remained in the ghost preparation, T4 at the same concentration ($1{\cdot}8 \times 10^9$ particles/mouse) served as a control.

As can be seen in Fig. 2, T4 particles bereft of their DNA do not make a contribution toward production of interferon in the mouse. The interferon that is produced obviously results from the intact T4 present in the preparation. Again a peak level of interferon production is reached 18 h after injection of the T4. It can be seen, however, that with the closer defined points during the early period, a separate early peak is produced by T4 at 2 h with a characteristic decay.

Early experiments in this study had indicated that extracted T4 DNA was not active as an inducer. It was examined again over a wider range of concentration and time. Quantities of phenol-extracted DNA equivalent

to that found in 1×10^9 to 3×10^{11} phage particles were injected into mice, and their sera were tested for interferon. Only at the high concentration equivalent to 3×10^{11} phage particles at 2 h after injection was any inhibition seen, and the quantity of interferon produced was relatively low.

Inducers of interferon are generally divided into two classes; the endotoxin or releaser type and the viral or inducer type which leads to *de novo* synthesis of interferon. Although endotoxin was found not to be involved in the production of interferon by T4, the early peak at 2 h indicated toxicity and possibly, therefore, release of interferon from the cells, rather than stimulating *de novo* synthesis. The kinetics of interferon production at a later time, with the main peak at 18 h, indicates that this interferon is newly synthesized. The appearance of interferon at 18 h could, however, be a reflexion of a slow uptake and degradation of the particle.

The best method for differentiating between release or synthesis of interferon is to test the ability of the inducer to produce interferon in tissue culture. Endotoxin is not capable of producing interferon *in vitro*, while viral inducers possess this capability. When T4 was tested in mouse L cells and challenged with VSV after 24 h, no evidence of protection was seen. But when it was applied to monolayers of primary rabbit kidney cells, a cell which takes up materials much more readily, inhibition of VSV was clearly demonstrated (Table 2). This system was not sensitive enough to detect exogenous interferon in the supernatant fluids. The cells were thoroughly washed (four times) before infection, however, to remove nonspecific inhibitors, and we relate the inhibition to interferon and conclude that the inhibitor that makes its appearance late in mice after injection of T4 coliphage is newly synthesized interferon.

Our data show that T4 coliphage is capable of inducing interferon. When the component parts of the phage particle, both the protein coat and the DNA, were tested separately, however, they failed to induce interferon. Thus there was no possibility of the presence of contaminating materials, such as endotoxin. These data require an explanation for interferon induction by T4 phage.

The protein portion of the phage in our estimation is not a strong contender as an inducer. This follows from the fact that T4 ghosts were not capable of stimulating interferon. The morphology of phage coats, as seen from electron micrographs of our preparations, is not altered appreciably by osmotic shocking. Coliphage ghosts retain their basic anatomical features and chemical constitution[13,14]. Their uptake should not therefore be altered from that of the phage particles themselves. If the coat of the intact phage was active initially as a stimulator of interferon, it should remain so. The lack of stimulation of interferon by T4 ghosts not only is evidence against the protein of T4 as the inducer but also rules against the particle *per se* being responsible solely because of its particular nature.

The situation with DNA is, however, entirely different. Inside the phage, the DNA is present as a closely packed unit and will readily be taken up as such. Outside the phage, the DNA occupies much greater space. Such expansion of the molecule would impede its uptake. The DNA in solution would also be exposed to degradation by DNase and by shearing. Encapsulation would protect the DNA against such degradation.

T4 phage, by virtue of its being a viral particle, may be channelled through a route generally taken by viruses after uptake. This would facilitate the delivery of the

Table 2. INHIBITION OF VSV BY T4 IN PRIMARY RABBIT KIDNEY CELLS

T4 particles/ml.	Per cent inhibition
$1{\cdot}5 \times 10^{12}$	72
$7{\cdot}5 \times 10^{11}$	50
$3{\cdot}0 \times 10^{11}$	14
$1{\cdot}5 \times 10^{11}$	11
$1{\cdot}5 \times 10^{10}$	0
$4{\cdot}7 \times 10^{9}$	0

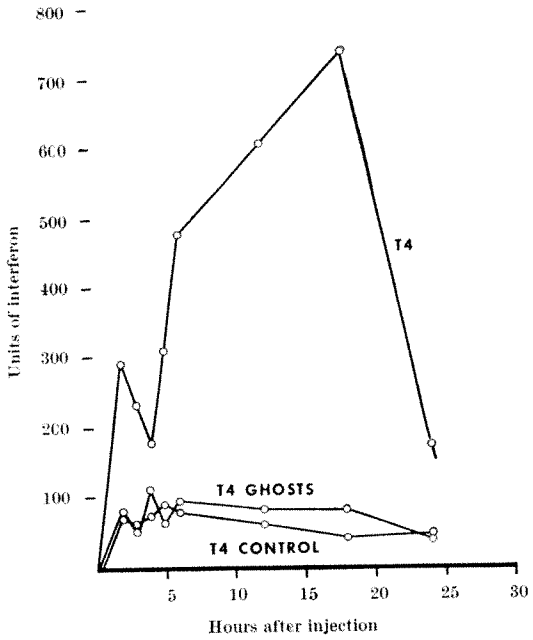


Fig. 2. Production of circulating interferon with T4 and T4 ghosts.

DNA to the inducer recognition site. On uncoating the phage at this stage, the DNA in its unaltered state would be able to initiate the sequence of events leading to interferon synthesis.

Colby and Duesberg[15] have recently reported the isolation of a double-stranded RNA from vaccinia-infected cells. Their observation has provided a mechanism for the stimulation of interferon synthesis by mammalian DNA viruses, namely, through the mediation of a double-stranded RNA. Jurale *et al.*[16] have also observed a double-stranded RNA in T4 infected *E. coli*. It is possible, although unlikely, that the DNA of T4 coliphage can also initiate the synthesis of a DS-RNA in mammalian cells.

Glucosylation of the T4 DNA may also be an important structural feature for activity, in distinction from other DNAs that have been tested. The extracted DNA, of course, remains glucosylated, but here the DNA may be decreased by degradation or by diminished uptake to a point below its effective concentration, while it remains intact while encapsulated.

The strict structural specificity required for a molecule to be active as an inducer of interferon[7] may be inherent in the structure of T4 DNA as it exists in intact phage. An exceedingly important observation pertinent to our argument is that of Maestre and Tinoco[14]. From optical rotary dispersion studies on a number of phages, including T4, and their nucleic acids, these investigators conclude that the DNA inside a phage does indeed possess a different conformation from the DNA in solution. Release of the DNA from the T4 phage before its reaching the recognition molecule may then result in a conformational change accounting for the failure of extracted T4 DNA to induce interferon. We therefore submit that DNA is the component molecule of T4 coliphage responsible for induction of interferon and that this is made possible through the delivery of the T4 DNA in the required configuration to the inducer recognition site, implemented through the encapsulation of the DNA in the intact phage. DeClerq *et al.*[17] have recently made an observation that supports this structural transition hypothesis. They have found that synthetic double-stranded polydeoxyribonucleotides acquire interferon-inducing capacity only after heating at 37° C before application to cells. They suggest that this activation is caused by changes in secondary structure of the polynucleotides.

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The Renin–Angiotensin System: Inhibition of Converting Enzyme in Isolated Tissues

by

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The converting enzyme content of isolated tissues suggests that the indirect action of angiotensin I involves the intramural generation of angiotensin II. Inhibition of the enzyme shows that there is also a residual, direct action of angiotensin I.

SKEGGS and his colleagues[1,2] have shown that there are two angiotensins, the decapeptide angiotensin I (A I), from which the second and more active octapeptide angiotensin II (A II) can be generated by a "converting enzyme" isolated from plasma. Ng and J. R. V.[3,4] found that *in vivo* the plasma converting enzyme was relatively inactive and that AI was converted to A II on transit through the pulmonary circulation. A I was also converted to A II in isolated lungs perfused with Krebs solution[5], so the enzymic activity was in the lung tissue and was not due to activation of the plasma enzyme on its way through the lungs.

In other vascular beds, such as kidneys or hind legs[3–5], there was only a net inactivation of A I and A II. Nevertheless, homogenates of various tissues show converting enzyme activity[6–10]. Thus A II might be formed in these tissues but in amounts too small to be detected in the fluid or blood perfusing them. Alternatively, converting enzyme activity of tissue homogenates might be due to endopeptidases liberated by maceration but not normally available to A I when the tissues are intact.

On isolated organs bathed or perfused with salt solutions, A I has similar effects to A II but is less potent[1–4,11–14]. In isolated organs such as the rabbit aorta[11], the rat colon[3,4] and the rat uterus[14], A I has 2–10 per cent of the potency of A II but in some perfused organs[12,13] and in guinea-pig isolated ileum[12] A I is up to 70 per cent as active as A II. This variation in the relative potency of A I may be due to one or more of three modes of action: (1) a direct action on specific A I receptors, (2) a direct but weak action on A II receptors, or (3) an indirect effect due to a variable conversion to A II within the isolated tissue itself.

We have now distinguished between these possible modes of action of A I by using an inhibitor of converting enzyme[5,8,15] found in an extract[16] of *Bothrops jararaca* venom. A pentapeptide in the extract with the structure pyroglutamyl–lysyl–tryptophyl–alanyl–proline[17] also inhibits converting enzyme *in vitro*[18]. A synthetic sample (SQ 20,475) of this pentapeptide was kindly supplied by Dr M. Ondetti and J. Pluscec of the Squibb Institute for Medical Research.

Angiotensin I used was the Ileu[5] synthetic decapeptide (Schwartz Biochemicals) and angiotensin II was the Asp[1]-β-amide, Val[5] angiotensin II (Hypertensin, Ciba). All drugs were dissolved in sterile physiological saline solution (0·9 per cent NaCl w/v) and administered by slow infusion (0·03–0·3 ml./min) into the perfusion or superfusion fluid. Drug concentrations are expressed as the free base or peptide and refer to the amount of substance in the perfusion or superfusion fluid. Contractions of isolated tissues were detected by isotonic transducers and recorded on a Watanabe 6-channel pen recorder.

Inhibition of Converting Enzyme

We first established that the synthetic pentapeptide inhibited converting enzyme of guinea-pig isolated lungs. Lungs were perfused through the pulmonary artery with Krebs solution at 37° C and 10 ml./min as previously described[5]. The effluent from the lungs superfused[19] up to six isolated tissues, including rat colons[20] and rat stomach strips[21]. These tissues were selected because they are contracted by low concentrations of A II but are relatively insensitive to A I. To increase the specificity of the bioassay for A II, a mixture of antagonists (methysergide bimaleate (200 ng/ml.), phenoxybenzamine hydrochloride (100 ng/ml.), propranolol hydrochloride (2·3 μg/ml.), hyoscine hydrobromide (100 ng/ml.) and mepyramine maleate (140 ng/ml.)) was infused into the Krebs solution after it had perfused the lungs. This had an additional beneficial effect of reducing the spontaneous activity of the bioassay tissues.

The pentapeptide strongly inhibited the converting enzyme activity of guinea-pig lungs as shown by contractions of a rat colon superfused with the effluent from the lungs (Fig. 1). The first three contractions were induced by A II (0·3, 1 and 3 ng/ml.) given directly to the assay tissues. When A II (3 ng/ml.) was infused through the lungs, its activity on the rat colon was scarcely reduced indicating, as shown previously[5], that A II was not inactivated in the pulmonary circulation. A I (10 ng/ml.) had little or no effect when infused directly to the assay tissues but when infused through the lungs at 3, 5 and 10 ng/ml. the contractions of the rat colon showed that there was about 50 per cent conversion of A I to A II. When the pentapeptide (SQ 20,475, 0·5 μg/ml.) was infused through the lungs, conversion of A I to A II was reduced to less than 10 per cent. During infusion of the

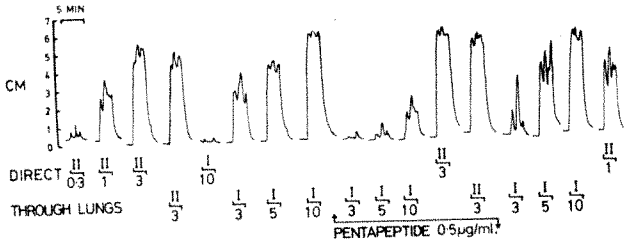


Fig. 1. The isolated lungs of a guinea-pig were perfused through the pulmonary artery with Krebs solution at 10 ml./min. The effluent superfused a rat colon, the contractions of which are shown. A I (I) or A II (II) was infused for 3 min into the Krebs solution to give the concentrations shown (ng/ml.). The sensitivity of the colon to A II (0·3–3 ng/ml.) was demonstrated by direct infusions into the pulmonary effluent. When A II was given into the pulmonary circulation (through lungs), there was only a slight reduction in size of contraction. A I (10 ng/ml.) given directly to the rat colon had no effect but caused graded contractions of the colon when infused (3, 5 and 10 ng/ml.) through the lungs. The conversion to A II represented about 50 per cent. When SQ 20,475 (pentapeptide, 0·5 μg/ml.) was infused through the lungs, the conversion of A I to A II was greatly reduced, but the effects of A II were unchanged. Conversion of A I in the lung recovered when the infusion of SQ 20,475 was stopped. Except for the first and last contractions, the trace was continuous but 3 to 5 min of the record between infusions has been removed to condense the figure. Vertical scale shows movement of pen recorder in centimetres with a ×4 magnification of contraction. The time scale was 5 min.

pentapeptide there was neither reduction in the direct effects of A II on the rat colon nor change in the removal of A II by the lungs. The generation of A II on passage of A I through the lungs returned when the infusion of inhibitor was stopped.

In four similar experiments different concentrations of the pentapeptide were used (0·1, 0·3, 0·5, 1·0 and 3 μg/ml.). There was a linear relationship between the degree of inhibition of converting enzyme and log concentration of inhibitor. SQ 20,475 gave 50 per cent inhibition of formation of A II at 0·15 μg/ml. and 100 per cent at 3 μg/ml. Thus the synthetic pentapeptide is a much more potent inhibitor of converting enzyme than the crude extract of snake venom which gave only partial inhibition[5] of the lung enzyme at 5 μg/ml.

Antagonism of Contractile Effects of A I

We next used the synthetic pentapeptide to find whether isolated arterial tissue contained converting enzyme activity. Arteries were removed from rats, rabbits and cats killed by a blow on the head, and from cats and dogs anaesthetized with pentobarbitone sodium and bled. The arteries were cut spirally into strips and superfused at 10 ml./min with Krebs solution at 37° C. The load applied to the strips was 2–4 g depending on their thickness (Table 1).

Both A I (100–1,000 ng/ml.) and A II (3–300 ng/ml.) contracted the arterial strips except those from cat renal arteries which were insensitive. The inhibitor of converting enzyme always reduced the contractions produced by A I without affecting those produced by A II. Fig. 2 shows this effect on strips of pulmonary artery (upper record) and thoracic aorta (lower record) from the same rabbit. Before adding SQ 20,475 the ratio of A I to A II to give equal activity was about 4 : 1 on the pulmonary artery and 20 : 1 on the aorta. In the presence of the pentapeptide (1 μg/ml.) the contractions induced by A I (400 ng) were substantially reduced, giving a new ratio of A I to A II of about 30 : 1 on the pulmonary artery and 65 : 1 on the aorta. After infusion of inhibitor was stopped the size of the contractions produced by A I increased again. This selective reduction in activity of A I strongly suggested that some of the activity of A I was due to its conversion to A II within the arterial strip itself. Furthermore, the conversion was much higher in the pulmonary artery than in the aorta.

The amount of intramural conversion was calculated as follows. The contraction of the pulmonary artery induced by A I at 400 ng/ml. was equivalent to A II at

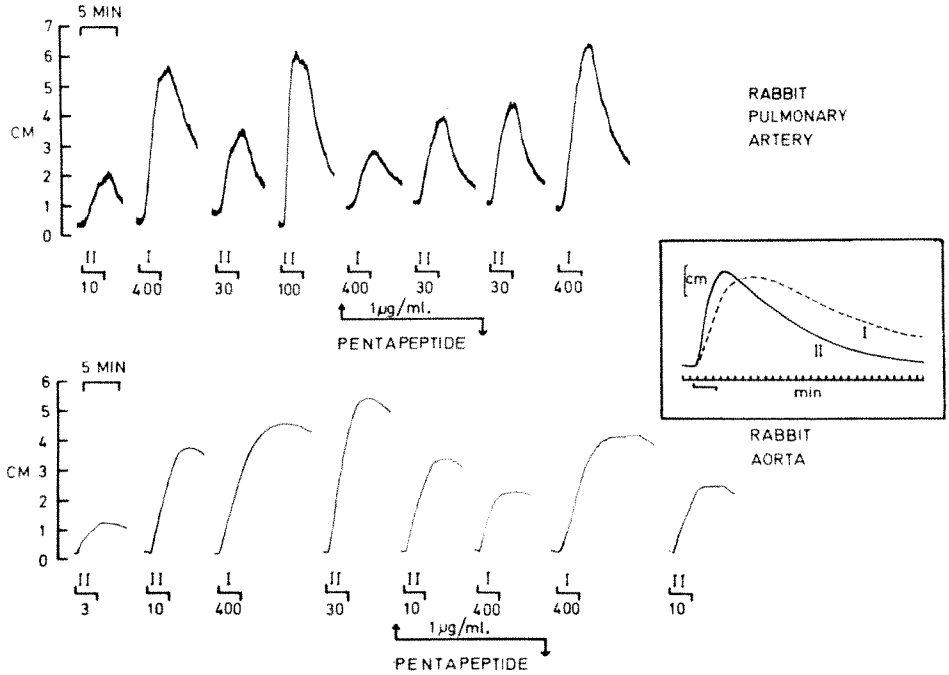


Fig. 2. The tracings show contractions of isolated strips of pulmonary artery (top) and thoracic aorta (bottom) from one rabbit. On the pulmonary artery an infusion for 3 min of A I (I, 400 ng/ml.) gave a contraction slightly less than that produced by A II (II, 100 ng/ml.). In the presence of the pentapeptide inhibitor (pentapeptide, 1 μg/ml.), the contractor activity of A I was selectively reduced. Similar results are shown for the thoracic aorta, except that in this preparation A I showed less activity (400 ng A I = 20 ng A II). Vertical scale in centimetres of pen movement (cm) ×15 (top) and ×10 (bottom) magnification of contraction. Time between infusions was 30 to 45 min. The time scale was 5 min. The inset shows the time course of contractions of a strip of rabbit aorta produced by A I (- - -) and A II (———). The vertical scale is centimetres of pen movement, with a ×10 magnification of contraction. The time scale is in 1 min divisions.

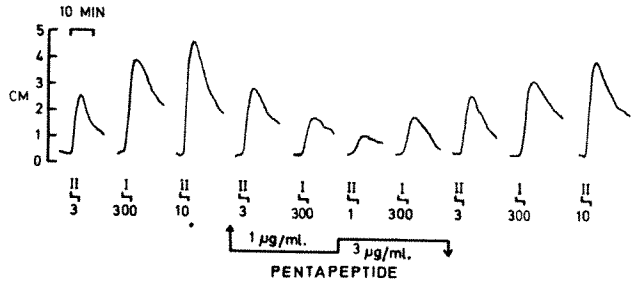


Fig. 3. This tracing shows contractions of an isolated strip of renal artery from a rabbit produced by A I (I, 300 ng/ml.) and A II (II, 1, 3 and 10 ng/ml.). Before the converting enzyme inhibitor, A I (300 ng/ml.) was equivalent to A II at about 6 ng/ml. When the inhibitor was infused (1 μg/ml.), the effects of A I at 300 ng/ml. were reduced, to become equivalent to A II at 2 ng/ml. No further reduction was achieved by increasing the concentration of inhibitor to 3 μg/ml. The vertical scale shows centimetres (cm) of movement of the pen recorder, with a ×20 magnification of contraction. The time scale is 10 min.

100 ng/ml. before inhibition and to A II at 10 ng/ml. during inhibition (Fig. 2, top). The reduction in activity was therefore equivalent to A II at 90 ng/ml. Assuming similar diffusion rates and distribution for A I and A II in the arterial strip, the concentration of A II reaching receptor sites during application of A I was equivalent to a conversion of 90 × 100/400 or 22·5 per cent within the tissue. This does not imply that 22·5 per cent of all the A I in the superfusion fluid was converted to A II, for the amount diffusing into the tissue can be only a minute fraction of the total dose applied.

When this calculation was applied to the experiment of Fig. 2, the conversion was 22·5 per cent in the pulmonary artery but only 4 per cent in the aorta. For more accurate estimates of conversion, taking into account the different molecular weights of A I (1,297) and A II (1,032), equipotent molar ratios were used (see later). These increased the conversion figure by about one fifth (27·5 per cent in the pulmonary artery and 4·9 per cent in the aorta).

In both vascular strips, A I produced a delayed onset and a slower decline of contraction than A II (see inset on Fig. 2). The fact that contractions induced by a 3 min infusion of A I sometimes took 7 to 9 min to reach a maximum suggested that A II continued to be formed within the tissue even after A I was no longer present in the superfusion fluid.

Another possible interpretation of the selective reduction of the activity of A I by SQ 20,475 is that the two angiotensins act on separate receptors and that the pentapeptide specifically blocks those for A I. This interpretation was rejected because cross tachyphylaxis showed that A I and A II acted on the same population of receptors. Contractions of a rabbit pulmonary artery were produced by noradrenaline, A I and A II. Repeated infusions of A II at 10 to 12 min intervals progressively desensitized the artery to A II. When the effects had almost disappeared, A I also had virtually no effect, whereas noradrenaline produced a greater response than before. In other experiments contractions produced by A II were selectively blocked when A I was used for desensitization. Similar results were obtained in strips of cat pulmonary artery and rabbit renal artery. When there are no specific antagonists, cross tachyphylaxis is good evidence for two substances acting on the same population of receptors[22]. Thus the specific inhibition of the activity of A I brought about by the pentapeptide SQ 20,475 must have been due to inhibition of conversion of A I to A II within the arterial strip.

Although SQ 20,475 reduced the contractions produced by A I, it never abolished them. For instance, in an isolated strip of renal artery from the rabbit (Fig. 3), A I (300 ng/ml.) produced a contraction equivalent to A II at 6 ng/ml. In the presence of the pentapeptide (1 μg/ml.) the contraction produced by A I was clearly reduced (A I at 300 ng/ml. now equivalent to A II at 2 ng/ml.). Increasing concentration of the inhibitor to 3 μg/ml., however, did not further reduce the contraction induced by A I (300 ng/ml.), showing that the effect of the pentapeptide was maximal at the lower concentration. Thus although the intramural conversion was only 1·5 per cent, about two-thirds of the total activity of A I on the rabbit renal artery in Fig. 3 was due to formation of A II; the remaining third was due to some other action of A I.

The maximum inhibition by SQ 20,475 (1–4 μg/ml.) of the effects of A I varied from artery to artery. Table 1 shows the relative potencies of A I and A II (±s.e. of

Table 1. CONVERTING ENZYME ACTIVITY OF VARIOUS VASCULAR STRIPS

Vascular tissue	Weight on lever (g)	n	Control equipotent molar ratio A I/A II (mean ±s.e.)	SQ 20,475 equipotent molar ratio A I/A II (mean ±s.e.)	Range of effective doses of A II (ng/ml.) Thresh.	max.	Degree of tachyphylaxis (0, +, ++)
Aorta (thoracic)							
Rat	2	5	8·0±2	28·0±5·2	1–6		0
Rabbit	4	6	20·0±4·3	69·7±11	1–100		0
Pulmonary art.							
Rabbit	2–4	12	5·5±1·6	25·6±3·2	1–300		+
Cat	3–4	5	5·2±2	35·7±4	1–100		++
Rat	2	4	3·8±2	27·0±8·2	1–100		+
Carotid art.							
Rabbit	3	2	14·0±6	59·0±19	1–100		++
Cat	2–4	5	12·5±3	47·3±9	1–100		+
Dog	4	4	11·3±4	46·0±11	0·3–100		0
Coeliac art.							
Rabbit	3	2	60±20	115±5	1–100		++
Cat	3	2	53±13	106±13	1–100		+
Dog	4	1	20	79			++
Renal art.							
Rabbit	2–3	4	46·5±6	84·0±13	1–100		+
Cat	2–3	4	No response to angiotensin				
Dog	4	3	22·3±5	53·0±14	3–100		+
Femoral art.							
Rabbit	2–3	4	36·0±5	48·0±6	0·3–30		0
Cat	3	3	52·0±0·7	83·0±3	0·5–100		0
Dog	4	3	24·0±2	57·0±12	1–100		+
Axillary art.							
Rabbit	3	2	40±7	100±15	1–100		+
Cat	3	3	90·0±10	113±9	0·3–30		0
Dog	4	3	51·0±24	60±26	0·5–30		0

The mean equipotent molar ratios for A I and A II are shown before and during inhibition of converting enzyme by supramaximal concentrations (1–4 μg/ml.) of SQ 20,475. A low ratio means a high activity for A I. The weight on the lever is shown in g. n = Number of experiments. The standard (s.e.) of the mean is shown, except when n = 2, where the range is given. The last column shows the degree of tachyphylaxis to the effects of A I and A II (see text).

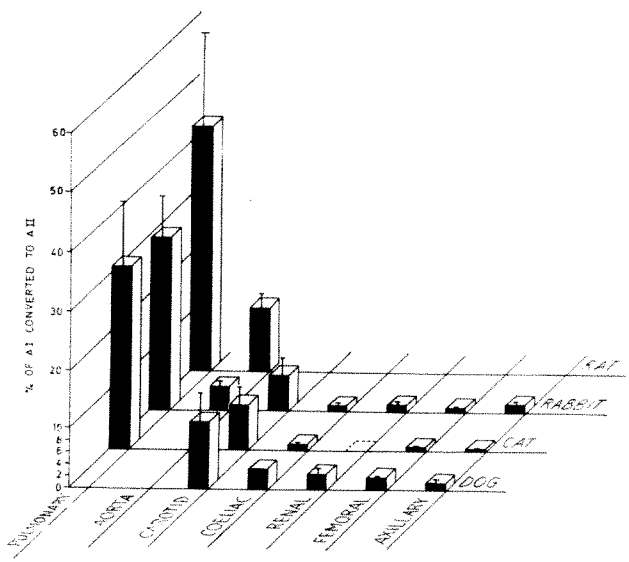


Fig. 4. The diagram illustrates the differences in converting enzyme activity shown by arterial strips from rat, rabbit, cat and dog. The activity is calculated from individual values, the means of which are shown in Table 1. The vertical lines at the top of the columns show either the s.e. or the range when n = 2 (see Table 1). Pulmonary arteries showed the highest activity, then rat aorta and then the carotid arteries. Coeliac, renal, femoral and axillary arteries had little or no activity. The cat renal artery did not contract to the angiotensins.

mean) before and during infusion of inhibitor. The relative potencies are expressed as the number of moles of A I equivalent to 1 mole of A II. Thus an "equipotent molar ratio" of 100 means that to produce the same contraction, one hundred molecules of A I were needed for each molecule of A II. The differences between the equipotent molar ratios before and during the infusion of SQ 20,475 were used to calculate, by the method described earlier, the amount of intramural conversion in each strip (Fig. 4).

Cat, rabbit and rat pulmonary arteries showed by far the greatest conversion (30–42 per cent). Dog pulmonary artery and dog and cat aorta were too thick to use. The aorta of the rat (10 per cent) and carotid arteries of the cat (8 per cent), dog (11 per cent) and rabbit (6 per cent) had relatively high converting enzyme activities. All the other arteries showed much less conversion (0·3–4 per cent).

The high intramural conversion in pulmonary arterial strips is an interesting corollary of the high conversion in blood-perfused[3,4] or saline-perfused[5] lungs. Converting enzyme might therefore be located in the vascular tissue; if it were present in all vessels down to the smallest pulmonary arterioles, the enormous surface area to which A I was exposed would explain its rapid conversion in the pulmonary circulation.

There was minimal conversion in renal, femoral and axillary arteries. It was in renal and femoral vascular beds that Ng and J. R. V.[3,4] were unable to detect conversion of A I to A II *in vivo*, although recently it has been suggested[23], on the basis of radioimmunoassays, that after massive doses of A I in the dog renal artery, about 8 per cent of the total immunoreactivity in the renal vein is A II. The significance of this figure is uncertain, for less than 30 per cent of the immunoreactive material in venous blood is in the form of A II (ref. 24). There was no striking variation in converting enzyme activity between species. There were some differences in the sensitivity of different arteries to A II. Whereas cat renal artery was insensitive and dog renal artery was relatively insensitive, femoral and axillary arteries were the most sensitive (Table 1). Thus the more peripheral arteries had greatest sensitivity to A II, but the least amount of converting enzyme. This suggests that, *in vivo*, the A II is delivered to these arteries rather than being formed in the walls.

Some arteries showed a gradual decline in response to repeated doses of A I or A II. With intervals of 30 min between infusions, tachyphylaxis was least evident in aortas, femoral and axillary arteries (graded 0 in Table 1) and most evident (+ +) in cat pulmonary, rabbit carotid, and rabbit and dog coeliac arteries. Tachyphylaxis occurred with all arteries when the interval between infusions was shorter (10 min).

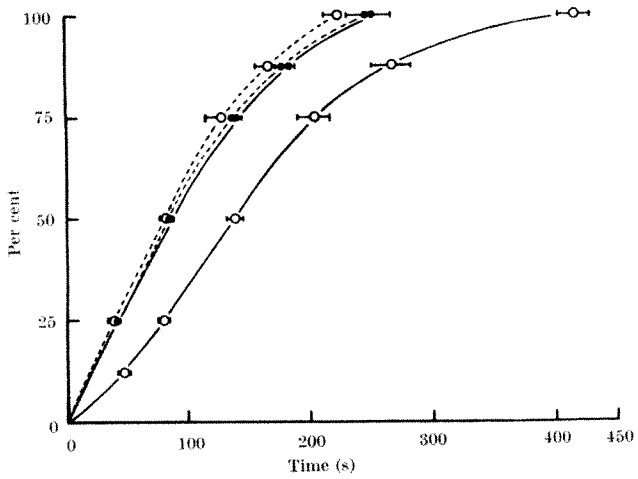


Fig. 5. The rates of development of contraction of strips of rabbit aortas ($n = 4$) are shown plotted against time in seconds. The closed circles are for A II and the open circles for A I. Before the converting enzyme inhibition (solid lines) the rate of contraction for A I was slower than that for A II. With the inhibitor present (dotted lines) there was no distinction in rates. To obtain the graphs, the time was measured for each contraction to reach 12·5, 25, 50, 75, 87·5 and 100 per cent of the final contraction height. The bars show the standard errors of the means.

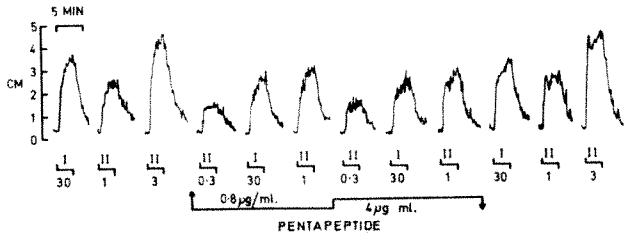


Fig. 6. The tracing shows the contractions of a rat colon superfused with Krebs solution at 10 ml./min. Before inhibitor, A I (I, 30) at 30 ng/ml. was equivalent to A II at 2 ng/ml. The inhibitor (pentapeptide 0·8 μg/ml.) selectively reduced the contraction induced by A I so that 30 ng/ml. was now equivalent to A II at about 0·8 ng/ml. Increasing the concentration of inhibitor to 4 μg/ml. did not further reduce the effects of A I. The vertical scale is in centimetres (cm), with ×4 magnification of contractions. The time scale is 5 min.

Residual Responses to Angiotensin I

In arterial strips, even with high concentrations of converting enzyme inhibitor present (Fig. 3), A I still produced small contractions. These could not have been due to contamination of the A I by a small amount of A II, because the residual activity of A I was equivalent to differing amounts of A II on different arteries (Table 1). These residual effects of A I might have been due to formation of A II by an enzyme not inhibited by the pentapeptide. This possibility has not yet been excluded. Our results suggest, however, that a more likely explanation is that A I has a feeble direct action (0·8–4 per cent) on A II receptors. First, on tissues such as pulmonary artery and aorta the rate of contraction induced by A I was slower than that produced by A II without the inhibitor but identical with the inhibitor present, as shown for rabbit aorta in Fig. 5. Rates of contraction are plotted (as a percentage of the contraction attained) for responses produced by A I and A II. The rate of contraction produced by A I was substantially slower than for A II, suggesting slow conversion of A I to A II within the vessel wall. When converting enzyme was inhibited, however, the rate of contraction induced by A I was as fast as that induced by A II, suggesting a direct action of A I and not one relying on an intermediate enzymic step. Similar effects were seen in pulmonary arteries. Further supporting evidence was found on the cat axillary artery, which has little or no converting enzyme activity; rates of contraction for A I and A II were similar without the inhibitor being present.

Our evidence suggests that the residual effects of A I are due to a direct but weak (0·8–4 per cent) action on A II receptors. In this context, it is interesting that in radioimmunoassays[25–27] A I reacts with the antibodies for A II to a similar extent (1–5 per cent). Such antibodies can be regarded as a form of receptor for A II.

Converting Enzyme in other Preparations

The contractile effects of A I on other isolated smooth muscle preparations were also reduced by the converting enzyme inhibitor. Fig. 6 shows this in an isolated ascending colon of the rat. A I at 30 ng/ml. caused a contraction of the colon approximately equivalent in size to one produced by A II at 2 ng/ml. (molar ratio A I : A II = 12:1). During infusion of SQ 20,475 (0·8 μg/ml.) the contraction induced by A I at 30 ng/ml. was reduced (molar ratio of A I : A II = 30:1). As in vascular tissue, contractions of the rat colon produced by A I could not be abolished by the pentapeptide. Thus increasing the dose of inhibitor to 4 μg/ml. had no further effect. Five minutes after infusion of pentapeptide was stopped the contraction produced by A I (30 ng/ml.) returned to near its original size. These results showed that, as in vascular tissue, part but not all of the response of the rat colon produced

Table 2. CONVERTING ENZYME ACTIVITY OF RAT NON-VASCULAR SMOOTH MUSCLE

Rat tissue	Wt. on lever (g)	n	Equipotent molar ratio A I/A II (mean ±s.e.)	SQ 20,475 equipotent molar ratio A I/A II (mean ±s.e.)	Range of effective doses. Thresh.-max. (ng/ml.)	Percentage of A I converted to A II
Colon	2	8	14·2±3	37·0±5	0·1–3	4·4±0·6
Ileum	1	6	15·5±3	45·5±7	0·3–3	4·2±0·1
Uterus	2	4	40·0±7	83·4±11	0·3–10	0·8±0·2

Details as in Table 1. The last column shows the calculated intramural conversion of A I to A II.

by A I was due to its conversion to A II within the isolated tissue itself. Results similar to those for the rat colon were obtained with rat ileum and rat uterus, but the amount of converting enzyme activity was different in the three tissues (Table 2).

It is difficult to compare our results on rat isolated organs with those with rat tissue homogenates. The converting enzyme activity of macerated tissues, as measured by Huggins and Thampi[7], is different from that measured by Roth, Weitzman and Piquilloud[10]. Like Huggins and Thampi, we found low activity in the rat uterus, but we also found relatively high activity in rat aorta, whereas Huggins and Thampi found none. Furthermore, we found rat aorta to be higher in activity than ileum, whereas Huggins and Thampi found ileum to contain as much activity as lung tissue. These results emphasize again that enzyme activity measured in macerated tissues may not correspond to that measured in intact tissues.

This is the first time that the direct and indirect effects of A I on smooth muscle have been distinguished. The converting enzyme content of many isolated smooth muscle preparations is sufficient to contribute to the contractor action of A I by the intramural generation of A II. Such intramural generation of A II probably explains the high activities[12,13] of A I relative to A II, for when it is abolished, A I has a residual direct action of about 1–4 per cent of that of A II.

How important physiologically is the intramural generation of A II by converting enzyme in vascular tissue? First, the slowness of the intramural conversion must be emphasized. Whereas A I is almost completely converted to A II in the 2 to 5 s that it takes to traverse the pulmonary vascular bed, responses due to intramural conversion in arterial tissue *in vitro* take 4 to 8 min to develop. Unless the intramural conversion is much faster *in vivo*, it cannot contribute significantly to the effects of intravenous injections of A I. Similarly, when renin is present in the bloodstream, the lungs are likely to be the only important site for formation of circulating A II.

Any physiological importance of intramural conversion may be associated with a local role of the renin–angiotensin system within the vessel wall. A renin-like enzyme has been extracted from vascular tissue[28,29]. If this enzyme is effective *in situ* the juxtaposition of converting enzyme might, through the intramural generation of A II, contribute to the local control of vascular tone.

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Calcium Induced Release of Calcium from the Sarcoplasmic Reticulum of Skinned Skeletal Muscle Fibres

by

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The repeated contractions which can be observed in skinned fibres in appropriate concentrations of caffeine, Ca^{2+} and chelating agent suggest that calcium release is a regenerative process in which calcium itself causes the release of calcium from the reticulum.

It is now generally accepted[1] that at the final stage of excitation–contraction coupling calcium ions are released from the sarcoplasmic reticulum into the sarcoplasm, and Ca^{2+} in turn activates the contractile protein system. We have now further investigated the mechanism of the release of calcium from the sarcoplasmic reticulum using Natori's skinned fibre[2]. Although originally Natori's fibres were prepared and used under a mineral oil, the skinned fibres can also be kept in the relaxed state in an aqueous solution if the latter contains sufficient MgATP and GEDTA (glycolether diaminetetraacetic acid, or EGTA)[1,3]. With this preparation Ca^{2+} can be released easily and repeatedly from or reloaded on to the sarcoplasmic reticulum with the aid of a Ca^{2+}-releasing drug, caffeine[4]. In this way it was found that the free sarcoplasmic calcium itself induced the release of Ca^{2+} from the sarcoplasmic reticulum[5,6].

Living single twitch fibres were isolated from the semi-

tendinosus or iliofibularis muscle of the frog, *Rana japonica*; and all or part of the sarcolemma, together with underlying myofibrils, was removed in a K_2SO_4–Ringer solution containing 4 mM MgATP and 4 mM GEDTA. The skinned fibres thus obtained were set up in a trough through which a solution could be perfused rapidly. The two ends of the skinned fibres (1·5–3 mm long) were fixed between pieces of 'Scotch' tape and isometric tension was recorded with a mechanoelectronic transducer (RCA 5734 or Toshiba 5734 A). The normal medium consisted of 22 mM K_2SO_4, 20 mM Tris-maleate buffer (*p*H 6·8), 4 mM ATP, 4 mM $MgSO_4$, 96 mM sucrose and 2 mM GEDTA with or without a specified amount of $CaSO_4$. Experiments were usually conducted at 4° C by perfusing ice-cold water below the glass base of the trough.

When caffeine was applied to skinned fibres in appropriate conditions, it induced contraction (Fig. 1*a*). Weber and Herz[4] have clearly shown that the caffeine-induced contraction was the consequence of the release of calcium from the sarcoplasmic reticulum by the drug. This was certainly the case in skinned fibres, and the following experiments seem fully to confirm this mechanism of action of caffeine. First, purified phospholipase C, which is known to destroy the ability of the isolated sarcoplasmic reticulum (the relaxing factor) to induce relaxation[7] and to accumulate Ca^{2+} (ref. 8), abolished caffeine-induced contraction of the skinned fibres, whereas the contractile ability of the fibre was largely retained (Fig. 1b_1 and b_2). Second, the concentration of free Ca^{2+} in and around the skinned fibres was measured with a Ca^{2+}-binding dye murexide[9], and shown to increase during caffeine-induced contraction.

If the mechanism of caffeine-induced contraction is really as we have now described, the contractile response should be modified by the concentration of GEDTA in the medium. This was found to be the case. When the concentration of GEDTA was as high as 8 mM, 5 mM caffeine, for example, was never able to induce contraction. In the presence of 2 mM GEDTA, skinned fibres usually responded to this concentration of caffeine with a transient contraction, but this was a single response and it never recurred even if the drug was persistently present or re-applied after having been washed away (Fig. 1*a*). In the medium containing a low concentration of GEDTA (for example, 0·05 mM), smaller concentrations of caffeine were able to induce contraction of the skinned fibres. This was also transient but spontaneously recurred repeatedly in the continued presence of the drug (Fig. 1*c*, *d*). This behaviour of skinned fibres can be explained in part as follows. In the presence of 2 mM GEDTA, (1) most of the released Ca^{2+} eventually became bound to GEDTA molecules, and (2) because the concentration of free Ca^{2+} in the medium was very low (about 10^{-8} M) the reticulum of the skinned fibre could not take up enough Ca^{2+} again. In agreement with this view, if the fibre thus treated was immersed in a medium containing a higher concentration of free Ca^{2+} for a certain period, the responsiveness of the fibre to caffeine in the presence of 2 mM GEDTA was restored. As expected from the time course of re-uptake of Ca^{2+} by the reticulum, the degree of restoration was a function of the concentration of free Ca^{2+} and the time of immersion (Fig. 2). In the case of 0·05 mM GEDTA, the free Ca^{2+} concentration became about 5×10^{-7} M because of contaminating Ca^{2+} so that the reticulum could again take up Ca^{2+} (see Fig. 2), a process which constitutes the basis of the repeated release of Ca^{2+} by caffeine.

This discussion is still not a complete explanation of the repeated contraction we have observed. It was noted that the peak tension during the repeated contrac-

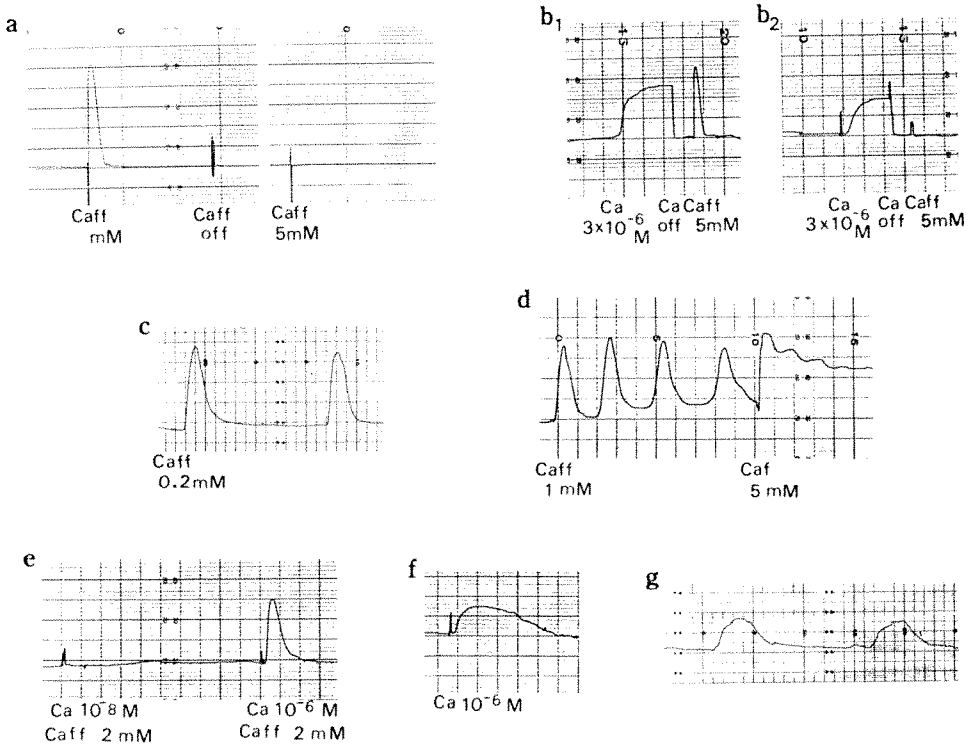


Fig. 1. Contractions of skinned fibres as a result of the release of calcium from the sarcoplasmic reticulum induced by various means. The experimental conditions were as described in the text except for the concentrations of GEDTA and Ca^{2+} and some additions. *a*, Transient contraction induced by 5 mM caffeine. GEDTA, 2 mM ($Ca^{2+} \simeq 10^{-8}$ M). Note that there was only a single response. *b*, The effect of phospholipase on caffeine-induced contraction. Between b_1 and b_2, the skinned fibre was treated for 30 min at room temperature with 2 mg/ml. phospholipase C, which was prepared from *Clostridium perfringens* and purified by column chromatography on DEAE-Sephadex G 50'. GEDTA, 2 mM. *c* and *d*, Oscillatory contractions induced by caffeine. GEDTA, 50 μM ($Ca^{2+} \simeq 5 \times 10^{-7}$ M). *e*, Contraction induced by a subthreshold concentration of Ca^{2+} in the presence of 2 mM caffeine. GEDTA, 2 mM. *f*, Contraction induced by a subthreshold concentration of Ca^{2+} in the absence of any drugs. GEDTA, 150 μM. *g*, Spontaneous oscillatory contractions. GEDTA, 25 μM ($Ca^{2+} \simeq 1 \times 10^{-6}$ M). Time: 24 s per division. Tension: 7 mg per large division.

tions of low frequency induced by caffeine was usually more than 80 per cent of the maximum tension that could be developed by the same fibre with the application of sufficient Ca^{2+}. This suggests that the characteristics of calcium release from the sarcoplasmic reticulum followed an essentially all-or-none pattern (albeit not genuine) along the entire length of the fibre. Indeed, microscopic observation revealed that sometimes during the rising phase of tension, a region of about 20–30 μm in which sarcomeres were highly shortened moved slowly from one end of the fibre to the other. If part of the effect of calcium release or its underlying processes were the induction of further release of calcium, a positive feedback loop would be formed and release of calcium would be according to an all-or-none pattern. We show later that free calcium causes the further release of calcium from the reticulum.

As shown in Fig. 1*e*, 10^{-6} M free Ca^{2+}, which by itself was usually below the threshold for contraction[1], evoked a transient contraction in the presence of a low concentration of caffeine (0·5–2 mM). It might be thought that 10^{-6} M Ca^{2+} would merely have brought the medium nearer to the threshold for contraction so that the amount of Ca^{2+} released by the low concentration of caffeine, which had been ineffective in inducing contraction in the presence of 2 mM GEDTA, could become effective. There are, however, a number of reasons why this is not possible, as the following experiments illustrate. The amount of calcium that remained in the reticulum after caffeine treatment in certain conditions was measured by applying to the fibre a higher concentration of caffeine, which released the remaining Ca^{2+}. The more Ca^{2+} was released by the first caffeine treatment, the smaller was the contraction in the second treatment. Fig. 3 demonstrates that more Ca^{2+} was released in the presence of 10^{-6} M free Ca^{2+} than with 10^{-8} M free Ca^{2+}. Thus free Ca^{2+} itself has an action on the reticulum which promotes the release of calcium. The effect was also observed in the absence of any drugs (Fig. 1*f*), indicating that it is an intrinsic property of the sarcoplasmic reticulum. This may also be reflected in the oscillatory contractions which occurred spontaneously in the absence of any drug (Fig. 1*g*).

Strontium ions showed a similar calcium-releasing action, although the effective concentration was about fifty times greater than for calcium. Free Mg^{2+} inhibited the release of Ca^{2+} from the reticulum, as was previously known[10].

The calcium-releasing action of Ca^{2+} which we have demonstrated may be the principal mechanism of the all-or-none type of calcium release from the sarcoplasmic

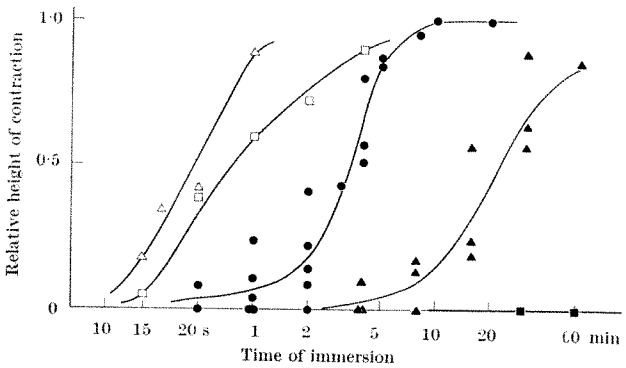


Fig. 2. Time course of restoration of the response of skinned fibres to caffeine for various concentrations of free Ca^{2+}: △—△, 10^{-5} M; □—□, 3×10^{-6} M; ●—●, 10^{-6} M; ▲—▲, 3×10^{-7} M; ■—■, 10^{-7} M. The experimental conditions were as described in the text. After calcium had been loaded on the sarcoplasmic reticulum of skinned fibres in a medium containing a certain concentration of free Ca^{2+} for a certain period, it was released by 5 mM caffeine in the presence of 2 mM GEDTA and the resulting transient contraction was recorded. Treatment with 10^{-6} M Ca^{2+} for 10 min was taken as standard, and the relative values of peak isometric tension in these contractions are plotted.

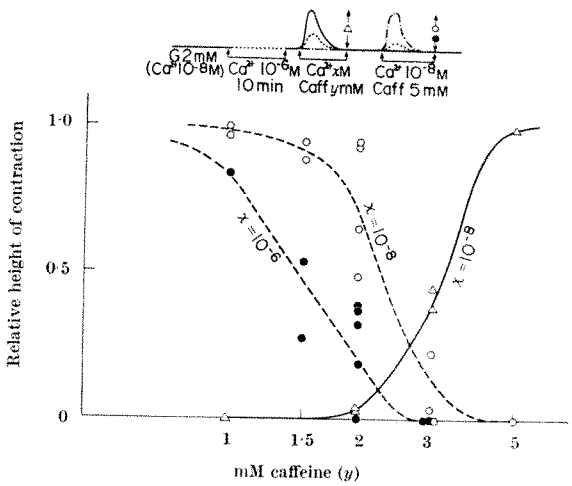


Fig. 3. Caffeine-induced release of calcium from the sarcoplasmic reticulum of skinned fibres and the effect of free Ca^{2+}. The experimental conditions were as described in the text. After calcium had been loaded on the reticulum by treating skinned fibres with 10^{-6} M Ca^{2+} for 10 min, caffeine was applied to the fibres with various concentrations of free Ca^{2+} and the peak tension of the resulting contraction was plotted (continuous line). The tension produced by 5 mM caffeine in the presence of 2 mM GEDTA (G) ($Ca^{2+}\simeq10^{-8}$ M) was taken as standard. The calcium remaining in the reticulum after this treatment was then released in controlled conditions, that is, with 5 mM caffeine in the presence of 2 mM GEDTA, and the peak tension of the resulting contraction was plotted as a function of the conditions of the first caffeine treatment (broken lines).

reticulum in the skinned fibre. It has not yet been possible to initiate contraction by other means, such as stretching the skinned fibre, or applying ADP or inorganic phosphate to the fibre.

It was recently demonstrated[11] that the physiological release of the activator of contraction occurs in a somewhat regenerative manner and this may be related to the well known sharp relationship between depolarization and tension in living muscle fibres. It is very probable that the Ca^{2+}-induced release of Ca^{2+} which we describe is the mechanism of the regenerative nature of the physiological contraction following depolarization. Adrian *et al.*[11] explained the regenerative nature of the release of the activator by assuming a kind of substrate inhibition in the re-uptake of the activator. But if we assume (as is reasonable) that the activator is Ca^{2+}, the rate of uptake of calcium by the reticulum in skinned fibres is never inhibited but is accelerated by increase in the concentration of Ca^{2+}, as indicated by experiments such as those illustrated in Fig. 2. A similar relationship is also obtained in the presence of caffeine. The rate of uptake of calcium by the isolated reticulum measured by Weber *et al.*[12] and Ogawa[13] also displayed monotonic dependence on the concentration of free Ca^{2+} in the medium. It therefore seems that the calcium induced release of calcium is a consequence of true activation of the release process, somehow produced by calcium itself.

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Lateral Inhibition between Orientation Detectors in the Human Visual System

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Experiment has shown that two lines of different orientation interact with each other so that they seem to be displaced from one another in orientation. This could be explained in terms of mutual inhibition between neighbouring columns in the visual cortex.

LATERAL inhibition is a very common property of sensory systems. Retinal ganglion cells receive antagonistic signals from an area of receptors surrounding the central zone of their receptive fields[1,2], and the same thing holds for somatosensory neurones with receptive fields on the skin[3,4]. Fibres in the auditory pathway also have inhibitory connexions with large areas of the basilar membrane adjacent to the region of their characteristic frequency[5,6].

Presumably lateral inhibition has important general functions. It has been pointed out, for example, that it sharpens up the positional information conveyed through a sensory neurone by strictly limiting the region of receptors that activate it[7,8]. Another important consequence, particularly in visual and somatosensory systems, is the elimination of spatial redundancy: any large stimulus that completely covers a receptive field will be relatively ineffective in exciting the neurone, because of the simultaneous inhibitory and excitatory signals. But the edge of a stimulus, falling on the skin or the retina, will be particularly exciting because it will cover only part of the inhibitory surround.

In the visual cortex of the cat and the monkey almost all the cells are selectively sensitive to the orientation of edges and lines in the visual field[9,10]. There is good reason to believe that there are orientation detectors in the human brain too[11–14] and now we present some psychophysical evidence that there is lateral inhibition between them.

Lateral Interaction in the Orientation Domain

Imagine that any orientation detector in the human visual cortex is excited by a relatively narrow range of orientations and is inhibited by a much broader band, with both the inhibitory and excitatory weighting functions being centred on the same optimal orientation. Then presentation of a single line would be expected to produce a distribution of activity among the population of orientation detectors. Those tuned to optimal orientations near that of the line would be excited; those tuned to rather different orientations would be inhibited. So the overall distribution of activity might look like one of the two individual functions shown in Fig. 1. An inhibitory process like this would, as Andrews[14] suggested, sharpen up the neural specification of the orientation of a single line. There are, however, serious consequences when another nearby line of a different orientation is simultaneously present.

Fig. 1 shows two hypothetical distributions of activity created by two individual lines of different orientation, and at the bottom of the diagram the two distributions are simply added together, assuming linear summation of inhibition and excitation. It is clear that the peaks of activity in this compound distribution are slightly shifted apart from the individual peaks produced by the single lines.

If the brain assesses the orientation of contours by inspecting this distribution and, say, identifying the most active neurones, then there should be distinct influences of one orientation on another. In fact, two lines forming an acute angle should appear to be shifted away from each other in orientation; the angle between them should seem to expand. This is true. Our explanation of this phenomenon in terms of lateral inhibition in the orientation domain should be contrasted with theories in which inhibition in the position domain is said to cause apparent "repulsion" of neighbouring parallel contours[8,15].

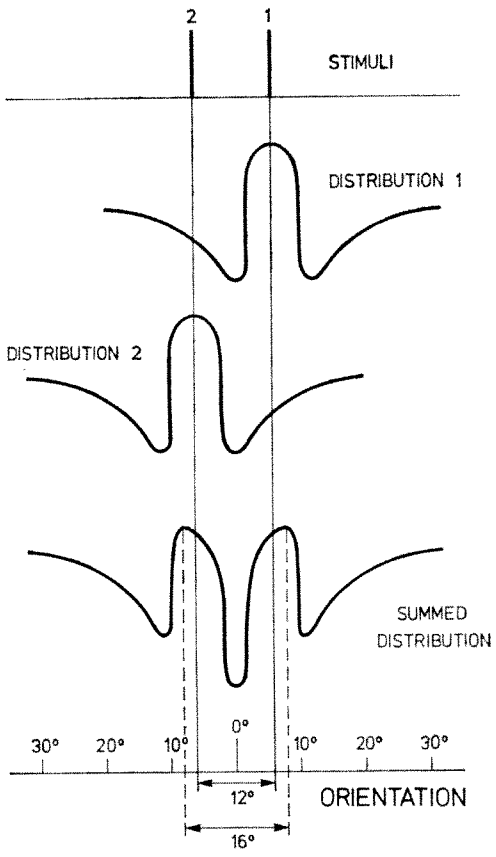


Fig. 1. This diagram represents, in a speculative way, the distributions of activity that might be set up in the orientation domain by two lines of different orientation. The abscissa of the figure is orientation, in degrees, as shown on the scale at the bottom. The two pulses at the top of the diagram represent the two stimulus lines, which differ in orientation by 12 degrees. Below them are the two profiles of activity that they would create individually in the population of orientation detectors. There is a broad peak of excitation among detectors tuned to orientations near that of the line and those further away from the line in orientation are inhibited. The bottom distribution shows what might happen if the two lines, forming an angle with each other, are shown simultaneously to the system. Addition of the individual profiles produces a compound distribution with the peaks of activity slightly displaced from each other.

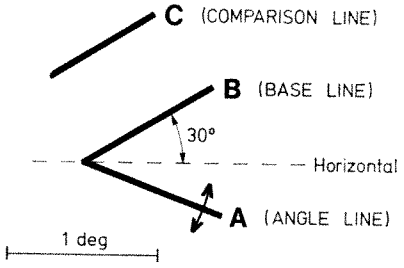


Fig. 2. The computer generated three bright lines on the face of the oscilloscope. The lines had a luminance of about 10 cd.m^{-2} and the dim background was about 0·1 cd.m^{-2}. Two of the lines, a "base line", *B*, and an "angle line", *A*, met at the centre of the screen to form an angle, so that they appeared to the subject like the hands of a clock. They were both 1·4 cm long so that they subtended 1 degree in length at the subject's eyes. Their width was about 2 min of arc. There was a similar "comparison line", *C*, 0·8 degrees long, with its centre on the normal through the middle of *B* and 0·6 degrees away from it. During all the experiments reported here *B* was fixed in the upper right quadrant of the screen at an angle of 30 degrees to the horizontal. Line *A* was rotated to form various angles with *B*.

Measuring the Phenomenon

An observer sat in a darkened room, 80 cm from an oscilloscope screen. A computer (Digital Lab-8) generated a pattern of bright, blue-white lines on the P-7 phosphor of this oscilloscope. The display took the form shown in Fig. 2 and described in the legend. The subject could alter the orientation of the "comparison line", *C*, by turning a knob that made it rotate about its centre. His task was to set *C* parallel to the "base line", *B*. Each time the knob was turned the display flickered slightly as the new commands were computed, but after a little practice this did not bother the subject.

We then tested the effect of the "angle line", *A*, on the apparent inclination of *B* as assessed by the setting of *C*. The subject bit on a dental impression to keep his head vertical and he was allowed to look wherever he liked within the display during the observations.

For the results shown in Fig. 3 the base line, *B*, was always set at 30 degrees anticlockwise from the horizontal, in the upper right quadrant of the screen. For the upper graph in Fig. 3, line *A* was set at different angles clockwise from *B*, up to 180 degrees, and line *C* was above *B*, as shown in the inset diagram. The subject made four pairs of settings of *C* for each value of the angle between *A* and *B*, in random order. At each setting the computer read the angle of *C*, in degrees, with respect to the orientation of *B*, anticlockwise being positive. For each value of *A* the mean of all eight settings was calculated along with its standard error.

The mean setting of *C* is plotted on the ordinate, zero being truly parallel to *B*, positive being anticlockwise error; negative, clockwise error. Results are shown for two subjects, with vertical bars representing one standard error on each side of the mean.

The whole experiment was repeated but with *A* rotated to different angles anticlockwise from *B* and with the comparison line, *C*, below *B*. The results are plotted in the lower graph of Fig. 3. It is evident that acute angles, particularly of the order of 10 degrees, appear to be very grossly expanded, each component orientation being displaced in orientation away from its partner. If both lines are equally displaced then the total enlargement of an angle of 10 degrees is about 4 degrees.

Now if the neural system for orientation detection were completely insensitive to position in the visual field, a line in the lower left quadrant should be indistinguishable to the system from its parallel in the upper right quadrant, and should have the same effect on *B*. So obtuse angles should appear to be contracted. The function should be symmetrical but inverted on each side of 90 degrees.

It is obvious from Fig. 3 that obtuse angles indeed seem contracted but the effect is not as great as that of acute angle expansion. The effect of an obtuse angle of 170 degrees in the upper graph of Fig. 3 is by no means as strong as the effect of an acute angle of 10 degrees in the lower one. It seems, then, as if the neural mechanism is somewhat, but not totally, specific for the position of the lines. This is only to be expected from the fact that there is considerable variation in the length and size of receptive fields even in the foveal projection area of the cortex[9,10]. A line of one orientation presented to different parts of the fovea will stimulate much the same, but not completely the same, group of neurones at each position.

We have illustrated results for just two subjects and at only one orientation of *B*, but we have confirmed the basic finding informally with several other subjects. We have also tried many other orientations of *B* and find roughly the same general effect. There is nothing dramatically special about angles with a horizontal or a vertical line as one of the component orientations. It is clear that an angle of 30 degrees in the upper graph of Fig. 3 (where line *A* is horizontal) and an angle of 30 degrees in the lower graph (where *A* is 30 degrees off-vertical) have similar magnitudes of effect on *B*, but in opposite directions, of course.

Orientation Detectors and Visual Illusions

Gibson[16] noticed this interaction between lines and described it in terms of "simultaneous contrast" between two orientations. He measured the influence of an off-vertical grating superimposed on a truly vertical line by rotating the line until it appeared vertical. In fact, Hering, von Helmholtz and Wundt had all concluded that acute angles must seem expanded because of the distortions that occur in certain of the geometric illusions. The Hering and Zöllner illusions, for example, in which lines seem to be twisted or bowed in the presence of other lines, could easily be explained in terms of angular distor-

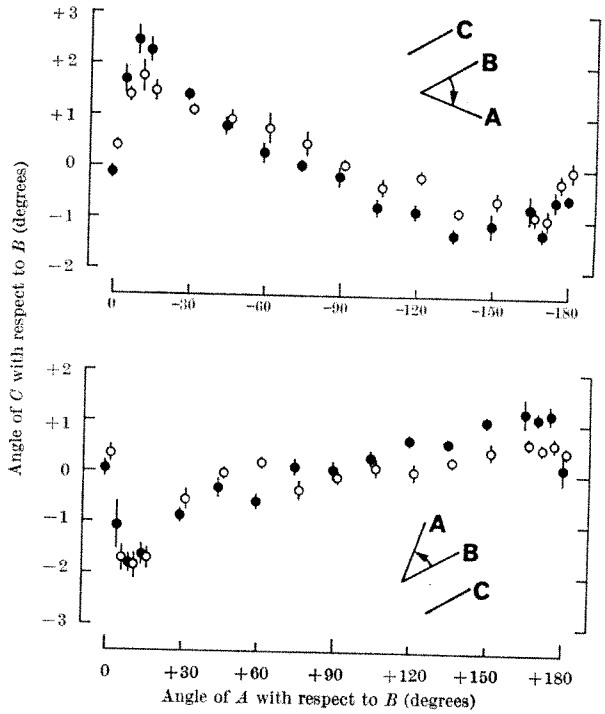


Fig. 3. The base line, *B*, angle line, *A*, and comparison line, *C*, were displayed as shown in the inset diagrams. *B* was always fixed at 30 degrees anticlockwise to the horizontal. For the upper graph, *C* was set above *B*, and *A* was rotated clockwise from *B* to form various angles with it. The abscissa is the angle of *A* measured from *B*, anticlockwise being positive. With the same sign convention the ordinate is the setting of *C* at which it appeared parallel to *B*. Each point is the mean of eight judgments, with vertical bars representing one standard error on each side of the mean. The data points for the two subjects have been slightly separated from each other horizontally to avoid overlap. The lower graph is much the same but in this case *C* was below *B*, and *A* was rotated anticlockwise to form various angles with *B*. ○, R.H.S.C.; ●, C.B.

tion. Jastrow[17] tried to measure this phenomenon by asking people to draw angles of particular sizes. Although he found big errors, there is, as Fisher[18] points out, a logical fallacy in this technique. Fisher himself asked subjects to estimate, in degrees, the size of angles shown to them. But there are obvious logical difficulties in that approach as well. We believe that our method, using a comparison line, overcomes the problem of reference to an internal image of verticality or a subjective scale of angle.

It is possible, of course, that the comparison line, C, is somewhat influenced by line A, and that we have therefore underestimated the phenomenon. This is something we must explore more closely in future experiments and we must certainly keep the possibility in mind when we attempt to predict the extent of geometric illusions from these basic data.

Inhibition or Adaptation?

It might seem that the results we obtain could be a product not of lateral inhibition but of "adaptation" or "satiation". As Sutherland[19] suggested, a reduction in the sensitivity of cortical neurones after prolonged stimulation could be responsible for such shifts in apparent orientation. Expansion of angles might be due to the same sort of adaptation process that is thought to cause the well known figural after-effects of orientation[16].

There are three reasons why we reject this hypothesis. First, we find angle expansion virtually unchanged if the subject makes his settings in 3 or 4 s, with long rest periods in between, implying a very fast build-up of the supposed adaptation. Second, because the observer generally glanced back and forth between B and C, recovery from adaptation must have been very fast to prevent C and B being equally influenced by A. These observations do not square with the long time-constants of known adaptation phenomena[11,12,16]. Finally, and perhaps most compelling, we have found that the effect of A on B is reduced by the addition of another line forming a small angle with A. "Disinhibition" of this type cannot be explained by any kind of adaptation.

In summary, we have found that two lines of different orientation interact with each other so that they seem displaced from one another in orientation. The effects could be explained in terms of mutual inhibition between neighbouring columns in the visual cortex, each of which contains cells tuned to one orientation. We must admit that, so far, there is no evidence that cortical units are inhibited by orientations away from their optimal, but we hope to test that hypothesis with neurophysiological experiments.

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New Stochastic Model for Geomagnetic Reversals

by

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A non-stationary model for geomagnetic reversals is proposed. From the model, a mean length of all polarity intervals of 1·6 m.y. is predicted.

THE occasional reversal of the Earth's dipole magnetic field is apparently a random process. To a first approximation, the occurrence of reversals resembles a stationary Poisson process, which led Cox[1] to propose a stochastic model for this phenomenon. In his model, the Earth's dipole field was assumed to oscillate regularly with constant polarity, reversing only when "triggered" into the opposite sense by the effect of random (normally distributed) non-dipole components, having mean zero and fixed standard deviation. The model suggested, physically, a number of time-varying eccentric dipoles in the vicinity of the core–mantle interface.

This model adequately explains the reversal pattern for the most recent 10 m.y. It was apparent to Cox, even then, however, that the same model could not apply to the Permian, where reversals were known to be much less frequent[2]. This has now been verified in the Cretaceous as well[3], making this simple stationary model inadequate. The partial success of Cox's model has led us to suggest a non-stationary extension of his principle.

In searching for the exact nature of the non-stationarity, we discovered the long-period regularity reported in a previous article[4], in which significant polarity oscillations with periods of about 300 m.y. and 75 m.y. were noted. Subsequently, we have performed spectral analysis of Simpson's mixed measurements and obtained periods of about 150 m.y. and 40 m.y. We are thus faced with the apparent paradox of a random process which exhibits regularities. We therefore propose the following non-stationary model.

Cox[1] considered in detail many physical reasons for suggesting a stochastic model involving an interaction of a regularly fluctuating dipole field and a random non-dipole component; these arguments will not be repeated here. The Earth's field is idealized by a sinusoidal variation of the form

$$F_e = \delta[A + B(1 + \cos(2\pi t/P_e + \varphi_e))] \qquad (1)$$

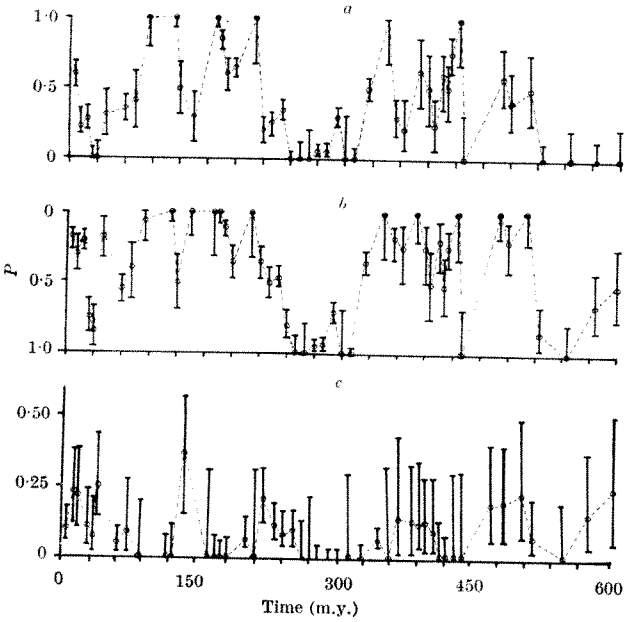


Fig. 1. Curves showing the proportion of normal (*a*), reversed (*b*), and mixed (*c*) palaeomagnetic measurements as compiled by Simpson. Approximate 95 per cent confidence limits are shown.

The minimum value, or bias of the field is A, the amplitude of the oscillation is B. The period and phase are denoted by P_e and φ_e respectively. The coefficient δ is either plus or minus one, depending on the polarity of the field; t denotes time.

The non-dipole or triggering field is a random variate from a normal distribution with mean given by

$$\mu = \alpha \cos(2\pi t/P_a + \varphi_a) \quad (2)$$

and standard deviation given by

$$\sigma = \beta|\cos(2\pi t(P_b + \varphi_b)| \quad (3)$$

The quantities α and β represent amplitudes, P_a and P_b periods and φ_a and φ_b phase angles of the oscillations. We introduce an additional parameter, C, a measure of the relative magnitude of the non-dipole field. Whenever at the moment of sampling, the following expression has negative sign, the field is reversed and the sign of δ is switched (that is, whenever the non-dipole component exceeds the dipole field in magnitude and is opposite in sign).

$$F_e[F_e + CN(\mu,\sigma)] \quad (4)$$

Where $N(\mu,\sigma)$ represents a random variate from a normal distribution with mean μ and standard deviation σ.

Several thousand realizations were generated by computer, over wide ranges of all the parameters. The random component was simulated by a series of discrete states generated from a normal distribution; from Cox's results, a 1,000 year spacing was used as a reasonable estimate of the growth and decay time of the non-dipole components. Obviously, we cannot reproduce the results of all the simulations here, but we will show the important results and summarize the effects of the various parameters.

A simulation for one full period (P_a) represents one individual of the total population of realizations of the model with a given set of parameters. For such an individual, curves of the proportion of normal polarity, P, reversal rate, RR, and the frequency of mixed measurements, M, can be plotted against time. A number of repetitions of the same experiment yield the mean time-variation of these factors. A mixed event was deemed to occur whenever the net effect of the dipole and random components was less than the bias level, A.

Fig. 1 shows the Earth's reversal history as compiled by Simpson[5], replotted on a linear time scale. Each point represents the proportion of the given polarity over approximately a 15 m.y. period. For comparison, polarity proportion curves produced by the model are also averaged over this period.

Fig. 2 shows the various mean behaviour curves for the "prime" model, that is, the model which gave the closest agreement with the known facts. The successful parameters are $A = 0{\cdot}1$, $B = 1{\cdot}0$, $\alpha = 1{\cdot}0$, $P_a = 300$ m.y., $\beta = 1{\cdot}0$, $P_b = 75$ m.y., $C = 0{\cdot}3$. Fig. 3 shows the curves for one typical individual realization of the model. Note the relatively large deviation of the individual from the mean, and the similarity in form between the model individual and the observed individual (Fig. 1).

Curves of mean reversal rate and maximum reversal rate as a function of various parameters are shown in Fig. 4. Predictably, the value of the bias, A, and the relative amplitude of the non-dipole component, C, are critically important factors. The period of the dipole oscillation, P_e, is unimportant if less than 2 m.y. Because of the extremely high ratio of P_a to P_e, the phase φ_e, is irrelevant. The amplitude of the dipole oscillation, B, also has little effect on the results. Cox[1] also gives reason for believing that the exact shape of the oscillation is unimportant.

The ratio of the amplitudes (α and β) of the random non-dipole oscillation affects the ratio of the primary and secondary peaks in the RR curves. Comparison of the computed curves with Fig. 1 puts limits on α/β as 0·5 to 2·0. We examined the effect of the periods P_a and P_b by spectral analysis. The model cannot resolve small differences in period, and to be discernibly different from the observed spectrum, the model parameters had to be outside the ranges 260 m.y. $< P_a <$ 380 m.y. and 65 m.y. $< P_b <$ 90 m.y.

The model predicts for periods of high reversal rate, such as the tertiary, a rate of about 7·5 per m.y., in good agreement with Harrison[6]. The predicted rate of 2·7 per m.y. for the last 80 m.y. agrees favourably with ocean floor spreading estimates[7]. The long-period behaviour is similar to the known pattern[4] and the polarity proportion curve of a typical individual has the same character as the observed curve (Fig. 1). The match of the mixed curve is only moderately good. There is some agreement

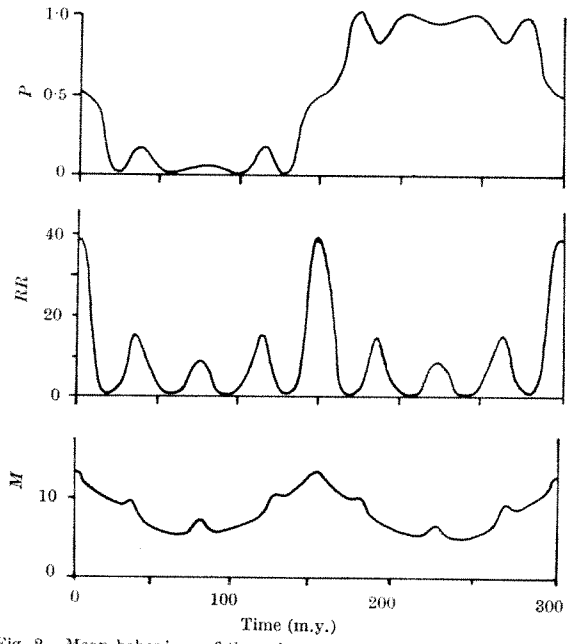


Fig. 2. Mean behaviour of the prime model. P = Polarity proportion, RR = reversal rate in reversals per m.y., M = mixed events. Scale of M curve is arbitrary.

between the reversal rate curve and the observed mixed curve, which suggests that a portion of these are caused by a number of reversals within a short time. A quasi-exponential distribution of interval length is obtained as observed by Cox[1]. A χ^2 test on the model produces a statistic of 35·62 (45 degrees of freedom), which is well below the critical region for rejection of the hypothesis (62·83).

Some physical interpretation of the parameters would certainly enhance the value of this stochastic model. The dipole oscillation portion is quite consistent with various physical models of self-excited dynamo behaviour, the susceptibility of which to triggering into a reversed state has been shown[8]. Associating a physical process with the random (but regularly fluctuating) component is difficult and must be speculative. The thermodynamics of the planet make an internal terrestrial source for such an oscillation unlikely. On physical grounds we reject Steiner's[9] hypothesis of an oscillating Earth's radius and contend that all major global processes are proceeding irreversibly on the time scale considered. The source must therefore be sought externally. The excitation medium is apparently polarized so that the mean direction of the random component alternates in sign with a long period of about 300 m.y., while becoming more variable about every 75 m.y. Our interpretation of this phenomenon is that it is correlated with the Earth's position in the galaxy, the rotational period of which is about 280 m.y.[9]. The excitation is apparently more uniform in the galactic plane, accounting for the change in variance corresponding to the vibration of the solar system perpendicular to the galactic plane with period 84 m.y.[10]. The galactic magnetic field is probably too small to be a direct cause (although only a small effect is required). Dauvillier[11] has suggested a possible solar–terrestrial interaction for magnetic reversals. Perhaps a ternary galactic–solar–terrestrial interaction should be proposed.

On the basis of the model we predict a mean length of all polarity intervals of 1·6 m.y., long intervals of constant polarity in the Silurian (normal) and Middle Cambrian (reversed), periods of frequent reversals in the Jurrassic, Carboniferous, Ordovician and Lower Cambrian, and that the palaeomagnetic history of other terrestrial

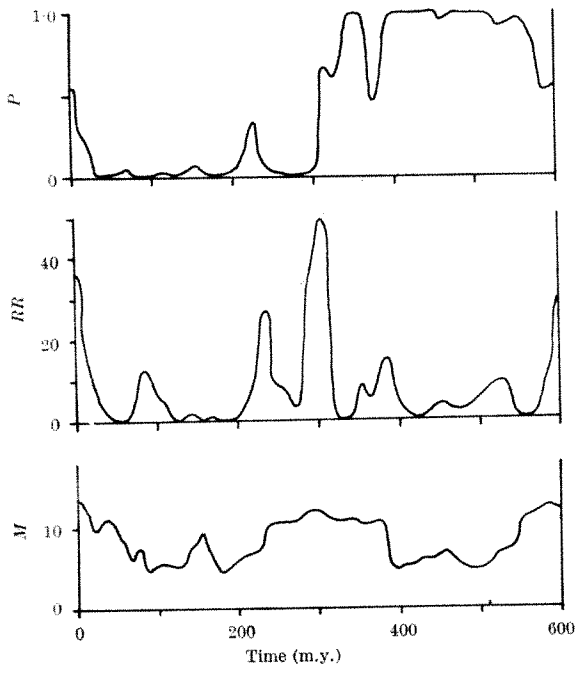


Fig. 3. A typical individual realization of the prime model. Axes denoted as in Fig. 2.

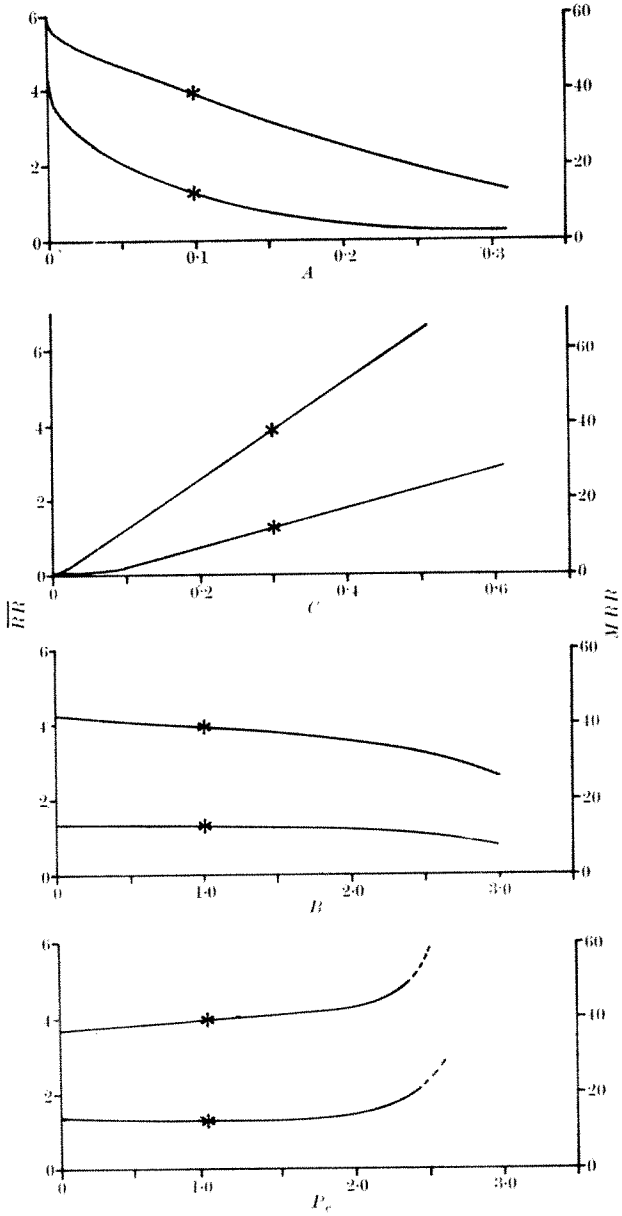


Fig. 4. The effect of varying the model parameters. $\overline{RR}$=Mean reversal rate, left-hand axis, lower curve; MRR=maximum reversal rate, right-hand axis, upper curve.

planets with Earth-like cores will show the same long-period behaviour.

Another implication is that magnetic anomaly patterns may be of limited use in delineating ocean-floor spreading which is older than Cainozoic. The search for older magnetic lineaments (in the north-west Pacific, for example) should be concentrated in ocean floor of Jurassic or Carboniferous age.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Pulsar Nulling Phenomena

DURING an investigation of the short-term behaviour of the radiation coming from pulsars during strong bursts, several objects were found to exhibit pulse nulling phenomena. This was first noted on analogue records of the pulsar radiation intensity where the pen deflexions are a rough measure of the pulse energy. PSR's 0834+06, 1133+16, 1237+25 and 1929+10 were found suddenly to miss from one to ten pulses at various times, termed "nulls", during otherwise very strong and steady bursts. A null means that the pulse energy decreased by a factor of ten or more over one or two pulse periods and then increased just as sharply after the null. The nulls can be divided into two types according to their duration and their spacing. Type I nulls are the most prominent with a width between three and ten pulses and recur with spacings of about fifty pulses. Type II nulls have a width of only one or two pulses and recur with spacings of three to ten pulses. Pulse sequences from 1133+16 and 1237+25 and possibly 0834+06 contain the Type I nulls while sequences from all four objects contain Type II nulls. A quick look at the spacings of these nulls suggests that they may recur periodically and hence contribute to responses at characteristic periods in pulse energy fluctuation power spectra. This possibility has been reported indirectly by Sutton *et al.*[1] and Hesse[2] for the Type II nulls in 0834+06 and directly by Lang[3] for Type I nulls in 1133+16 and 1237+25.

The phenomena have been observed using linearly polarized feeds at 318 MHz and 606 MHz and a circularly polarized feed at 430 MHz. Further inspection of dual frequency recordings (318 MHz/111·5 MHz) from 0834+06, 1133+16 and 1237+25 shows that both types of nulls are correlated over hundreds of MHz.

The short duration, the correlation over a wide frequency range and the possible periodicity indicate that the radiation-producing structure is undergoing some rapid changes. Further interpretation will be presented following several detailed analyses.

First it should be stated that the Type II nulls in all but 1929+10 are most likely related to the class two effect which is observed in other pulsars. This has been suggested by Sutton *et al.*[1] for 0834+06 and will be shown to be true for 1237+25 in a separate paper. The type I nulls and the 1929+10 nulls could result from a particular form of the class two effect, but they are sufficiently different to warrant an independent discussion.

One measure of the significance of these nulls in a sequence of pulses is the distribution of pulse energies. Fig. 1 contains pulse energy distributions for pulses within a strong burst for three of the pulsars. A pulse energy is defined as the sum of the samples taken in a window on a pulse minus the sum of the samples taken in an equal length window adjacent to the pulse. The root-mean-square (r.m.s.) spread about the mean of the sums taken off the pulse is shown on each of the plots. The average signal-to-noise ratio, defined as the mean pulse energy divided by the off r.m.s. spread, is about twenty for each of the distributions. Because of this large ratio the effects of background and receiver noise are negligible. The peaks at zero pulse energy in the 1133+16 and 1237+25 distributions (Fig. 1*a* and *b*) indicate that the null pulses constitute a distinguishable group of pulses in these pulsars. For 1929+10 there is no peak at zero which means the nulls in a sequence of pulses are the sites of weak pulses. The significance of the 1929+10 nulls thus depends on other properties of the sequence.

Investigation of the intensity-time sequence sampled digitally from these pulsars shows that not only does the pulse energy drop suddenly at a null, but also that there is typically no pulsed radiation in the null pulses above a threshold intensity set near the noise level.

To determine the significance of the spacing of these nulls the consecutive pulse numbers of the null pulses, defined as those pulses during which the intensity was everywhere below a fixed threshold, were noted. A sequence of numbers was then formed with a positive integer at elements whose index was equal to a null pulse number and zeroes elsewhere. The sequence was transformed using a Cooley–Tukey algorithm to find a fluctuation power spectrum. The spectrum for 1929+10 resulting from the incoherent addition of twenty 256 point transforms is shown in Fig. 2. The response at 0·094 cycles/pulse period (*P*1) suggests that the nulls recur in this object with a characteristic spacing of 10·6 *P*1. This feature occurs near the frequencies of the feature reported for pulse energy spectra by Huguenin (unpublished) and Lang[4]. Thus either the zeroes in the sequence of pulse energies give rise to the feature or there are characteristic variations in the pulse energies synchronized with the nulls. For 1237+25 there is a feature in the null spacing spectrum resulting from a characteristic spacing of the Type II nulls of 2·8 *P*1 which agrees with the feature reported in the literature for pulse energy spectra. There may also be a longer characteristic period related to the Type I nulls, but the data were insufficient to make a definite conclusion.

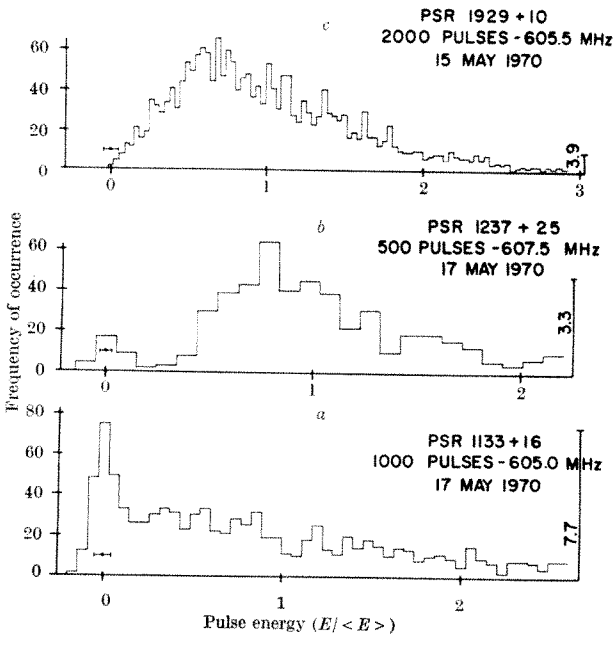


Fig. 1. Pulse energy distributions for *a*, 1133+16; *b*, 1237+25; and *c*, 1929+10. Zero indicates the average of the off sums and unity indicates the average of the on sums. The r.m.s. spread of the off sums is shown as error bars centred on zero. The number of pulse energies beyond the horizontal scale is shown as a vertical line on the right hand side of each plot; the number by this line is the maximum pulse energy in the sample.

For 1133+16 there is a feature due to the Type I nulls at 0·04 cycles/$P1$ in the null spacings spectrum which agrees with the feature reported in the literature for pulse energy spectra. There may also be a weak feature at 0·23 cycles/$P1$ due to the more frequent Type II nulls.

In order to determine the relation of the periodic nulls to the periodicity of pulse energies the pulses near 49 nulls in a sequence of 4,700 pulses observed at 606 MHz from 1929+10 were averaged together. As mentioned already, the nulls in 1929+10 tend to last only for one or two pulses. The sequence of nine pulses preceding and following the central pulse of the nulls were averaged together to obtain an average sequence (Fig. 3*a*). Although the null results from selecting the central pulse numbers for each member of the average sequence, it was found that 97 per cent of the pulse energies of the pulses averaged for a sequence number outside the null ($|N|>1$) were larger than the largest of those that constituted the central null pulse ($N=0$). The root-mean-square scatter from the mean of the pulse energies of the pulses averaged for a particular N was $\sim 0{\cdot}6$ times its mean for each N.

The average of all the pulses going into the average sequence was subtracted to see whether there were significant differences between the pulses preceding and following the nulls (see Fig. 3*b*). The positive residuals for $N>1$ indicate that the 1929+10 nulls are followed by an increase in pulse energy. Thus the pulse energy spectra would have a feature due not only to the periodic zeroes of the null pulses, but also to an enhancement that follows them.

The variation with N of the average pulse energy for a given N in the sequence is shown in Fig. 3*c*. The enhancement following the null is only marginally significant on the basis of this result. There are significant decreases in the average pulse energies at ± 12 and $+22/-24$ pulses. These were found to be only partially due to the periodicity which was detected in the power spectra of the null positions. The decreases were due also to the occurrence of weak pulses which do not satisfy the null criterion. This is further evidence of the source of the characteristic period in 1929+10.

As suggested earlier, this effect must be related to the structure producing the radiation. Most authors find that the radio emission must arise from the correlated motion of a group of charges, because coherent radiation is required to explain the inferred high brightness temperatures. Gold[5], for example, suggests that the group of charges are held in the magnetosphere of a rotating neutron star.

The Type I nulls and possibly the Type II nulls in 1929+10 indicate that some cause is periodically destroying most of the correlation for a small number of pulse periods. Within Gold's model this could arise from a perturbation of the magnetospheric trapping field which would dump the charges from the radiating region or from the onset of a plasma instability which would disperse the charges from the radiating region. As mentioned earlier, the Type II nulls are most likely related to the class two effect. Narrow marching subpulses with a spacing greater than the pulse profile could periodically cause a null pulse. This aspect of the phenomenon may be due to slow drifts of the group of radiating charges in the magnetosphere.

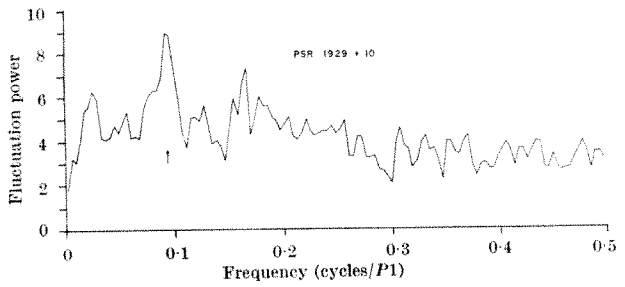


Fig. 2. Fluctuation power spectrum of the spacings of null pulses in twenty sequences of 256 pulses from 1929+10 smoothed over two frequency components.

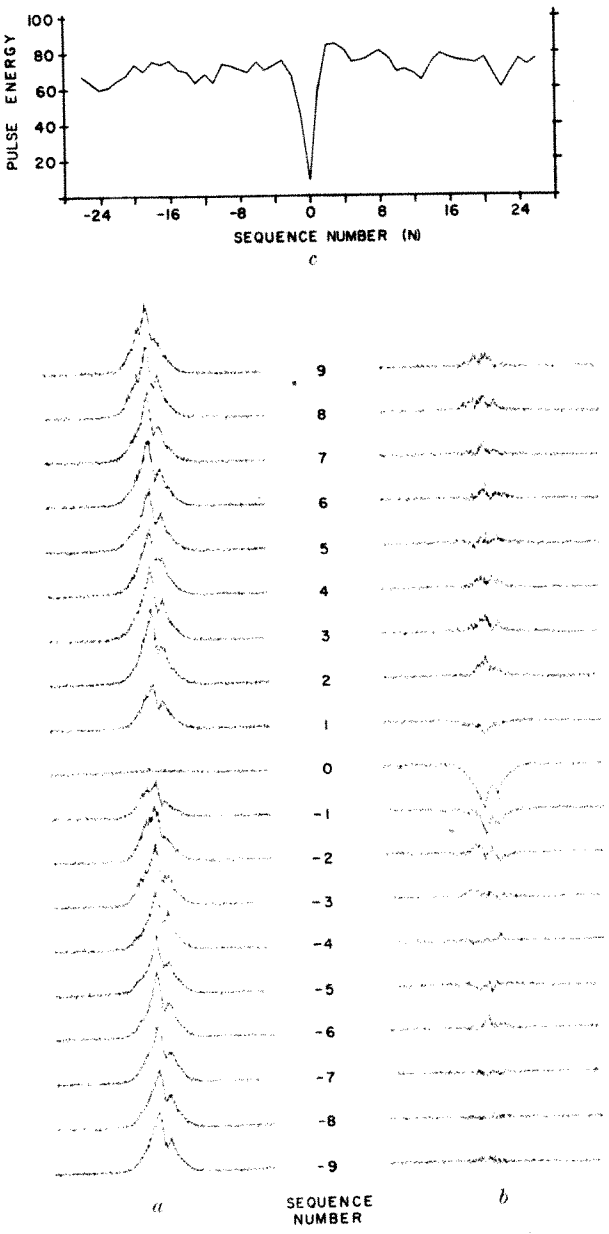


Fig. 3. *a*, Averaged sequence of pulses centred on deep nulls for 1929+10; *b*, averaged sequence with the average pulse profile subtracted from each member of the sequence; *c*, mean pulse energy versus pulse sequence number (N) relative to null centre.

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Fine Structure of Nebular Emission Lines

THE fact that nebular lines may have significant fine structure seems to have been neglected by nebular spectroscopists who have been considering the turbulent and thermal broadening of these lines. For example, Aller[1] and Courtès *et al.*[2], who have measured or interpreted the widths of the lines, do not mention this effect.

In particular Hα is composed of four components. Kent *et al.*[3], Lewis and Spedding[4] and Spedding *et al.*[5] showed experimentally, for a laboratory source, that only the two principal components, of nearly equal brightness, were significant. They are separated by 0·14 Å. Sommerfeld and Unsöld[6,7] and Schrödinger[8] calculated theoretically the relative brightnesses of this fine structure. These depend strongly on the conditions of excitation.

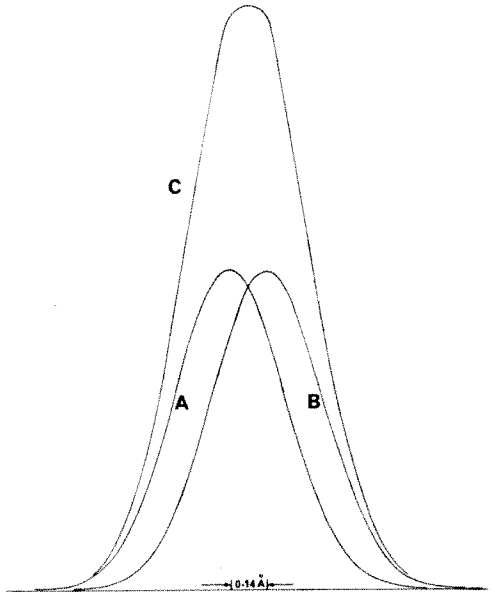


Fig. 1. Thermally broadened component of the Hα line composed of two Gaussians A and B.

It is interesting to estimate the error introduced into the measurements of the electron temperatures of HII regions from the width of the Hα line if the fine structure is neglected. Consider the case shown in Fig. 1 where the thermally broadened component of the Hα line is composed of two Gaussians A and B of equal brightness, where each has a thermally broadened width of 0·506 Å corresponding to a nebular temperature of 10,000 K and where these two components are separated by 0·14 Å. The thermally broadened component of the Hα line would then appear as the summation, C, of these two curves. This has a half-width of 0·545 Å. If this were inadvertently considered to be a single Gaussian representing the thermal component of the Hα line, then the electron temperature would be given wrongly as 11,880 K. This indicates an error of around +2,000 K. It is then possible that every measurement of temperature of gaseous nebulae that has relied on the width of the Hα line and which has neglected this splitting is then 2,000 K overestimated. Those methods that depend on the widths of [NII] and Hα (ref. 2) are even further uncertain if the fine structure of this forbidden line is also neglected.

It would seem necessary to consider the fine structure of all the nebular lines in the conditions existing in nebulae when making exact measurements of their widths and shapes.

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Apex of Solar Motion of Neutral Hydrogen Concentrations at Different Velocities

De Vaucouleurs and Peters[1], Kerr and Sullivan[2] and I[3] derived the solar motion with respect to the high velocity HI clouds (HVCs) with a view to examining the suggestion by Verschuur[4] that these cloud complexes and the Local Group of galaxies form part of the same extragalactic system of objects. They concluded that the solar motion solution does not seem to justify Verschuur's hypothesis. Kerr and Sullivan[2] also derived the solar motion with respect to a sample of 125 intermediate velocity clouds (IVCs) and concluded that they seem to be kinematically distinct from the HVCs.

Dieter[5] used the data from the Berkeley neutral hydrogen survey (2 km s^{-1} velocity resolution) to show that in almost every case high velocity condensations occur where there is evidence of excess gas at more moderate velocities. Using these data she also found evidence for physical interaction between gas masses at different velocities at the same location in the sky. This relation between gas at different velocities was based on her study of the velocity dispersion of the IVCs associated with the HVCs and also on the column densities of neutral hydrogen atoms.

In order to see if her data, which showed a physical relation between gas masses at different velocities, also showed a kinematic relationship, I divided her list (Table 1 of ref. 5) of 139 hydrogen condensations into two groups—(1) 68 with velocities greater than 70 km s^{-1} and (2) 71 with velocities less than 70 km s^{-1}—and derived the solar motion with respect to the local standard of rest separately for the two groups. The solutions are shown in Table 1. These results may be compared with those derived by de Vaucouleurs and Peters[1], Kerr and Sullivan[2] and me[3] (Table 2).

Table 1. SOLAR MOTION SOLUTIONS RELATIVE TO LOCAL STANDARD OF REST

Group	N	$l^{II}_{\odot}$	$b^{II}_{\odot}$	$V_{\odot}$ km s^{-1}
> 70 km s^{-1}	68	129°±4°	−12°±3°	272±16
< 70 km s^{-1}	71	148°±13°	−12°±12°	91±9

Table 2. RESULTS OF EARLIER WORK

	N	$l^{II}_{\odot}$	$b^{II}_{\odot}$	$V_{\odot}$ km s^{-1}
de Vaucouleurs and Peters[1] (HVCs)	17	139°±16°	−13°±13°	184±66
	16	148°±7°	−12°±5°	234±29
V. R. V. (HVCs)	17	139°±20°	−13°±16°	184±66
Kerr and Sullivan[2] (HVCs)	62	136°	−4°	222±19
Kerr and Sullivan[2] (IVCs)	125	138°	+42°	58±3

Kerr and Sullivan's solution for IVCs is different from my solution for gas at less than 70 km s^{-1}. This may be due to the inclusion in their sample of 125 of a large proportion of IVCs that are not associated with HVCs.

It is seen from my solution (Table 1) that the apex of solar motion of gas with velocities greater than 70 km s^{-1} is not very different from that for gas with velocities less than 70 km s^{-1}. The apices are in the galactic plane which seems to suggest that the gas concentrations are related to the galaxy. The close agreement between the direction of the solar motion of the two groups suggests that they

may have a common dynamic origin which, perhaps, is a super-supernova explosion as proposed by Rickard[6].

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Penetration of Solar Particles to Ionospheric Heights at Low Latitudes

Ganguly and Rao recently presented evidence for the possibility of the penetration of high energy solar particles to ionospheric heights at the equator[1]. Their letter, based on ionospheric absorption data obtained at Calcutta (geomagnetic latitude 12° 15′ N), together with optical observations of solar flares and tabulated proton flux data, is very interesting in the light of extensive work recently on the penetration of solar particles to low magnetospheric L-values ($L \sim 4$–$7\ R_E$) at the equator[2–7]. Because the observations of Ganguly and Rao would have a profound and far-reaching impact on current ideas of the mechanisms for the access of such solar particles to the geomagnetic equator[2–4,6], the ionospheric and solar evidence presented by the Calcutta group has been examined carefully. We conclude that there is little basis for their contention that solar particles can penetrate directly to low altitudes and thus influence ionospheric absorption at the equator.

Ganguly and Rao present perhaps their most conclusive evidence in their Fig. 2. They plot, as a function of time, the night-time absorption (for 4·7 MHz) and the "proton flux ($E > 60$ MeV) measured by Explorer 33" for the period 1400 UT August 1 to 0300 UT August 2, 1967. Proton data ($E \sim 60$ MeV) measured by the Bell Laboratories instrument on ATS-1 at the equator (150° W geographic longitude) at synchronous altitude were examined for evidence of a solar particle enhancement occurring around the time reported in ref. 1. No evidence for such an enhancement was found.

The $E > 60$ MeV proton data from the solar proton monitoring experiment on Explorer 34, published routinely in the ESSA bulletin *Solar Geophysical Data*[8], were examined for a proton enhancement as presented by the Calcutta group. During the time period discussed by Ganguly and Rao, Explorer 34 was at perigee and thus measuring the electron radiation belts by electron pile-up in the proton channels[9]. It was found that the $E > 60$ MeV data presented by Ganguly and Rao were equal to the fluxes reported by the Explorer 34 solar proton monitoring experiment during its perigee-pass through the radiation belts. Hence we conclude with respect to the "correlation" presented in Fig. 2 of the letter by Ganguly and Rao that: (1) ATS-1 data give no evidence of a solar proton enhancement at high altitudes at the equator; (2) Ganguly and Rao were evidently using Explorer 34 and not Explorer 33 data in their comparison; (3) the data they plotted were the perigee-pass data when the satellite detector was measuring the radiation belts.

We have examined the optical flare data and the solar proton data from ATS-1 and Explorer 34 during the three periods of anomalous absorption presented in Fig. 1 of Ganguly and Rao. The optical flare data which we used were those in the master list of flares and subflares compiled by World Data Center A and published in *Solar Geophysical Data*[10]. Although not stated explicitly in their caption to Fig. 1, Rao informs us that the data were plotted in Indian Standard Time (IST = UT + 5·5 h).

The flare that occurred between 0337 and 0352 UT, February 11, 1968, was classified as −N (ref. 10). The flare that occurred between 0416 and 0439 UT, February 14, 1968, was classified as 1B (ref. 10). No evidence of high energy solar protons was observed at synchronous altitude on ATS-1 at the time of either of the two absorption events which Ganguly and Rao associated with these flares. Explorer 34, located in the magnetotail, also measured no high energy solar protons. A small flux of low energy (≤ 5 MeV) solar protons was observed on both ATS-1 and Explorer 34 throughout February 14. The flare that Ganguly and Rao reported as occurring between 0820–0840 UT, February 7, 1968, was classified as 1F in the list of "Small or Unconfirmed" flares[10]. No solar protons in any energy range were observed on either satellite during this day.

We conclude with respect to the three February 1968 flare events in Fig. 1 of Ganguly and Rao that the solar proton data do not support the authors' contentions that high energy protons contribute to low altitude equatorial absorption at Calcutta. We further note that the three February 1968 flares used by Ganguly and Rao were smaller than the flares that generally produce high energy proton fluxes at the Earth.

In summary, we find the evidence for correlation of proton events with low altitude equatorial absorption to be invalid, and find the supporting flare data to be unconvincing and unsupported by spacecraft measurements.

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Transform Faulting in the Thai Peninsula

RECENT mapping by a joint UK/Thai team of geologists[1], operating under the Colombo Plan, has shown that the angular elbow-like feature in the Phuket area of peninsular Thailand projecting into the Andaman Sea is the physiographic expression of two major transcurrent faults with a total sinistral displacement of at least 200 km.

A zone of rich tin-bearing granites stretching up the Malay Peninsula into Thailand as far as Surat Thani has been offset by both faults. Across the largest of these, the Khlong Marui Fault, the belt has been displaced a distance of at least 150 km to the south-west to Phuket Island, whence it continues up the western part of the Thai Peninsula to Ranong (Figs. 1 and 2*c*). Across the Ranong Fault the tin-bearing zone has again been displaced at least 20 km to the south-west and the offset northern part of the zone passes through the islands of

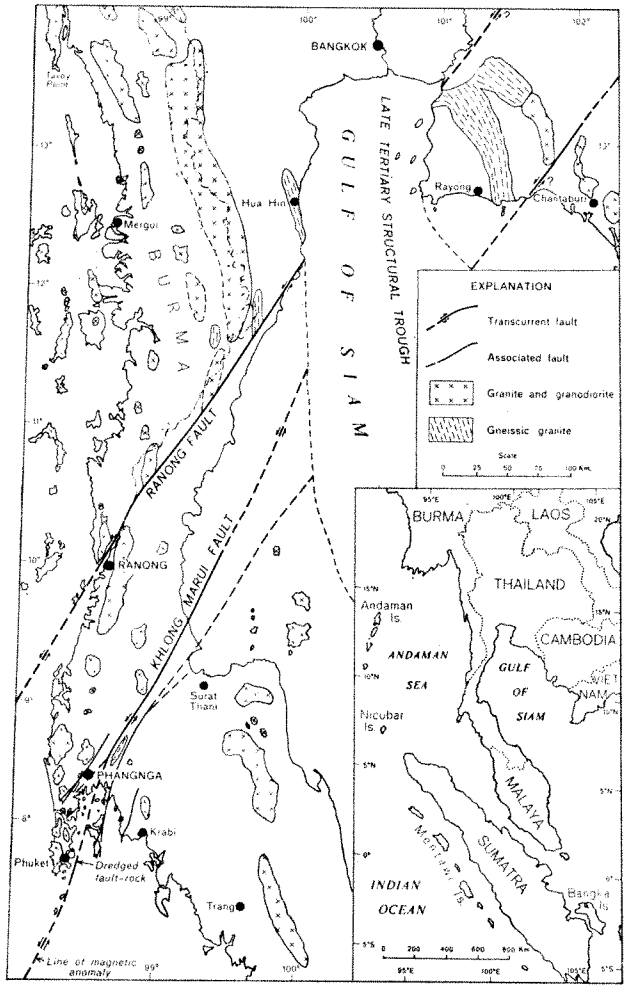


Fig. 1. Location of transcurrent faults and granitic rocks in peninsular Thailand and Burma.

the Burmese archipelago and the granitic hills adjacent to the coastline.

The Khlong Marui and Ranong Faults probably converge in the Gulf of Siam under a thick cover of late Tertiary sediments within a structural trough[2] to form a single fault which displaces the Rayong gneissic granites to the Hua Hin area. These sediments are currently being investigated by several oil companies; the location and age of the faults in the gulf are therefore of major significance. Projected to the south-west of the peninsula, the principal Khlong Marui Fault coincides with the submarine slope at the edge of the Malacca Straits[3] and also with a NE-trending magnetic anomaly[4].

In the Phuket–Phangnga area there is a close relationship between the NE-trending transcurrent fault system and a parallel swarm of tourmaline-pegmatites and lepidolite-pegmatites, both worked extensively as tin ores. The latter are possibly the largest unzoned lepidolite-pegmatites yet recorded anywhere in the world, and are apparently unique in south-east Asia[5]. Related lineated granites occupy a graben in a fault-valley traced across the peninsula from Phangnga to north of Surat Thani.

The age of the faulting is not yet established with certainty. It, however, certainly post-dates emplacement of the late Carboniferous to Triassic granites of the Chantaburi[6] area which are affected by these transcurrent movements. At Ranong, adamellites dated at 111 ± 6 m.y. and 113 ± 8 m.y. using the Rb : Sr method[6] are displaced by the Ranong Fault. It is considered that the NE trending tourmaline-granites are a late phase of these granites and were intruded into fractures in the zone of transcurrent faulting. A significant period of mica rejuvenation is recorded at 65 m.y. in the Thai–Malay Peninsula[6,7] and we believe this may be related to the final consolidation of the transcurrent fault system.

The location of the faults in a zone of Mesozoic plutonic activity and on the concave side of the arcuate Sunda mobile belt of Cainozoic and late Mesozoic age suggests that the movements were related to the development of the Sunda arc. Large lateral displacements terminating at one end in a mobile belt can most simply be interpreted in terms of the ocean floor spreading hypothesis[8] and the concept of plate tectonics[8,9] as movements along transform faults[10,11].

One of the more genetically significant features of the faults is that the south-western end of the Khlong Maru Fault, and probably that of the Ranong Fault, lies at or near the junction of the Sumatra and Andaman–Nicobar segments of the Sunda arc. The Sumatra arc lies on the south-western margin of the Sunda Shelf, the western margin of which continues northwards as a major bathymetric and topographic feature through the Andaman Sea and Shan Scarp in Burma. The shallow depth of the Sunda Shelf and the presence of broad positive gravity anomalies[12] suggest that its crust has a thickness approaching that of continental crust. The crust on the concave side of the Andaman–Nicobar island arc is of oceanic type[13,14]. Thus the faults terminate at or near the point where a mountain arc lying on crust of continental type meets an island arc located on oceanic crust.

According to the concept of plate tectonics, the seismic and volcanic activity in the Sunda arc is related to the descent of north-eastward spreading lithosphere along an inclined Benioff zone[15,16]. The trend of the actively volcanic east of the Mentawi Island chain therefore suggests that lithosphere was descending beneath the Mentawi Islands at that time. Thus the eposide of lithospheric descent which in the Andaman–Nicobar–Mentawi island belt ended in the late Cretaceous had started beneath the Mentawi Islands segment of the belt by early Cretaceous times.

Most of the Andaman–Nicobar arc is below sea level, and there is no evidence for the time at which lithosphere started to descend beneath the zone now occupied by these islands. We suggest that the lateral displacements along the Khlong Marui and Ranong Faults can most simply be explained if descent of lithosphere beneath this zone began before that beneath the Mentawi Islands, probably in late Jurassic times. We also suggest that, before this episode of differential movement between the plates, the area of continental-type crust to the south-east of the present faults lay 200 km south-west of its present position (Fig. 2*a*). During the late Jurassic to early Cretaceous episode of lithospheric descent this crustal segment remained coupled to the spreading ocean floor, moving north-eastwards as spreading continued (Fig. 2*b*). The resulting differential horizontal movement between this southern plate, which included Sumatra, Malaysia and and the Sunda Shelf, and the northern plate, which included the Thai Peninsula and Andaman Basin, led to transform faulting, most of the displacement occurring along the Khlong Marui and Ranong Faults.

By the early Cretaceous the margin of the Sunda Shelf plate had moved to its present position, approximately in line with the Andaman–Nicobar islands, and became uncoupled from the ocean floor. Lithosphere then began to descend beneath the zone now occupied by the Mentawi Islands (Fig. 2*c*), resulting in the early Cretaceous volcanic activity in the Gumai and Garbai ranges. Adamellites were intruded at Ranong at about the same time. Loss of lithosphere beneath this zone and beneath the belt now occupied by the Andaman–Nicobar islands ceased in the late Cretaceous, resulting in infilling of the submarine trench, which was uplifted during the late Tertiary to form the present islands. Since the beginning of the Tertiary,

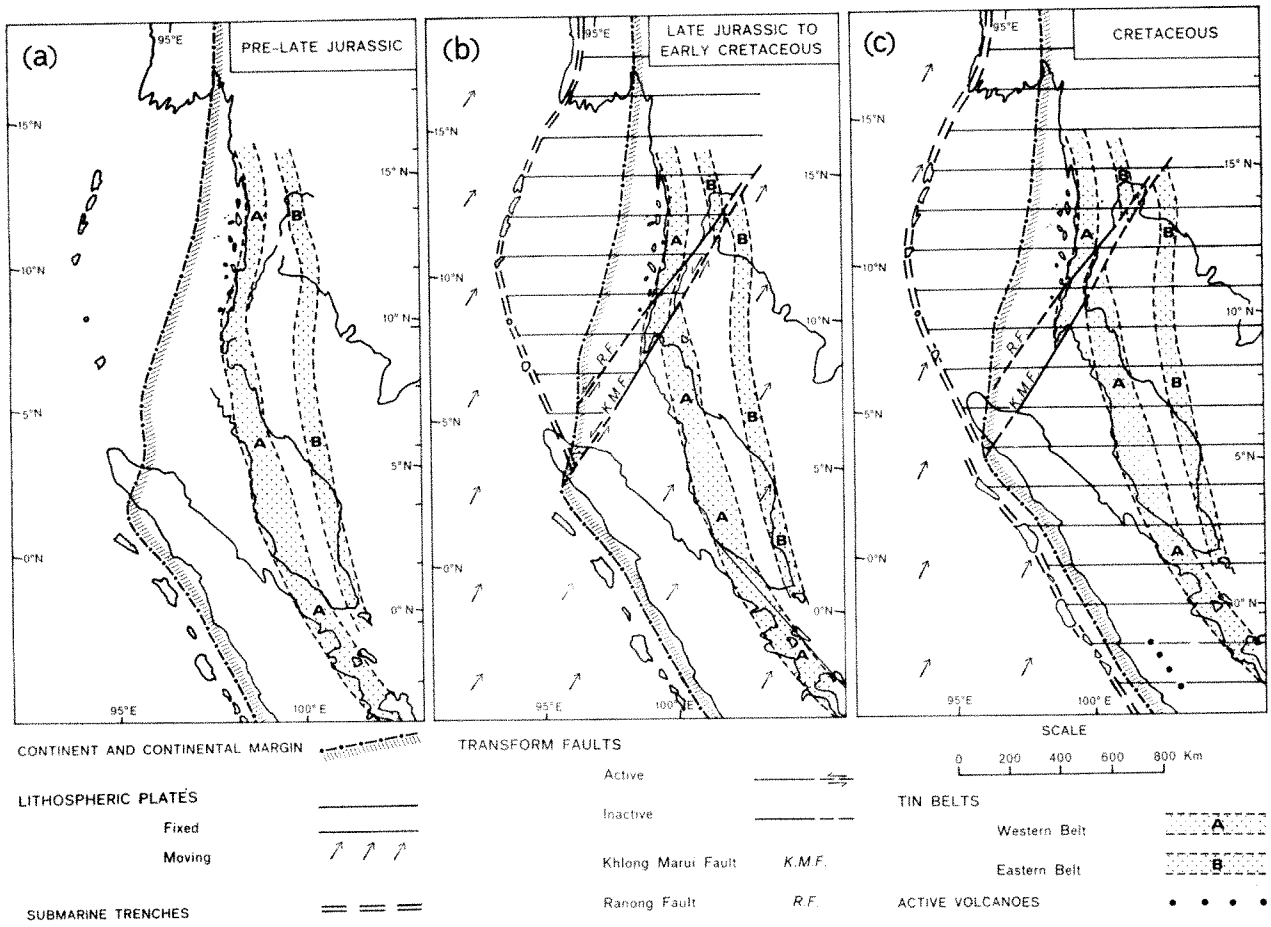


Fig. 2. Proposed relationship of ocean floor spreading and descent of lithosphere to fault movements in the Thai-Malay Peninsula in the late Mesozoic.

spreading lithosphere has been lost, either continuously or intermittently, along a Benioff zone which approaches the surface beneath the Sumatra trench and to the west of the Andaman–Nicobar Islands, and transform fault movements have not been renewed.

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Bottom Melting of Ice Shelves and the Mass Balance of Antarctica

The world sea level is estimated[1,2] to be rising by approximately 1 mm yr^{-1}. Attempted explanations[3,4] have considered heating of the oceans, release of juvenile water, a reduction in atmospheric humidity and melting of the polar ice sheets. Existing evidence[5] does not indicate any pronounced warming of the deep ocean waters, and most of the rise in sea level probably results from wastage of glaciers.

Giovinetto[4], however, has concluded that much of the Antarctic ice sheet has a positive net balance. He assumes appreciable net accretion of ice on the bottom surface of the principal floating ice shelves, which make up about 7 per cent of the area of the ice sheet. Here we present experimental data from the Brunt Ice Shelf which cannot be reconciled with this assumption (a detailed analysis will be published elsewhere). Bottom melting rates of 0·8 m yr^{-1} are measured at 70 km from the ice front, and we consider that significant bottom freezing can take place only in very special conditions.

By estimating the rate of change of thickness of the ice shelf and using continuity and hydrostatic equilibrium equations, Swithinbank[6] and Crary[7,8] deduced bottom melting rates of 0·87 and 0·63 m yr^{-1} near the ice fronts of the Maudheim and Ross ice shelves respectively. Another method, analysis of the temperature/depth curve, has been applied to the "Little America" bore hole through the Ross Ice Shelf (refs. 7, 9, 10 and unpublished results of G. de Q. Robin in a paper at the Antarctic Symposium, Buenos Aires, 1959); but the necessary data are difficult to collect and are relevant only to the site of

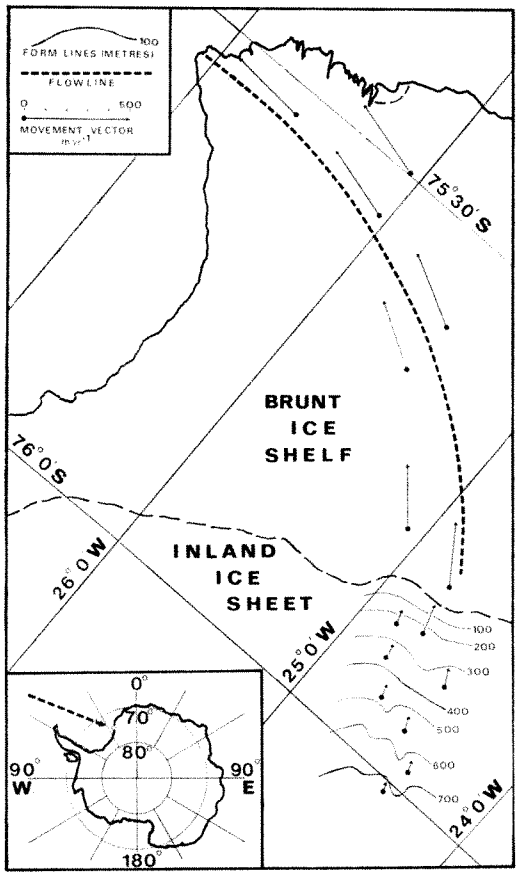


Fig. 1. The western part of the Brunt Ice Shelf, showing the flow line along which measurements were made of snow accumulation, strain rates, ice shelf velocity, thickness and surface elevation. Inset shows position of Brunt Ice Shelf.

the bore hole. The kinematic method can be applied to determine bottom melting rates at frequent intervals across an ice shelf.

Following Shumskiy and Zotikov[10] we adopt a system of fixed rectangular coordinates with x-axis along a flow line at sea level and z-axis downwards. We assume the ice shelf to be in steady state (elevation, thickness and mean density are functions of x only), and that the seawater density, ρ_w is a constant.

The continuity and hydrostatic equilibrium equations then reduce to

$$\dot{A}_b + \dot{A}_s + Z_b \dot{E}z\, \rho_w - u\rho_w \tan\beta = 0$$

where subscripts s and b refer to the upper and lower surfaces; $\dot{A}$ is the accumulation rate (bottom melting rate $= -\dot{A}_b$); $\dot{E}z$ the vertical strain rate ($-$ve for compression), equal to $-(\dot{E}_1 + \dot{E}_2)$ where E and $\dot{E}_2$ are the principal strain rates at the surface; u the ice shelf velocity along the flow line; and β the slope of the lower surface.

The Brunt Ice Shelf is one of several small ice shelves fringing the eastern coast of the Weddell Sea (Fig. 1). During 1966–70 the net accumulation of snow on the surface, the rate of movement of the ice shelf and horizontal strain rates were measured at points on a flow line which extended for 72 km from the junction of the ice shelf with the inland ice sheet to the ice front[11]. Surface elevations were measured to $\pm 0{\cdot}1$ m by optical levelling. Ice thickness was measured by radio echo sounding from an aircraft in January 1970 (information made available by the joint SPRI/US National Science Foundation programme of airborne echo sounding, 1969/70). A thickness profile to ± 10 m was obtained, and bottom elevations were calculated by subtracting the measured thickness from known surface elevations.

To simplify computation of a continuous profile of bottom melting rates along the flow line, polynomials $y = P(x)$ were fitted to the measured parameters (y) (see Fig. 2). By fitting polynomials of low order to the upper and lower surface elevation profiles, the upper surface undulations and the lower surface "noise" could be smoothed out to leave the overall slope characteristics of the profiles.

The bottom melting-rate profile is shown in Fig. 2 with the first order line of regression superimposed. Large errors are likely in the values of bottom slope taken over small distances; although the mean bottom melting rate is within $\pm 0{\cdot}15$ m yr^{-1}, individual points may be in error by 0·5 m yr^{-1}. This source of error, however, cannot account for the larger deviations from the regression line, which we believe to result from non-steady-state conditions. Near the ice front increased melting takes place, though not to the extent implied by the profile. Because of the intermittent calving of icebergs, steady-state conditions do not apply, and ice shelf thinning can be accurately estimated only by repeated measurements. Surface elevations were regularly measured at points near the Brunt Ice Front; corresponding bottom melting rates show a rapid approach to steady state conditions over the first 10 km from the ice front (Fig. 2).

Other published estimates of bottom melting rates are given in Table 1. All except Crary's results are based on measurements of surface slope (α) and an assumed value of the variable ratio β/α, the uncertainty of which has limited the accuracy of the calculations.

Behrendt[14] concluded that the extreme melting rates calculated for the Filchner Ice Shelf were incorrect because of non-steady-state conditions near the ice front; but he

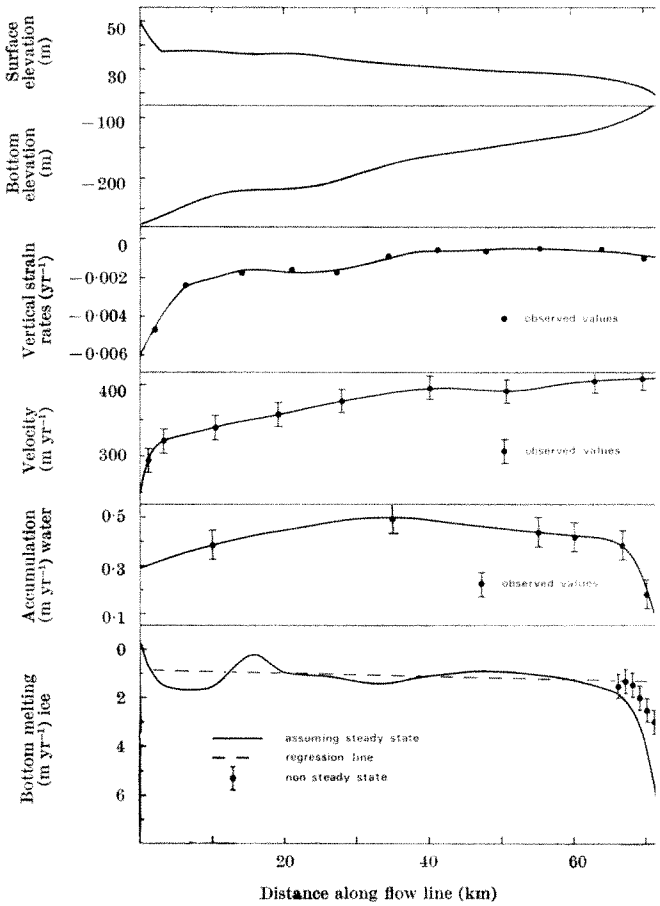


Fig. 2. Plots of polynomials fitted to the various parameters measured on the Brunt Ice Shelf, and a profile showing melting rates beneath the ice shelf assuming steady state flow.

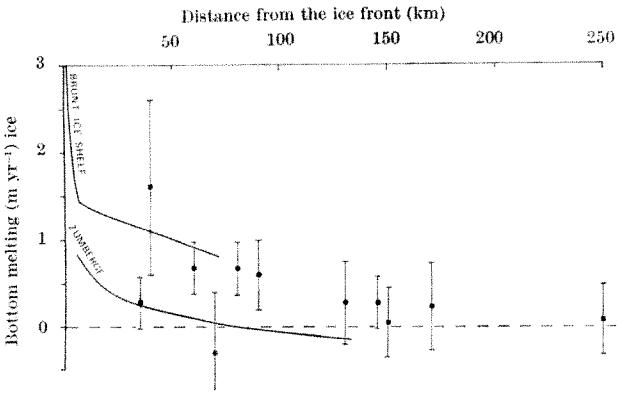


Fig. 3. Melting rates computed by the authors for the Amery (■) and Ross (●) ice shelves. A profile of bottom melting beneath Brunt Ice Shelf is included together with that proposed by Zumberge[17] for the Ross Ice Shelf.

found a high melting rate even 50–100 km back from the ice front.

Budd[12] used measurements of surface slope calculated from elevations determined by pressure altimetry. Measurements by optical levelling (W. Budd, personal communication), however, make it possible to compute more accurate bottom melting rates for the Amery Ice Shelf. Similarly, on the Ross Ice Shelf, velocity measurements[15], radio echo sounding of the ice thickness (B. M. E. Smith, personal communication), measurements of snow accumulation[16] and strain rates (E. Dorrer, personal communication) have been used to compute the bottom melting rates shown in Fig. 3, together with those for the Amery and Brunt ice shelves.

Table. 1. PUBLISHED ESTIMATES OF BOTTOM MELTING RATES. THE VALUE FOR THE ROSS ICE SHELF NEAR ROSS ISLAND WAS COMPUTED BY THE AUTHORS FROM THE DATA OF PAIGE[13]. THE NEGATIVE VALUE REPRESENTS BOTTOM FREEZING.

Source	Position	Bottom melting rate (m yr⁻¹) of ice
Swithinbank[6]	Maudheim Ice Front	0·9
Crary[8]	Ross Ice Front (Little America V)	0·6
Budd[12]	Amery Ice Shelf: 150 km from ice front	−0·1
	250 km from ice front	0·2
Paige[13]	Ross Ice Front (near Ross Island)	1·3
Behrendt[14]	Filchner Ice Front	9·6

To fit earlier data from the Ross Ice Shelf, Zumberge[17] proposed a bottom melting profile (Fig. 3) that required bottom freezing at distances from the ice front greater than 80 km, with a minimum freezing rate of 0·1 m yr^{-1} at 130 km. Robin[18] questioned this figure on the grounds of steady-state heat flow. He assumed that all heat absorbed by the ice shelf was used to warm downward moving ice, and concluded that the maximum freezing rate beneath the Ross Ice Shelf would be about 0·02 m yr^{-1}. This would correspond to a situation in which the ice shelf had trapped a body of seawater[19], isolating it from any supply of heat from ocean currents.

We conclude that there can be very little bottom freezing on any ice shelf; it probably occurs only beneath thin ice shelves, such as the ablation area of the Ross Ice Shelf in McMurdo Sound[20], or where the ice shelf has trapped beneath it a body of seawater.

Until more data are available it would be unwise to offer a quantitative assessment of the total loss of ice from Antarctica by bottom melting, but we are led to question the validity of recent estimates[4] of a positive balance for the major drainage basins.

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Optical Transmission and Reflexion Gratings by Laser Evaporation

We wish to report a new technique for the manufacture of optical transmission and reflexion gratings. The gratings are formed by the action of standing light waves in a ruby laser cavity on thin metallic films deposited on glass substrates within the cavity (Fig. 1).

We used two ruby laser systems, one a Bradley laser type 302 which had a very inhomogeneous beam; the other was a more refined system, with a Brewster angled ruby rod. At first two mirrors of 100 per cent reflectivity were used in the cavity, but we found that more controlled evaporation was possible with one of the mirrors only partially reflecting. Rough stack mirrors[1] were also used with success. In both laser systems gratings were produced over an area of about 10 mm^2, with rulings up to 2,000 lines mm^{-1}.

Most of our experiments have been carried out with gold films 20·0 to 10·0 nm thick, deposited on smooth float glass. Gratings were also produced using silver films. The films cause a loss in the cavity which vanishes when oscillations start the Q of the cavity suddenly increases and the laser output is a high power giant pulse. This is

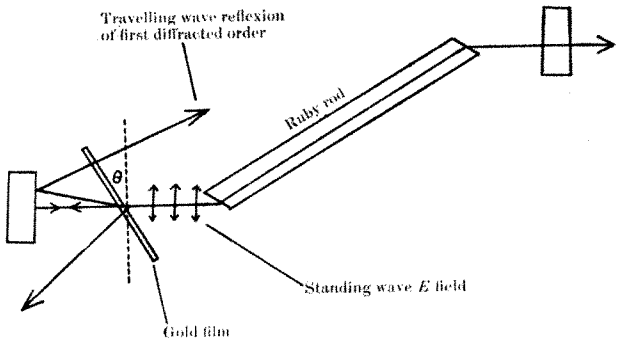


Fig. 1. Technique for producing diffraction gratings by laser evaporation.

a standard Q-switching method[2,3] producing peak powers of the order of 10 MW in our experiments.

When oscillations start, evaporation takes place at the anti-nodes[4] of the electric field forming a grating within the laser burn. On two films the diffracted first order travelling wave after one reflexion in the cavity mirror also burnt the film. As expected, this burn did not show any diffraction effects.

The number of lines per mm, n, can be varied by changing the angle θ between the film and the normal to the cavity axis, because

$$n = \frac{\sin\theta}{\lambda_R/2}$$

where λ_R is the wavelength of ruby light. Gratings were produced for various values of θ, and n was calculated from the results of normal incidence first order diffraction experiments. The graph (Fig. 2) is a straight line, showing good agreement with theory.

The gratings were examined using a helium–neon laser. Fig. 3 shows the reflected and transmitted first and second order diffracted beams. Spraying the gratings with a protective clear lacquer did not seem to affect their performance. Some of the glass substrates were examined after the metal film had been dissolved, and the glass was found to be etched, giving faint diffraction patterns. We intend to investigate the latter phenomenon as part of the current programme of work at this college on laser damage.

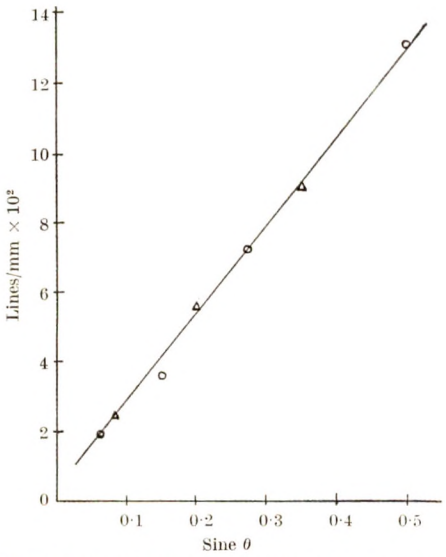


Fig. 2. Laser evaporated gratings. Graph of lines mm^{-1} as a function of film angle, θ, within the cavity. ○, Bradley type 302 laser; △, Brewster angled laser system.

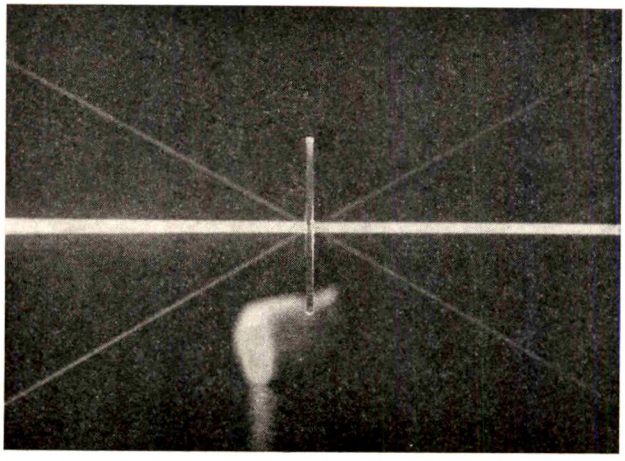


Fig. 3. Transmitted and reflected, first and second diffracted orders by a laser evaporated grating with 715 lines mm^{-1}. Incident radiation 632·8 nm.

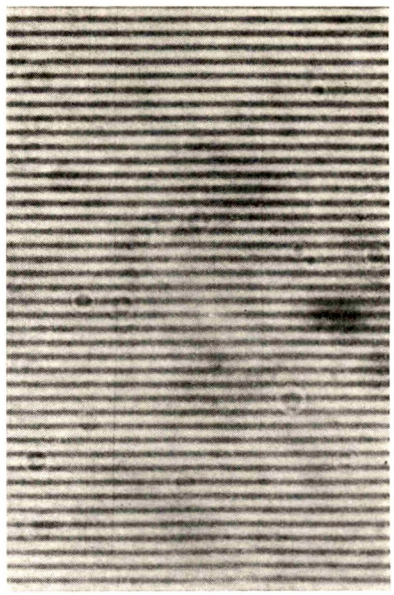


Fig. 4. Laser evaporated grating photographed by transmission microscopy. The grating period is 2,500 nm.

The uniformity of the rulings of this kind of grating can be assessed from Fig. 4. In this case the line spacing was 2,500 nm.

Using this technique the length of grating which can be made seems to be limited only by the length of the laser cavity and the available laser power. The width of the grating is determined by the width of the laser beam—usually only a few mm. The grating spacing could conceivably be as small as 250 nm if frequency doubled ruby laser light were used, and there is no reason to believe that the quality of the rulings would be any worse than those shown in Fig. 4. Development work is proceeding along these lines.

We thank Professor S. Tolansky and Mr M. Thyer for Fig. 4 and Mr D. Hambley for preparing suitable gold films. R. W. and D. M. R. are supported by Science Research Council research studentships.

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Liquid Crystals in Molten Salt Systems

Because of the strong long range forces acting between the molecules, molten salts constitute a highly distinctive class of liquids. Up to now, however, the variety of stable ionic melts studied has been largely confined to

the simplest ions, such as inert gas type halides, nitrates, and network forming oxides and fluorides. Through the establishment of procedures for achieving chemical stability in molten salts with organic anions[1,2], the variety of these ionic melts available for investigation and use is potentially greatly increased. Distinctive microstructures and unusual properties might be expected for at least some of these liquids. We illustrate these possibilities here with new observations on liquid crystals, stable over a wide interval of temperatures, formed by melts of sodium isovalerate and related salts. This group of substances differs in several ways from well known classes of liquid crystals, which include chiefly rod-shaped molecules, with long rigid parts[3].

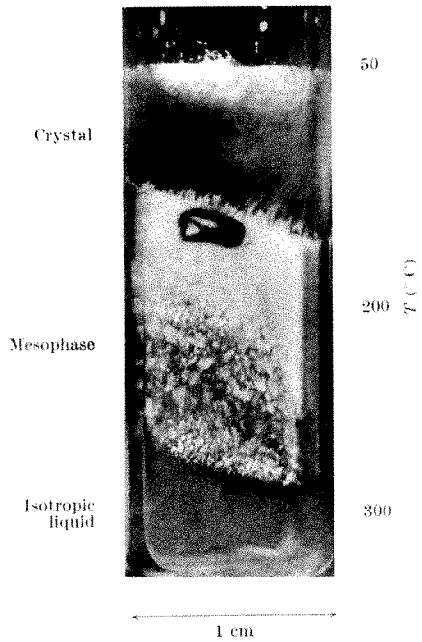


Fig. 1. Macroscopic view of 3 phase sample of sodium isovalerate in polarized light.

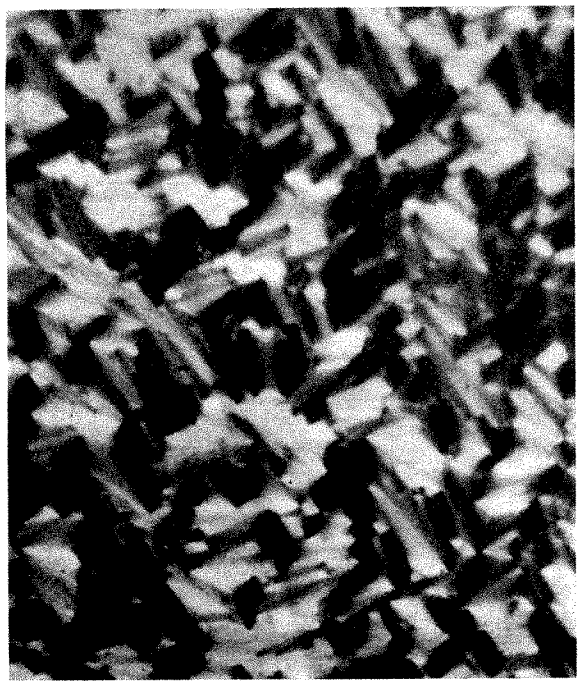


Fig. 2. Microscopic view of sodium isovalerate. Polarized light; temp = 200° C; ×50 (original magnification ×100).

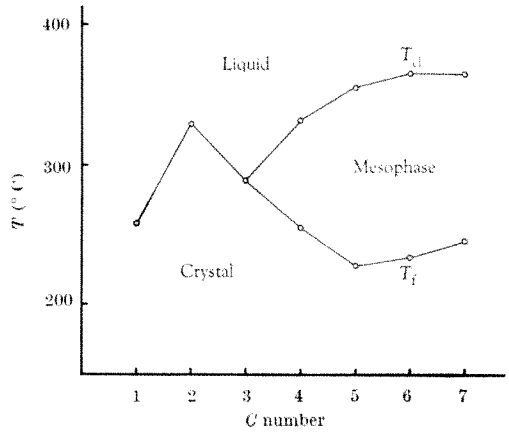


Fig. 3. Transition temperatures of sodium salts of fatty acids against C number.

In systematic studies of the physico-chemical properties of organic ionic melts, we have observed thermotropic mesomorphic behaviour in sodium salts of fatty acids even of low carbon number ($n < 6$). Anions in these compounds are quasiglobular, and do not show any obvious geometrical anisotropy, although this had generally been considered a condition for the formation of liquid crystals[4]. Although the mesomorphic behaviour of these salts was briefly reported some sixty years ago[5], to our knowledge no attention has been given since that time to this behaviour in compounds of low molecular weight. Mesomorphic behaviour is, of course, well known for alkali salts of longer chain fatty acids ($n > 10$) (ref. 6).

The thermal stability of molten sodium isovalerate and the extended temperature range ($\Delta T > 100°$ C) of its mesomorphic phase provide good examples of recent developments in our researches. We also include here brief data on sodium propionate, which show a fugitive mesophase extending only 1° above the melting point, and on sodium *n*-butyrate the mesophase of which is stable over a wider range of temperature, but the melts of which are less stable than those of sodium isovalerate. All these salts were prepared following a procedure described elsewhere[2].

Birefringence is one of the most conveniently identifiable properties of liquid crystals. Fig. 1 shows sodium isovalerate contained in a 'Pyrex' cuvette (1 cm depth) observed in plane-polarized light travelling towards the observer normal to the container face. A temperature gradient within the sample allows the three phases to co-exist (isotropic liquid, mesophase, and solid). The mesophase is seen to be strongly birefringent. When the melt is slowly heated towards the mesophase–isotropic transition, it breaks up into strongly coloured domains (second order of colours). This phenomenon occurs in the bulk of the liquid, and can therefore hardly be attributed to the wall orientation effects which are sometimes found for liquid crystals.

Chromatic polarization is usually observed in birefringent crystals cut in thin plates with faces parallel to the optic axis. Because the birefringence observed for sodium isovalerate is relatively high, comparison with other mesophases that show this property[7] would suggest that an extensive two dimensional ordering of its molecules occurs spontaneously.

Some additional information about the nature of the mesophase has been obtained from hot-stage (200° C) microscopic examination (×100) in polarized light of sodium isovalerate mounted as a film between a glass slide and a cover slip. The pattern observed (Fig. 2) resembles the "A type" exhibited, for instance, by the classical smectogenic compound di-*n*-hexyl-4,4′-azoxycinnamate. This type has been described as a "Fächerstruktur"[8].

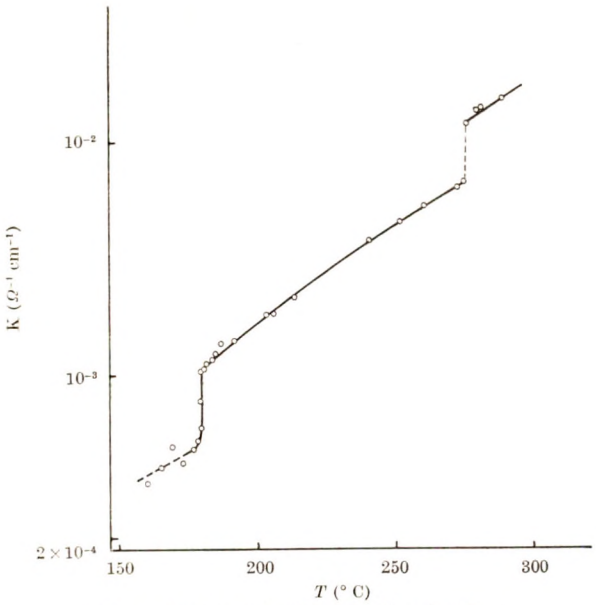


Fig. 4. Specific conductivity of sodium isovalerate

Fig. 3 records the transition temperatures we observed for some sodium carboxylates plotted against the number of carbon atoms in the organic anion. As the number of carbon atoms in the anion is increased, the strong long-range electrostatic forces are progressively diluted by the organic fraction, for which interaction forces are of much shorter range. This trend brings with it mesomorphic phases stable over extended regions of temperature.

Table 1. MELTING T_f AND CLEARING POINTS T_{cl} FOR LIQUID CRYSTAL IONIC MELTS (°C)

Compound	T_f	H_f kcal/mole	S_f e.u.	T_{cl}	H_{cl} kcal/mole	S_{cl} e.u.	(S_f+S_c)*
* Sodium propionate	293	2.1	3·71	–	–	–	3·71
* Sodium *n*-butyrate	256	1·48	2·80	332	0·35	0·58	3·38
* Sodium isovalerate	187	1·0	2·17	283	0·177	0·32	2·49
† Ethyl-*p*-azoxybenzoate (smectic)	114	4·73	12·2	122·6	1·19	3·01	15·2
‡ *p*-azoxyanisole (nematic)	118	7·06	18·1	135	0·137	0·34	18·4
§ Cholesteryl myristate (cholesteric)	71	11·22	32·6	85	0·269	0·75	33·4

* This work.
Assuming first order transitions at melting and clearing points.
† Ref. 12.
‡ Ref. 13.
§ Perkin–Elmer data.

Calorimetric data have been obtained using a Perkin–Elmer differential scanning calorimeter (model DSC-1B). The heating rate used was 16°/min. Estimated enthalpies and entropies for the various melting mechanisms are recorded in Table 1. Although the results are not yet sufficiently precise for evaluation of specific heats, they may be usefully compared with data for typical non-ionic liquid crystals, included in Table 1 for convenience. Although our new data are provisional, it is clear that entropies of fusion of the salts are far smaller than for molecular liquid crystals of classical types, so far as these have been determined. Small solid–solid transitions were also observed at about 78° C for sodium isovalerate and at 178° C and 246° C for sodium *n*-butyrate. These probably resemble the transitions found in solid acetates[1], but will not be further discussed here.

In the interval of temperature between the onset of melting T_f and the clearing point T_{cl}, mesophase ionic melts exhibit remarkable transport properties. For convenience of description, this intermediate state of these molten salts has been provisionally referred to above as "liquid crystal". Measured with a conventional capillary viscometer, a high viscosity of about 100 poises at 260° C has been found for sodium isovalerate in the mesomorphic state. Above its clearing point, the viscosity drops to 0·7 poise. These results may resemble the viscosity changes from 200 poises for smectic ethyl *p*-azoxybenzoate, to $<0{\cdot}05$ poise at its clearing point[10]. Such sharp decreases may be contrasted with the change in viscosity on passing from nematic to isotropic *p*-azoxyanisole[11]. Detailed measurements, however, are needed to characterize the molten salt mesophases more precisely in this respect.

Electrical conductance changes observed for sodium isovalerate are recorded in Fig. 4, in a semi-logarithmic plot against temperature. These values were determined using a low constant cell (0·263 cm^{-1}) with an a.c. bridge at 1 kHz. The jump in specific conductivity $\varkappa$ in passing from crystals to the "liquid crystal" melt (a factor of about 160) is comparable with the jump on passing from "liquid crystal" to isotropic melt (a factor of about 100). Nominal activation energies in Arrhenius plots of log $\varkappa$ against $1/T$ are 8·15 kcalories/mole for the mesophase and 12·2 kcalories/mole for the isotropic ionic melt. Both are lower than for crystalline sodium isovalerate (about 30 kcalories/mole). Present findings indicate that changes in conductivity do not run parallel to the observed changes in macroscopic viscosity of the molten salts. More information is needed to elucidate the mechanisms of ion-drag in the various phases of sodium isovalerate.

An alternative explanation for the remarkable mechanical and optical properties now observed might be that the cation lattice in crystalline sodium isovalerate becomes positionally disordered at T_f while the anion lattice retains long range correlations between neighbours, until the clearing point T_{cl} is reached. By way of comparison, certain silver salts show positional melting of the cation lattice many tens of degrees before their anion lattice melts[14]. Jumps in conductance on attaining positional disordering of the cation lattice in a salt such as silver iodide are comparable with those now observed for sodium isovalerate, and there may thus be an analogous sequence of melting mechanisms in the two groups of salts. If this alternative suggestion about the microstructure of these organic ionic melts is well founded, the "liquid crystal" state of sodium isovalerate might in fact be more correctly described as a plastic solid with extremely low elastic limit. Even for moderate shear stresses, a plastic crystal with almost zero elastic limit is likely to resemble a "liquid" of very high viscosity, with certain customary methods of measuring this property. More detailed studies on the properties and microstructure of these remarkable liquids, which are being made, should help to clarify these problems. In the meantime, the ready availability of these novel anisotropic "liquids" may prove to be of use in a wide variety of studies.

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MIRVs and the Strategic Balance*

BELLANY[1] claims that the introduction of multiple independently targeted re-entry vehicles (MIRVs) could increase the stability of the strategic balance between the US and the Soviet Union. This conclusion is not warranted, and in fact quite the opposite is true.

The measure of stability adopted by Bellany is based solely on the number of warheads that survive a first strike by the opposing power. I shall present several arguments to show why this criterion is unsatisfactory. In the model in which the forces of the two major powers are assumed to be equal, Bellany's basic equation is

$$X = nN(1-C)^{fnR} \qquad (1)$$

Here N is the number of launchers on each side; n is the MIRV ratio (number of warheads per missile); R is a reliability factor (probability of successful launch); C is the probability that a target missile silo is destroyed by a single warhead; and f is the fraction of its launchers that country A employs in a hypothetical (counterforce) first strike. X is then the number of B's warheads expected to survive such an attack. By maximizing X with respect to n, holding all other parameters fixed, Bellany obtains an "optimum" MIRV ratio.

Does the "optimum" situation thus derived actually entail an increase in stability? Consider the numerical example used by Bellany: $R = 0{\cdot}75$, $f = 1$, $C = 1/2$; for simplicity, take $N = 1{,}000$. The situation with and without MIRV is then as follows

	$n=1$ (no MIRV)	$n=2$	$n=3$
Initial warheads on each side	1,000	2,000	3,000
Warheads B has left after A's first strike	625	706	645

$n = 2$ is the optimum MIRV ratio according to Bellany's criterion. The introduction of MIRV has achieved a modest (I am tempted to say, trivial) increase in the number of surviving warheads, at the cost of doubling the initial number of warheads on each side. Perhaps a would-be first striker will feel somewhat more deterred. But a much more important consideration has been ignored by Bellany. Imagine a crisis situation, in which each side fears the other may attack, even though neither has a true first-strike capability. (I regard this as a more likely prelude to nuclear war than a coldly premeditated first strike.) In such a situation, military leaders will weigh the risks and advantages of a pre-emptive attack against those of waiting and perhaps letting the opponent get in the first blow. Even with a MIRV ratio of only 2, pre-empting will reduce the opponent's force by a factor of almost 3; if the opponent should strike, one's own force will be similarly depleted. The advantage of striking first is always much greater with MIRV than without. This is the very essence of instability.

Bellany has also maximized X with respect to only one parameter, n. If we complete his calculation by similarly finding the optimum value of N, obviously, this value is infinite and, according to Bellany's criterion, the most stable situation is one in which each side possesses an infinite number of launchers, each missile containing some finite number of warheads. If the number of launchers is held fixed, say, by international agreement, the procedure of maximizing only with respect to n becomes more justified. But the other criticisms remain equally valid even in this case.

Equation (1) can be used to demonstrate another reason why MIRV is destabilizing. The values for accuracy, hardness, and so on used by Bellany are fairly representative of present capabilities. The trend in missile technology, however, has been toward ever improving accuracy, and we must take account of the likelihood that targeting errors will be reduced substantially below their present values. The parameter C is extremely sensitive to missile accuracy; it is given by the relation

$$1 - C = 2^{-K/CEP^2} \qquad (2)$$

where CEP is the r.m.s. targeting error and the constant K depends on the yield of the weapon and the hardness of the silo. If $C = 1/2$ when $CEP = 0{\cdot}25$ miles (these are the numbers used by Bellany), then a reduction of CEP to 0·1 mile (not at all outside the range of possibility) increases C dramatically, to more than 99 per cent. Even by Bellany's criterion, the optimum MIRV ratio then falls well below unity. More important, when C is close to unity, X rapidly approaches zero as n increases. The combination of high accuracy and MIRV is extremely dangerous: It can lead to a situation in which each side has a first-strike capability with respect to the other's fixed missiles. This would be about the most unstable situation imaginable.

One can hardly expect countries to deploy MIRVs now and voluntarily dismantle them later, when improved accuracy makes them a potential first-strike weapon. Any agreement to refrain from incorporating higher accuracy in an existing MIRV installation would be subject to the well known difficulties in verification.

I conclude that the introduction of MIRVs, at any ratio, would have a highly destabilizing effect on the strategic balance.

I thank S. Weinberg and P. Morrison for helpful suggestions.

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[1] Bellany, *Nature*, **226**, 412 (1970).

MAY I reply to Professor Sartori's points roughly seriatim?

Let me say first that I was careful not to be dogmatic about what actually constituted a more or a less stable balance. My choice of the number of surviving warheads as a measure of stability was made because it seemed plausible that decision makers would think, even in periods of crisis, in terms of numbers of targets threatened—roughly proportional to number of warheads—and of how well they might defend these targets with ABM defences—again closely related to the number of attacking warheads to be engaged. Professor Sartori wishes to substitute this criterion by one where the incentive to strike first is measured by the inverse fraction of total warheads expected to remain after a first-strike attack. He may be right.

Second, I am glad Professor Sartori has been good enough to illustrate my contention that there is an optimum value for the number of MIRV warheads per launcher. That he has gone farther and demonstrated that the number of warheads surviving a first strike is, near the turning-point, a slow moving function of the MIRV number adds weight to my general contention that MIRVs do not necessarily threaten a serious upset to the strategic balance.

Third, my justification for maximizing X with respect to n is simply that the number of launchers, N, is the only parameter at all likely to be fixed at some future date by political agreement, as Professor Sartori himself concedes.

Finally, the fact that, by my criterion, as missile accuracy increases the optimum MIRV number falls below unity merely bears out what Professor Sartori has to say about the effects of increasing accuracy and does not strike me as giving him much cause for complaint on that point. Might I, however, complain that while Professor Sartori's discussion of the effects of increasing accuracy is, as far as it goes, unexceptionable, it does not go far enough? He

* An article on this subject was also published in *Nature*, **227**, 696 (1970)—*Editor.*

has forgotten to allow for the effects of ABM defence of, in this case, offensive missile installations. As he may know, even the crude current techniques of ABM defence are likely to be at their most effective when employed in defence of targets already partially protected against nuclear attack, such as offensive missile emplacements. This factor alone will prevent the probability of an offensive missile emplacement (a silo) being destroyed by a single warhead rising, as missile accuracy increases, anywhere near as fast as he suggests.

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BIOLOGICAL SCIENCES

Mutagenic Methods for determining which DNA Strand is transcribed for Individual Viral Genes

THIS work describes two mutagenic methods for determining which strand of the DNA double helix is transcribed. The approach is applicable to viruses which contain single-stranded DNA in the mature form. The analysis identifies which strand is transcribed for a given viral gene.

The usual method for determining the transcribed DNA strand is by DNA–RNA hybridization. Using phage φX174, Hayashi, Hayashi and Spiegelman[1] compared the ability of phage-induced mRNA to hybridize with the single-stranded mature phage DNA and with the double-stranded replicative form. Because the bulk of the mRNA hybridized with the double but not the single-stranded DNA, most of the mRNA must be copied from the complementary strand of the replicative form. But the hybridization data do not exclude the possibility that a small portion of mRNA hybridized with the single-stranded DNA, which would mean that one or a few genes may be transcribed differently. Because the mutational analyses we describe are gene specific, we have re-examined this problem using phage S13, which is closely related to φX174[2].

The transcribed DNA strand can be identified for individual genes of single-stranded DNA phages by studying the induction of *ochre* (UAA)[3] mutants because two of the three possible transition mutations of the *ochre* codon produce other nonsense codons (Fig. 1). This situation makes it possible to determine from mutagenesis experiments alone whether the DNA triplet in the mature virus and the corresponding RNA codon are complementary or homologous (with T replacing U).

The first method used to identify the transcribed strand was to study whether *ochre* mutants could be induced from wild type by particular transition mutations. The only sense codon related to *ochre* by a transition is CAA, which codes for glutamine (Fig. 1). If the mRNA and the mature phage DNA are homologous, then C→T (CAA→TAA) is the only transition which can produce an *ochre* from a sense codon; if they are complementary, G→A (TTG→TTA) is the only transition which can produce an *ochre*. To identify the transcribed strand by induction of *ochres* from sense, it is necessary to use a mutagen that may induce G→A or C→T but not both. The mutagen can also induce other mutations provided they are transitions; the qualification is necessary in order to restrict the origin of the *ochre* to the glutamine codon.

Hydroxylamine, which induces only C→T transitions[4], was used to study the induction of *ochres* from wild type in bacteriophage S13 (Fig. 2, method *A*). Induction of an

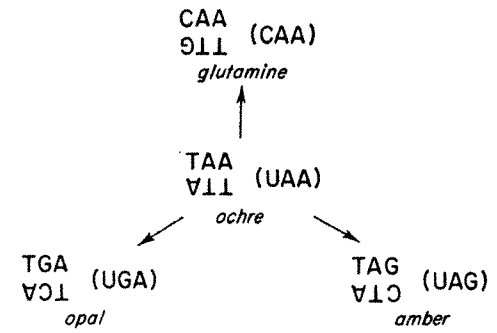


Fig. 1. Codons related to the *ochre* by one-step transitions. Both the homologous and complementary DNA triplets are shown next to the corresponding RNA codons which are in parentheses.

ochre in a given gene with hydroxylamine would prove that the mRNA and mature phage DNA are homologous for that gene. But failure to induce an *ochre* with hydroxylamine would not prove that the mRNA and phage DNA are complementary because there are other reasons why an *ochre* might not be observed even if the mRNA and phage DNA are homologous. One reason for this is that the gene might not contain the CAA triplet anywhere in its DNA.

The second method used to identify the transcribed strand was to study whether *ochre* mutants could be induced from *opal* (UGA) nonsense[5] mutants. If the mRNA and DNA are homologous, then only a G→A (TGA→TAA) transition can produce an *ochre* (Fig. 1). When complementary, only C→T (TCA→TTA) produces an *ochre*. Any mutagen which induces a G→A or a C→T transition, but not both, can be used to identify the transcribed strand. Hydroxylamine was again used to study the inducibility of *opal*→*ochre* in S13. An *ochre* is inducible from an *opal* only when the mRNA is complementary to the mature phage DNA (Fig. 2, method *B*). The same argument applies to the induction of *amber* (UAG)[3]→*ochre* but it is more difficult to study this mutation because of the problem of selection; *ochre* suppressors in the host suppress both the *amber* and *ochre* codons. It was possible, however, to study one example of *amber*→*ochre*.

Method *B* is more general than method *A*, because *B* can be used successfully to identify the transcribed strand for a gene either when the mRNA and DNA are complementary or when they are homologous, while *A* can succeed only when they are homologous.

To apply these mutagenic methods, it is necessary to be able to distinguish the *amber*, *opal* and *ochre* nonsense mutants from each other. Table 1 shows that the three types of mutants can be identified by their growth patterns on different cell strains.

To identify genes having mRNA homologous to the mature phage DNA by method *A*, *ochres* were induced with hydroxylamine[4] from S13 wild type[6]. *Ochres* were found in genes I, II, IIIa-IIIb, IV and VI (Table 2). Establishing that an *ochre* occurs in either gene IIIa or IIIb is sufficient to establish for both genes that the mRNA is homologous

Table 1. GENERAL GROWTH PATTERNS OF PHAGE NONSENSE MUTANTS ON VARIOUS STRAINS OF *E. coli*

Cell:	CA165·2		C600·1	T_3A-2·1[8]	WWU[9]		C	
	str^s	*str*^{r}			*su*$^-$	*su*$^+$	*str*^{s}	*str*^{r}
Phage								
Ochre	+	+/0*	0	0	0	0	0	0
Amber	+/0	+/0	+	0	0	+	+/0	0
Opal	+/0	+/0*	+	+	+/0†	+/0†	+/0	0

* The *str*^{r} allele greatly decreased the suppression of many *opal* mutants but only slightly affected suppression of *ochres*. For several allelic *opal-ochre* pairs the efficiency of plating (eop) of the *opal* was relatively low (eop of *opal*/eop of *ochre* $\leqslant 10^{-5}$). Thus it was easy to select mutations from *opal* to *ochre* by using CA165·2 *str*^{r}.

† A given *opal* mutant had the same eop on both *su*$^-$ and *su*$^+$ strains.

+/0 indicates that some phage mutants grow and some do not.

Other cell strains showed at least some suppression of S13 *opal* mutants. *E. coli* KB, KT*su*$_{UAG}$, AB1157*su*$_{UAG}$, CR63*su*$_{UAG}$, KMBL146(CR34)*su*$_{UAG}$ and *Shigella dysenteriae* Y6R*su*$_{UAG}$.

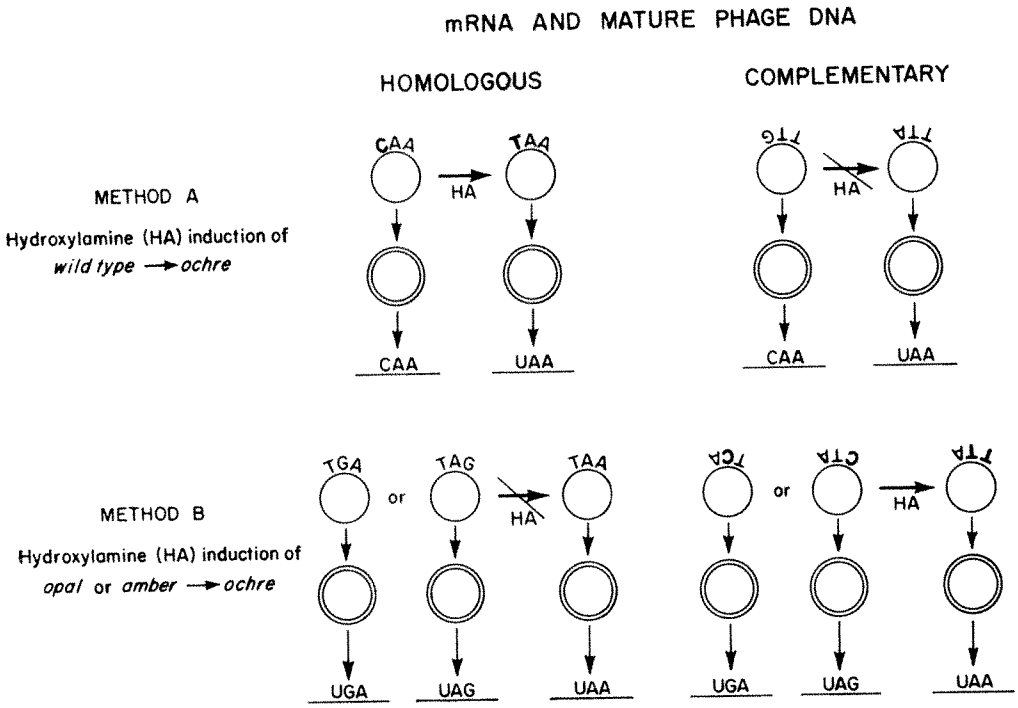


Fig. 2. Two mutagenic methods identifying the transcribed strand of phage S13. The diagram represents the conversion of single-stranded mature phage DNA (single circle) to double-stranded, intracellular replicative-form DNA (double circle) and the production of phage specific mRNA (straight line). The DNA triplets are shown on the single circles and the mRNA codons are on the straight lines. The heavy arrows indicate mutagenesis. The bases C and T are shown in heavy letters wherever they are involved in hydroxylamine-induced mutations. Hydroxylamine, which is specific for C→T transitions, can induce an *ochre* mutant from a sense codon only when the mRNA and mature phage DNA are homologous (method *A*). When the mRNA and DNA are complementary, two DNA triplets, TCA and CTA, could be mutated by hydroxylamine to an *ochre*, but both correspond to nonsense. An *ochre* can be induced from an *opal* or an *amber* by a C→T transition if the mRNA and phage DNA are complementary. If they are homologous, a C→T transition cannot convert an *opal* or an *amber* to an *ochre*.

to the phage DNA because polarity of nonsense mutants in gene IIIa has shown that both genes are translated from the same mRNA[7]. No conclusion can be reached about the two remaining genes, V and VII[2], because no *ochres* were found.

Confirmation of these results was obtained by method *B*. The ability of hydroxylamine to induce *opal*→*ochre* and one *amber*→*ochre* was tested (Table 3). These *opal* and *amber* mutants were at the same sites as the *ochres* induced in the preceding experiment. Although the *ochres* had been induced by hydroxylamine, none of the *amber* and *opal* mutants could be mutated. Nitrous acid was used as a control mutagen because it can induce both G→A and C→T transitions[4] and therefore should induce the *ochres* regardless of which DNA triplet corresponds to the UGA or UAG codon. As expected, all the mutants were induced to *ochre* with nitrous acid. The mutants used in these experiments were in genes II, IV, IIIa-IIIb, and VI. The homology of the mRNA with the viral DNA was therefore verified for these genes.

Table 2. INDUCTION OF WILD TYPE → *ochre* BY HYDROXYLAMINE

Gene	Induction frequency × 10⁸ Hydroxylamine*	Spontaneous
I	9·6	< 0·1
II	23·5	< 0·1
IIIa–IIIb†	4·5	0·2
IV	3·5	0·2
V‡	< 0·1	< 0·1
VI	16·5	< 0·1
VII‡	< 0·1	< 0·1

* The induction frequency is expressed per lethal hit.
† It was not necessary to determine whether these *ochres* are in genes IIIa or IIIb (see text).
‡ No *ochres* were isolated in gene V or gene VII.
Wild-type phage were treated[4] with hydroxylamine to a survival of 0·2. Fifty-five mutants were isolated by replica plating and placed into complementation groups[10]. The spontaneous frequency was measured by replica plating of unmutagenized phage.

Table 3. INDUCTION OF *opal*→*ochre* AND *amber*→*ochre*

Gene	Mutant	Induction frequency/lethal hit × 10⁷ Nitrous acid	Hydroxylamine
II	*opal*	400	< 0·7
IIIa–IIIb	*amber**	320	0·8
IV	*opal*	340	< 0·3
VI	*opal*	350	< 2·6

* It is not known if this mutant is in IIIa or IIIb (see text).
Although *ochre* suppressors in the host suppress both *ambers* and *ochres*, the *amber* mutant grows poorly enough on the *ochre* suppressor strain for the *ochre* to be selected. Each *amber* or *opal* mutant tested was induced with 5-bromodeoxyuridine[11] from the *ochre* mutants described in Table 2. CA165-2 *str*^{r} was used to select *ochres* from *opals*, and CA165-2 was used to select the *amber* from the *ochre*. *Ochres* were identified by spot testing on appropriate cell strains (Table 1). The induction frequencies for hydroxylamine are not significantly higher than the spontaneous frequencies.

We made several attempts to isolate mutants in other genes so that hydroxylamine inducibility of *opal*→*ochre* could be studied. *Opals* were induced from the wild type by nitrous acid, but it was found that all these *opal* mutants occurred in previously tested genes. Also, no *opals* were found after treatment of the wild type with hydroxylamine. Finally, *ochres* were isolated with nitrous acid. It was intended to convert the *ochres* to *opals* and then test as in method *B*. But again, all the mutants observed were in genes that had already been tested.

From this mutational analysis it can be concluded that for six of the eight[2] known genes of S13-φX174, the transcribed RNA strand is homologous to the mature phage DNA. These results extend the earlier hybridization studies[1] which did not exclude the possibility that one or more genes may be read on the complementary strand. Because the genetic analysis is gene specific, this possibility can be eliminated for six genes.

These genetic or mutagenic methods for determining the transcribed strand have been applied to a single-stranded DNA phage, but they also can be applied to any organism in which viable complementary DNA strands can be separated and single-base substitutions can be identified. They can also be applied to RNA viruses.

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Specific Inhibition of Influenza Replication by α-Amanitin

α-AMANITIN, a bicyclic polypeptide from the toadstool *Amanita phalloides*[1], is known specifically to inhibit the DNA-dependent RNA polymerase of mammalian cells by interaction with the enzyme rather than with the DNA template[2,3]. No influence of the toxin on the replication of a number of DNA or RNA-containing viruses has been found so far (ref. 4 and personal communication from L. Philipson). Actinomycin, which reacts with the DNA template and so impairs the DNA-dependent RNA polymerase reaction, depresses the multiplication of influenza viruses but does not interfere with the replication of most of the other RNA-containing viruses[5–7]. Because the mode of action of this antibiotic on the synthesis of influenza viruses is still obscure, we extended our studies to the action of α-amanitin on the multiplication of this virus group.

As a model system for influenza virus the fowl plague virus (FPV) strain "Rostock" and, as a control, a parainfluenza virus (Newcastle disease virus, NDV) were propagated in chick fibroblasts. The plaque test and the determinations of viral neuraminidase, haemagglutinin and the inner component (RNP-antigen) were performed according to standard methods. Viral plus and minus strand RNA were determined by the hybridization technique[8]. Viral RNA polymerase activity was assayed in a cytoplasmic extract prepared 5 h after infection[9].

FPV multiplication is inhibited completely by 50 μg/ml. of α-amanitin, whereas NDV multiplication is not affected (Table 1). FPV replication is even more efficiently blocked when the inhibitor is added to the cultures 2–3 h before infection. This holds true not only for the infectivity, but also for the amount of haemagglutinin, neuraminidase and RNP-antigen (Table 2).

Table 1. DOSE EFFECT OF α-AMANITIN ON THE YIELD OF FPV AND NDV

α-Amanitin (μg/ml.)	HA units		P.f.u. $\times 10^{-6}$	
	FPV	NDV	FPV	NDV
0	256	32	110	140
1	224	32	110	250
10	32	32	14	150
25	4	32	8	—
50	< 2	32	2	—
100	< 2	32	1·9	150

The toxin was added immediately after infection. The cell-associated virus-specific activities were determined 8 h after infection after breaking the cell by freezing and thawing three times.

Table 2. EFFECT OF α-AMANITIN ON THE SYNTHESIS OF VIRUS-SPECIFIC RNA *in vivo*, AND ON THE PRODUCTION OF OTHER VIRAL COMPONENTS

	No α-amanitin	25 μg/ml. α-amanitin added 2·5 h before infection
Radioactivity (d.p.m.) in:		
Total RNA	71,500	34,000
Plus strand	5,700	1,700
Minus strand	4,300	320
Cell-associated parameter:		
HA units	128	1
P.f.u. $\times 10^{-5}$	33	1·6
RNP-antigen titre	64	4
Neuraminidase units	520	38

To each of three FPV-infected cell cultures 100 μCi of ^{3}H-uridine was added 2·5 h after infection. When α-amanitin was investigated, 200 μCi ^{3}H-uridine was investigated. Total RNA was extracted by sodium dodecyl sulphate plus phenol 3·5 h after infection. Viral plus and minus strand RNA was determined by hybridization[8].

Up to 50 μg/ml. of α-amanitin had no influence on the incorporation of ^{3}H-uridine or ^{3}H-leucine into the corresponding macromolecules of uninfected cells, when the isotopes and the toxin were administered simultaneously. If the pulse was given 3 h after the addition of α-amanitin there was also no significant effect. Six hours after addition of the inhibitor, however, the uptake and phosphorylation of ^{3}H-uridine and RNA and protein synthesis reached only about 50 per cent of the values for untreated cells.

The results in Table 2 demonstrate that a substantial amount of viral plus strand RNA is still being synthesized in conditions when the plaque test indicates a twenty-fold inhibition of FPV multiplication. The production of viral minus strand RNA, however, is barely measurable. A similar result has been obtained recently using mithramycin as an inhibitor of RNA synthesis[10]. FPV-specific RNA polymerase, which synthesizes chiefly minus strand RNA[11] *in vitro*, was not inhibited by 180 μg/ml. α-amanitin. There is no measurable inactivation of FPV by incubating the virus for 16 h with 60 μg/ml. α-amanitin at 4° C.

The results indicate that α-amanitin inhibits specifically influenza virus replication without interfering significantly with the overall synthesis of RNA and protein in the host cell. The delayed effect on these activities might be rather a non-specific toxic alteration of the metabolism in our cell system, which is essentially different from the systems used by others[2,3]. At this time the multiplication cycle of FPV is in any case completed, so that it is not very probable that α-amanitin inhibits influenza virus multiplication by interfering with cellular RNA synthesis as has been discussed for the action of actinomycin[5,7]. Because much viral RNA is still being synthesized in the presence of α-amanitin, it could be that this inhibitor also affects some step of the translation of viral RNA.

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Tryptophan tRNA of *Escherichia coli*

THE tryptophan tRNA of CAJ64, a UGA suppressing strain of *Escherichia coli*[1], has an altered structure compared with that isolated from the wild type (CA244). A complete account of this work will be published elsewhere, but the sequence shown in Fig. 1 has two points of immediate interest. First, both the su$^+$ and su$^-$ structures contain an adenosine at position 15 and a uridine at position 48. In most other tRNAs guanosine and cytosine, respectively, occupy homologous positions in the sequence. Another exception with adenosine and uridine in these positions has recently been found in a leucine tRNA of *E. coli*[2]. The three dimensional model proposed by Levitt[3] has hydrogen bonds between the bases in these positions; these coordinate base changes are consistent with the structure and lend support to this feature of it.

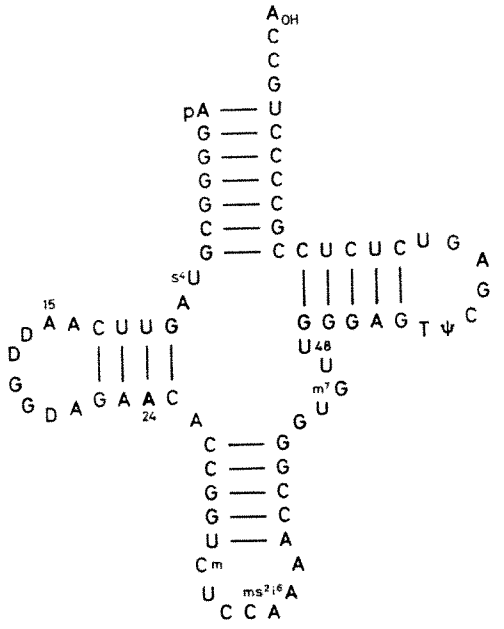


Fig. 1. Nucleotide sequence of *E. coli* (strain CAJ64) tryptophan tRNA. The unusual nucleoside 3′-phosphates are abbreviated as s^4U for 4-thiouridine, D for dihydrouridine, Cm for 2′-O-methyl cytidine, Ms^2i^6A for 2-methylthio-6-isopentenyl adenosine, m^7G for 7-methyl guanosine, T for ribosyl thymine, and Ψ for pseudouridine (5-ribosyl-uracil).

Second, both the su$^+$ and su$^-$ sequences have the same anticodon sequence, CCA, which according to the predictions of the wobble hypothesis[4] should recognize the codon UGG alone. The only difference between the two sequences is that the su$^-$ has a guanosine instead of an adenosine at position 24. Evidence has been obtained to show that there is no other tryptophan tRNA (less than 3 per cent of the amount found) in the su$^+$ strain, that both the su$^+$ and su$^-$ tryptophan tRNAs can read UGA, and that the mutant alteration allows the su$^+$ sequence to do this more efficiently.

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Amino-acid Composition of Crayfish Trypsin

THE homology in primary structure of mammalian pancreatic serine proteases is well established[1,2]. Progress has now been made in the elucidation of the evolutionary history of this group of proteases. Neurath and his co-workers[3] studied the amino-acid composition of trypsinogen isolated from the pancreas of the spiny Pacific dogfish and presented a preliminary examination of the primary structure of this enzyme. From these data a high degree of homology as compared with bovine trypsinogen can be inferred. It is now of interest what will be found beyond the gap that separates vertebrate and invertebrate animals. Invertebrate proteases are difficult to investigate because of the small amounts of material obtainable from these animals. In the crayfish *Astacus leptodactylus* we found an enzyme resembling mammalian trypsin in many respects[4,5]. It exhibits a distinct tryptic cleavage specificity when tried on the oxidized B-chain of insulin and is inhibited by all natural and synthetic trypsin inhibitors including TLCK. It is a serine protease and possesses the sequence –Asp–Ser–Gly– around the active centre serine residue[6]. In addition, extended immunological studies have been carried out on the crayfish trypsins of three species which revealed that they do occur as isoenzymes varying in number and electrophoretic mobility[7].

Table 1. AMINO-ACID COMPOSITION OF TRYPSINS

	Invertebrate	Vertebrate	
	Crayfish	Dogfish[3]	Bovine[11,12]
Lysine	5	5	14
Histidine	5	8	3
Arginine	2	7	2
Aspartic acid	30	24	22
Threonine	15*	7	10
Serine	17*	17	33
Glutamic acid	21	16	14
Proline	10	10	9
Glycine	28	28	25
Alanine	16	16	14
Half-cystine	6†	12	12
Valine	18‡	17	17
Methionine	2	8	2
Isoleucine	15‡	14	15
Leucine	16	15	14
Tyrosine	11	12	10
Phenylalanine	7	1	3
Tryptophan	3§	5	4
NH_3‖	17		
No. of residues	227	222	223

* Calculated from 20 h and 70 h hydrolysates by first order approximation to zero time.

† Determined after performic acid oxidation[9].

‡ Values for 70 h hydrolysis.

§ Determined spectrophotometrically[10].

‖ An approximative value for the amide content of crayfish trypsin was derived from the 20 h ammonia value by subtraction of the ammonia peak from a blank hydrolysate, the ammonia being the result of the decomposition of serine and threonine.

Purified *Astacus leptodactylus* trypsin is composed of two closely related bands which are both active against BAEE. They were separated in a final purification step by disc electrophoresis[8] and the amino-acid content of each component was analysed quantitatively. Four analyses of component I (which moved fastest to the anode) were done from 20 h and 70 h acid hydrolysates at each time interval. An analysis of bovine trypsin which was run for comparison in the same conditions gave correct values. The examination of component II did not reveal significant differences in amino-acid composition. This is in concordance with our observation that both components are cross-reacting immunologically[7].

Crayfish trypsin was shown to have 227 amino-acid residues (dogfish and bovine trypsin have 222 and 223 residues respectively). This accounts for a molecular weight of 24,060.

From the amino-acid composition of the three trypsins it may be concluded that the invertebrate crayfish trypsin is homologous in primary structure to vertebrate trypsin. The proportions of proline, alanine, valine, isoleucine, leucine and tyrosine are essentially the same in all three trypsins. On the other hand, the high lysine and serine content of bovine trypsin is not shared by the two other trypsins. Crayfish and dogfish trypsin likewise exhibit only a third of the lysine and a half of the serine content of bovine trypsin. In dogfish trypsin the histidine, arginine and especially the methionine values are increased, whereas the other two enzymes are similar in this respect. This may reflect specific evolutionary features of dogfish trypsin, as well as the increase in lysine and serine content of bovine trypsin. Outstanding differences of this kind were not found in crayfish trypsin.

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Iodination and the Biological Activity of Gastrin

RADIOIODINATED polypeptide hormones are widely used as tracers for both radioimmunoassay and *in vivo* metabolic studies. The process of iodination may induce gross chemical changes which are detectable by physico-chemical techniques, but such techniques may not reveal more subtle changes reflected only in decreased biological activity. Such a situation arises with gastrin, in which iodination of the synthetic hormone by the chloramine-T method[1] results in almost total loss of biological activity. This is due to oxidation of the methionyl residue at position 15, which is in the biologically active C-terminal tetrapeptide sequence, and can be prevented by modifying the iodination procedure. The resulting iodinated gastrin has full biological activity, is labelled to the same specific activity as in the original method, and has an increased affinity for antibodies to the hormone.

Synthetic human gastrin I (SHG; Pyr-Glu-Gly-Pro-Try-Met-$(Glu)_5$-Ala-Tyr-Gly-Try-Met-Asp-PheNH_2) and pentagastrin (ICI 50, 123; *t*-butyloxycarbonyl-β-Ala-Try-Met-Asp-PheNH_2) were obtained from ICI Ltd, Alderley Park, Cheshire. SHG was iodinated either with carrier-free ^{125}I (Radiochemical Centre, Amersham) or with ^{127}I using the chloramine-T method, as modified by McGuigan[2]. Iodinated SHG was separated from unreacted iodide on a 1 × 15 cm column of 'Sephadex G-10'.

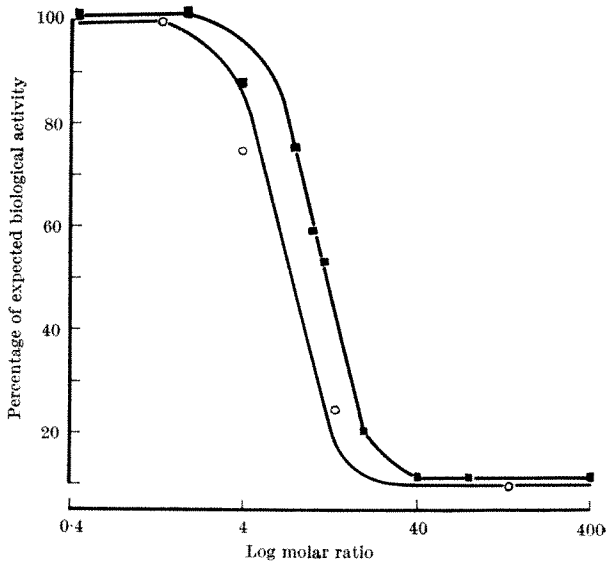


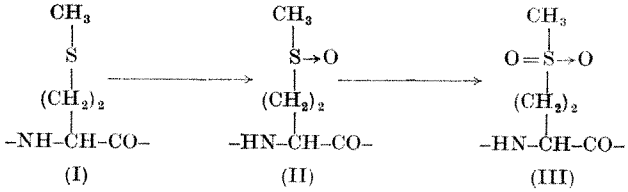
Fig. 1. The biological activity of synthetic human gastrin I (■) and pentagastrin (○) after treatment with chloramine-T. 1 μg quantities of SHG and pentagastrin were treated with increasing amounts of chloramine-T as described in the text. The biological activity, measured by bioassay in the anaesthetized rat, is expressed as a percentage of the activity of the same amount of untreated SHG or PG. On the abscissa is plotted the logarithm of the molar ratio of chloramine-T to SHG and PG. In the amounts used in the radioiodination of SHG, the log molar ratio of chloramine-T to SHG is 140.

The biological activity of SHG and pentagastrin samples was measured by bioassay in the anaesthetized rat[3].

Iodination with either ^{125}I or ^{127}I reduced the biological activity of SHG to less than 10 per cent of its original value. This was not due to the introduction of iodine atoms into the molecule because a similar effect was produced on exposure of SHG or pentagastrin to chloramine-T and metabisulphite in the absence of iodide.

To investigate this, varying amounts of chloramine-T (0·05–50 μg) were added to 1 μg SHG followed, after exactly 1 min, by sodium metabisulphite (five times the weight of chloramine-T). The same buffer and volumes were used as for iodination and the reaction mixtures were again fractionated on 'Sephadex G-10' columns. The first 10 ml. of the eluates were collected as pools. Similar experiments were carried out with the substitution of an equimolar amount of pentagastrin for SHG. The biological potencies of SHG and pentagastrin were reduced to similar extents (Fig. 1), and so we concluded that one of the reagents used in the iodination procedure was exerting an effect on the C-terminal tetrapeptide sequence which the peptides have in common.

Morley[4] has shown that the methionyl residue (I) at position 15 in the gastrin molecule is readily oxidized to the sulphoxide (II) and sulphone (III) derivatives



This process leads to loss of biological activity. Chloramine-T is an oxidizing agent and is present in considerable excess in the iodination reaction mixture, so it seemed possible that the observed effects on SHG and pentagastrin were the result of oxidation of the methionyl side-chain sulphur atom by chloramine-T. We confirmed that such an oxidation reaction occurred by amino-acid analysis of a sample of SHG exposed to chloramine-T. Methionine was absent and an equivalent amount of

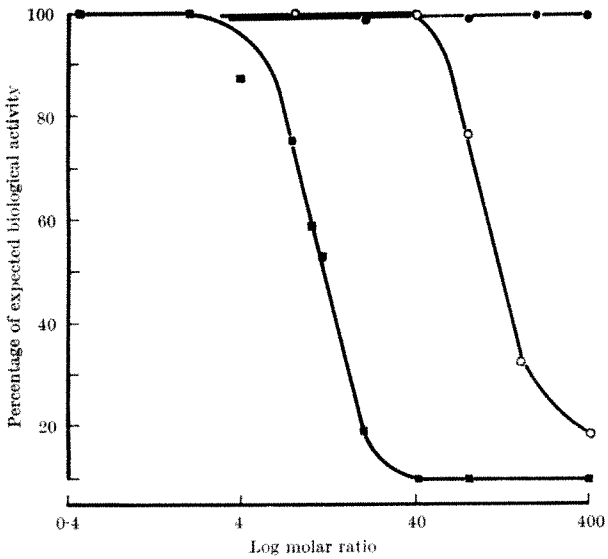
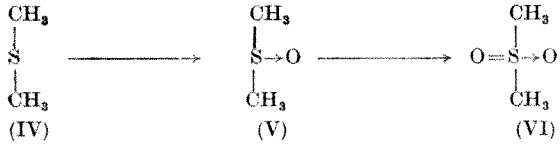


Fig. 2. The protection of SHG by dimethyl sulphoxide (DMSO) from oxidation by chloramine-T. ○, Biological activity of 1 μg SHG after treatment with varying amounts of chloramine-T in the presence of a 700 molar excess of DMSO with respect to SHG (25 μl. DMSO/μg SHG); ●, results obtained when DMSO was present in a 1,400 molar excess (50 μl. DMSO/μg SHG); ■, curve obtained when no DMSO was present, shown for comparison. The scales are the same as in Fig. 1.

methionine-S-oxide was present, corresponding to a methionine-S-oxide: leucine standard ratio of 1 : 1.

We therefore examined methods for protection of the methionyl residue from the oxidative action of chloramine-T, by use of a "scavenger" which would compete with methionine for chloramine-T. Dimethyl sulphoxide (DMSO; V), a stable, relatively non-volatile liquid, miscible with water, was selected for trial. This is the intermediate in the oxidation of dimethyl sulphide (IV) to dimethyl sulphone (VI)



When 25 μl./μg of SHG was incorporated in the reaction mixture (without iodide), the curve showing reduction of biological activity was shifted to the right; with 50 μl./μg of SHG, full biological activity was observed at all concentrations of chloramine-T tested (Fig. 2). The inclusion of DMSO at the latter concentration in iodinations of SHG with ^{125}I yielded a radioiodinated peptide with full biological activity and without reduction of specific activity.

Antiserum to SHG (residues 2–17) was produced in rabbits by immunization with the peptide conjugated to bovine serum albumin by carbodiimide condensation[5]. ^{125}I-SHG prepared in the presence and absence of DMSO was tested for its ability to react with this antiserum, using a double antibody technique. The binding of the biologically active ^{125}I-SHG was consistently greater than that obtained with ^{125}I-SHG prepared in the absence of DMSO. In a typical experiment, the percentage of binding was increased from 34 to 58. Gastrin antibodies were raised against the native hormone so it is not surprising that the antibody binding of biologically active ^{125}I-SHG was significantly greater than that of the inactive ^{125}I-SHG. This observation supports McGuigan's suggestion[6] that one of the principal antigenic determinant sites of the gastrin molecule is the C-terminal tetrapeptide sequence.

The procedure we have developed for the protection of gastrin during radioiodination may be applicable to other polypeptide hormones, and may have significance for both radioimmunoassay and *in vivo* tracer studies.

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Anthropological Significance of Gamma Globulin (Gm and Inv) Antigens in Bougainville Island, Melanesia

The known distribution patterns of Gm and Inv antigens reflect remarkably the accepted historical relationships of different human groups[1]. In this communication we report new developments which modify the previously recognized pattern of Gm and Inv variation in Melanesian populations and its implications for Melanesian prehistory.

The linguistic distinction contrasting the Melanesian (hereafter MN) with the residual category of non-Austronesian languages (hereafter NAN) forms the most pervasive dichotomy throughout Melanesia. Most of the perhaps 500 NAN languages are located in the interior of New Guinea, although some are found on islands near by. While it has proved difficult to establish clear relations for languages within the NAN group, the sketchy archaeological record from Melanesia suggests that the distributions of some artefacts roughly agree with the total extent of NAN languages. At least we can assume that human populations have occupied New Guinea for the past 20,000 years[2].

The later advent of MN languages in the area has been equated by some workers with a widespread pottery tradition, the so-called Lapita style, which is dated at about 750 BC in the Philippines and about 500 BC in Tonga. These languages have been described as being quite diverse while having an underlying structural uniformity. There are three theories concerning their origins: (1) (emphasizing the similarities) they were brought into Melanesia from Indonesia by groups belonging to the same stock and diversified in place; (2) (emphasizing the diversities) they were brought in by groups from different parts of Indonesia, and pidginized varieties of these languages developed at the sites of the new habitation, so that the grammar represents a simplified Indonesian, while the vocabulary consists of a core of simplified Indonesian and a large number of less basic words derived from indigenous (NAN) languages; (3) (attributing all the diversity to a long period of development in isolation) they were a local development, perhaps representing the earliest branching of the Austronesian stock[3].

Giles *et al.*[4] were able to distinguish clearly between MN speakers in the Markham Valley, New Guinea, and NAN speakers there and on nearby Bougainville Island by the almost total lack of Gm (2) individuals among the

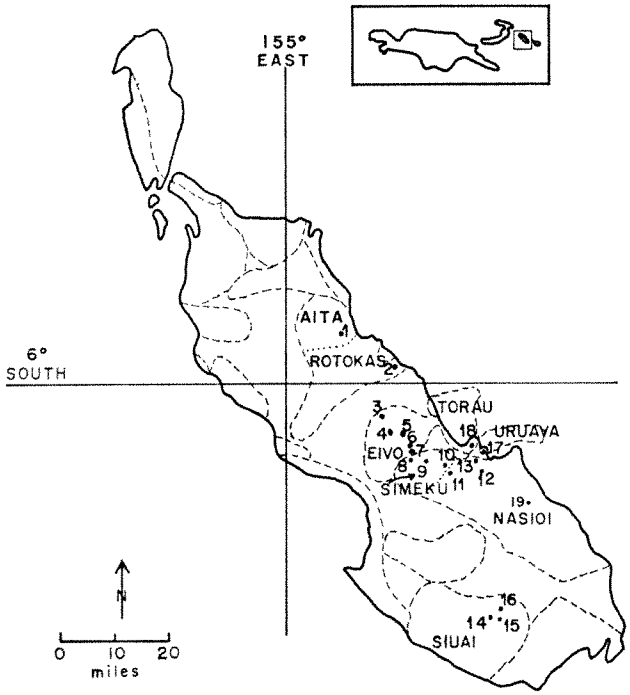


Fig. 1. Geographical location of the sampled villages on Bougainville Island. - - - -, Language boundaries; · · · ·, relevant dialect boundaries.

MN speakers, as opposed to their presence in low to medium frequencies among the NAN groups. These MN groups also had consistently lower frequencies of Gm (1) and higher frequencies of Gm (5) than the NAN groups. It seemed, too, that the MN speakers of the Markham paralleled south-east Asians in Gm phenogroup frequencies more closely than did the NAN peoples. Giles *et al.* concluded: "The Gm frequencies imply the existence of two populations with origins separate in time or space. One of these populations, the MN speakers, appears closely related to south-east Asians and not an autochthonous Melanesian differentiation"[4]. This amounts to a refutation of the third hypothesis of the origin of MN languages.

Steinberg[5] later retested 836 of the Gm (1, 5) positive samples from the Markham Valley for Gm (3), Gm (13), and Gm (14) and found that all were Gm (13) and Gm (14) positive, but that only 38·5 per cent of these were Gm (3) positive in the NAN group, while 53·2 per cent were positive in the MN group. The data indicate the presence of a $Gm^{1,5,13,14}$ phenogroup which had not been postulated for any of the few other series from Asia or Oceania. In terms of the *Gm* phenogroup frequencies, the MN speakers in the Markham could possibly be interpreted as hybrids of indigenous NAN New Guinea groups having $Gm^{1,5,13,14}$ and of some outside stock lacking that phenogroup.

Here we examine the possibility that this linguistically related biological division on New Guinea holds for NAN and MN groups on Bougainville Island as well[6]. There are seventeen mutually unintelligible languages indigenous to the island[7], of which six languages and two dialects were represented in this study (Figs. 1 and 2). The eighteen villages covered in the survey, and the additional group previously tested by Giles *et al.*[4], are all located within a very small area, so that environmental differences are minimal. An effort was made to collect blood from all residents over 2½ years old. Serum samples were typed for Gm and Inv factors by established methods[8] in the same laboratory which participated in the Markham Valley study, and tests for the Gm (3), Gm (13), and Gm (14) antigens were added to the earlier results. (All samples were tested for Gm (1) (Wils./251); Gm (2) (Tay./Ham.); Gm (3) (E.W./Jac.), (Joh./3196); Gm (5) (Dra./31369); Gm (6) (Deb./War.); Gm (13) (Tho./21369) Gm (14) (Bur./21369); and Inv (1) (Mak./Roe.). The entries in the brackets show the reagents used; the antibody to immunoglobin is shown first, and the antibody to D second.)

Table 1. Gm PHENOTYPE AND PHENOGROUP FREQUENCIES BY VILLAGE

No. and village	Sample size	Gm phenotype frequencies 1	1,2	1,2,3,5, 13,14	1,3,5, 13,14	*Gm* phenogroup frequencies Gm^{1}	$Gm^{1,2}$	$Gm^{1,3,5,13,14}$
Section A. NAN speakers								
1. Nupatoro	99	1	2	21	75	0·076	0·121	0·803
2. Okowopaia	94	18	0	0	76	0·438	0·000	0·562
3. Kopani	190	0	1	2	187	0·046	0·008	0·946
4. Kopikiri	70	1	0	3	66	0·108	0·021	0·871
5. Nasiwoiwa	128	1	0	3	124	0·082	0·012	0·906
6. Atamo	124	0	1	6	117	0·000	0·032	0·968
7. Uruto	90	3	0	1	86	0·179	0·006	0·815
8. Karnavitu	133	4	0	5	124	0·163	0·019	0·818
9. Boira	93	0	0	0	93	0·000	0·000	1·000
10. Korpei	181	8	0	0	173	0·210	0·000	0·790
11. Sieronji	62	0	0	0	62	0·000	0·000	1·000
12. Pomaua	116	4	0	2	110	0·181	0·009	0·811
13. Bairima	59	0	2	4	53	0·000	0·068	0·932
14. Turungum	88	0	2	13	73	0·000	0·097	0·903
15. Moronei	112	0	0	13	99	0·000	0·058	0·942
16. Old Siuai	26	1	0	3	22	0·164	0·058	0·778
19. Rumba*	148	3	0	17	128	0·109	0·057	0·832
Section B. MN speakers								
17. Arawa	108	1	0	6	101	0·082	0·028	0·890
18. Rorovana	243	1	0	14	228	0·050	0·029	0·920

All samples were tested for Gm (1), Gm (2), Gm (3), Gm (5), Gm (6), Gm (13) and Gm (14). Only the positive reactions are recorded.
* Sampled by Giles *et al.*[4].

The Gm results are extremely heterogeneous (Table 1) There are, however, no consistent distinctions in *Gm* phenogroups or phenogroup frequencies between the MN and NAN villages. Note that while 351 MN speakers were typed, these were taken from only two villages which may be distinct from MN speakers in northern Bougainville, for the founders of group 18 emigrated from a southern off-shore island only 100 years ago and the founders of group 17 sometime before that.

All typed Bougainvillians who were Gm (1, 5, 13, 14 were also Gm (3) positive, that is, the $Gm^{1,5,13,14}$ phenogroup is absent. This same constellation holds for the other circum-Pacific groups that have been surveyed thus far for these antigens[5], with the aforementioned exception of the Markham Valley New Guineans.

Bougainvillian Inv frequencies (Table 2) from a clear step cline from north to south, the first such cline so far noted. The most pronounced shift in frequency occurs among the Simeku (8–10), the transitional population between the northern and southern NAN groups. The Inv frequency for village 1 is higher than any previously reported, being approached only by a few South American Indian tribes[9]. The lowest frequencies from the current series belong to the 2 MN villages which, because of their recent migration from the south, may be viewed as a simple continuation of the cline. In very sharp contrast New Guineans from the Markham Valley[4] uniformly have among the lowest Inv^{1} frequencies of any known group

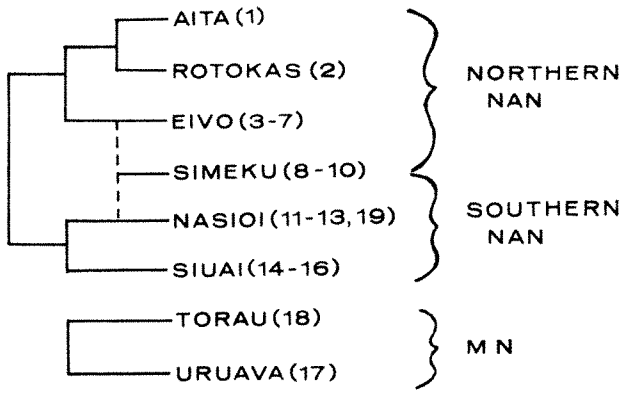


Fig. 2. The relationships of the language groups sampled from Bougainville in terms of shared cognate percentages reported in ref. 7.

Table 2. Inv OBSERVED PHENOTYPES AND PHENOGROUP FREQUENCIES BY VILLAGE

No.	Sample size	Inv (1) observed phenotype	*Inv*[1] phenogroup frequency
	Section A. NAN speakers		
1	99	96	0·826
2	94	87	0·727
3	190	165	0·637
4	70	64	0·707
5	128	116	0·694
6	124	109	0·652
7	90	71	0·541
8	133	97	0·480
9	84	55	0·412
10	181	119	0·415
11	62	42	0·432
12	116	85	0·183
13	59	43	0·479
14	88	63	0·467
15	112	69	0·380
16	25	18	0·529
19*	148	97	0·413
	Section B. MN speakers		
17	108	59	0·326
18	243	149	0·378

All samples were tested for Inv (1). Only positive reactions are recorded.
* Samples by Giles *et al.*[4].

The emerging pattern of Gm and Inv distributions reflects the extraordinary diversity of the NAN speaking groups and the primary importance of the local geographic region as the unit of isolation. The biological separation of the two linguistic stocks within the Markham Valley remains clear, but its extension to Bougainville NAN and MN speakers is not, nor is the affinity of MN speakers with peoples in south-east Asia. A linguistically related division in Inv frequencies does exist within Bougainville, but this concerns the two divisions of NAN which presumably diversified in place.

These data, then, cannot be said to rule out any of the three hypotheses concerning the origins of the MN languages. If linguistic affinities do reflect population movements in this area, we believe that a model which allows for significant interbreeding of neighbouring MN and NAN groups over time and which stresses isolation by distance is required to explain the data. Such interbreeding over short distances has obliterated most of the clear biological distinctions which may once have existed between the NAN and MN speakers within Melanesia, but such a process evidently has not been completed in New Guinea.

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Membrane-complement Fixation Test for Hepatitis Associated Antigen

We describe here an analytical test for hepatitis associated antigen (HAA)[1], originally called the Australia antigen[2] or S.H. antigen[3], that combines the advantages of the Ouchterlony technique[3] in its simplicity, the complement fixation technique[4] in its sensitivity, and the electrophoretic technique[5] in its rapidity and accuracy.

Antigen was detected in seventy-eight of one hundred coded sera obtained from patients with acute viral hepatitis within 1 month after onset of the disease. The test was based on the technique previously described[5] in which immunoprecipitation was promoted by the electrophoretic movement of antigen through a preformed microporous cellulose acetate membrane saturated with antibody, and which detected 38 per cent HAA positive reactions in unconcentrated sera. To increase the sensitivity of the original test, it was reasoned that the standard complement fixation procedure was more sensitive as it detected soluble antigen-antibody complexes that washed out of the cellulose acetate membrane in the electrophoretic technique and out of the agar in the immunodiffusion technique. After the electrophoretic movement of HAA into the antibody saturated cellulose acetate membrane, complement was therefore added to the unwashed reaction medium. The complement probably fixed to the soluble and insoluble immune complexes "locking" them in the membrane. An antibody made to the serum of the species from which the complement was derived was added to the membrane after a brief washing of the membrane to elute unreacted proteins. After incubation and washing, the resulting immunoprecipitates were stained. The entire procedure took $2\frac{3}{4}$ h with constant current supplies and $1\frac{1}{2}$ h with the application of pulsed constant power (Ortec).

A microporous cellulose acetate membrane (95 mm × 75 mm × 0·01 mm thick, Millipore Filter Corporation or Gelman Instrument Company) was saturated with 1 ml. of a dilution of anti-HAA antiserum (1 : 16 was used in these studies) obtained by plasmaphoresis with informed consent from a haemophilic patient. The membrane was sandwiched between a top lucite template and a 'Parafilm' covered neoprene base bolted together by a torque wrench preset at 5×10^6 dyne cm.

Unknown serum samples (2 μl. each) were applied to conical wells found in the template open to the membrane below. Both HAA positive and HAA negative sera were included as controls in each test.

Barbital buffer, 0·05 M, *p*H 8·2 was used for dilution of the antibody and for soaking sponge wicks connecting the membrane to the electrophoresis chamber. 6 mA of constant current (approximately 250 V) were applied for 2 h; 18 to 20 mA of pulsed constant power gave comparable results in 30 min.

At the end of the electrophoresis, the membrane was placed directly in a plastic container and gently overlaid with a 1 : 400 dilution of guinea-pig complement[6] and was incubated at room temperatures for 15 min with intermittent shaking. The membrane was then washed with buffered saline for 15 min to remove unreacted human serum proteins and unreacted guinea-pig complement. The washed membrane was placed in a 1 : 4 dilution of rabbit anti guinea-pig serum for 15 min at room temperatures; it was then washed and the precipitates were stained ('Ponceau S' dye fixative followed by acetic acid clearance and 'Nigrosin' counterstain is routinely used in our laboratory).

The presence of rocket-shaped immunoprecipitates showed positive HAA antigenaemia. The antigen was measured using the immunoprecipitate planar area[7]. Fig. 1 shows the results of testing five sera previously HAA negative by either the Ouchterlony or electrophoretic techniques, and positive and negative control sera. The control sera were placed at 4° C for 1 week before testing

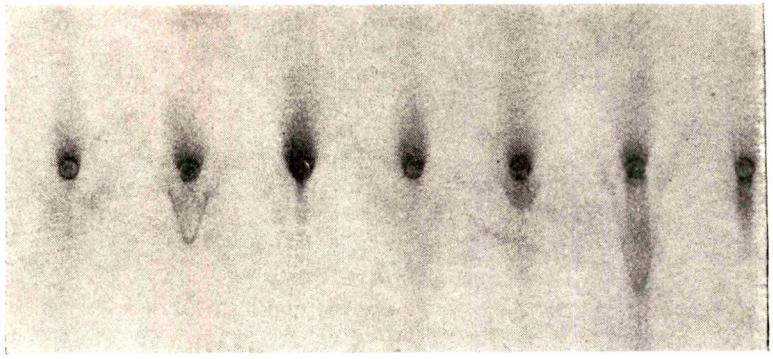


Fig. 1. Electrophoretic immunoprecipitates (or negative reactions) (reading from left to right), associated with acute viral hepatitis (samples 1, 2, 3, 4, 5), HAA positive control (sample 6), HAA negative control (sample 7). Positive samples are those with rocket-shaped immunoprecipitates pointed downwards towards the anode (samples 1, 2, 4, 5, 6).

to allow prote in-protein interactions making them more comparable with patient sera subjected to storage or adverse shipping conditions. Positive HAA reactions were now found in four of the five patient sera. Rocket-shaped immunoprecipitates were clearly distinguishable from the aggregated proteins found in the controls (which are not usually seen in fresh or frozen sera).

Thus the addition of complement reveals more of the soluble and insoluble immunocomplexes found in membrane media.

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Cell Mediated Immunity induced *in Vitro*

A CELLULAR immune response to antigenic stimulation is frequently accompanied by a humoral immune response. This conjugal behaviour has complicated an understanding of the specific steps of a cellular immune response. Humoral immunity is easily measured apart from cellular immunity. Specific measurement of cellular immunity, however, is complicated by the ability of some cells in lymphoid organs to produce both complement[1] and antibody[2]. Brunner *et al.*[3] have introduced a ^{51}Cr release technique for measuring cellular immunity, and our modifications[4] have increased the sensitivity of the procedure and the certainty that cellular immunity is being detected. We have now used this method to detect the *in vitro* induction of cellular immunity.

Normal mouse spleen cells (2×10^7/dish were sensitized by incubation for five day with allogeneic lymphoid tumour cell (1×10^4/dish) according to the procedure o Mishell and Dutton[5]. Cellular immun activity was measured apart from humora immunity by incubating replicate culture of the sensitized cells (5×10^6/dish) for 3 h with ^{51}Cr-labelled target cells (5×10^4/dish in the presence of carrageenan (200 μg/ml.) Between sensitization and measurement o cellular immunity, spleen cells were washe once by centrifugation and suspended i fresh media. Details of the cellular immun ity assay are described elsewhere[4].

The cellular immune response elicited b *in vitro* immunization was specific for th sensitizing antigen (Table 1*a*). Non-sensitize spleen cells were not reactive. Sensitize spleen cells did not react with target cell unrelated to the sensitizing cells. Sensitized spleen cell reacted not only with the sensitizing tumour cells bu also with normal lymphoid cells syngeneic with th sensitizing cells ((Table 1*b*). The immune cells therefor seem to recognize and react against histocompatibilit antigens. The effectiveness of the *in vitro* immunization i apparent from the similarity of the *in vitro* and *in viv* induced responses (Table 1*c*). If cellular immune activit were expressed as a function of viable cells instead c total cells (as is done here), the comparison would be eve closer. The viability (dye exclusion) of freshly explante spleen cells was consistently higher than 90 per cent spleen cells cultured *in vitro* for five days, however, ha an average viability of 67 per cent.

Table 1. CHARACTERISTICS OF CELL MEDIATED RESPONSE INDUCED *in vit*

	Immunogen	BALB/c spleen cells Target cells	Per cent lysis ± s.e. Exp. 1	Exp. 2
(*a*) Specificity of cytotoxicity				
	None	EL-4	−0·86 ± 0·41*	Not done
	EL-4†	LSTRA‡	0·22 ± 0·48	0·20 ± 0·36
	EL-4	P388§	0·33 ± 0·23	0·10 ± 0·39
	LSTRA	EL-4	−0·86 ± 0·48	0·35 ± 0·50
	EL-4	EL-4	7·52 ± 0·80	11·58 ± 0·70
(*b*) Reactivity with normal allogeneic cells				
	EL-4	EL-4	7·52 ± 0·80	11·58 ± 0·70
	EL-4	C57Bl/6 lymph node cells	7·52 ± 1·21	4·96 ± 1·37
(*c*) Comparison with immunity of *in vivo*				
In vitro	None	EL-4	−0·86 ± 0·41*	Not done
	EL-4	EL-4	7·52 ± 0·80	11·58 ± 0·70
In vivo	None	EL-4	−0·06 ± 0·46*	0·17 ± 0·45
	EL-4	EL-4	11·18 ± 0·53	14·02 ± 1·14

* Expressed relative to ^{51}Cr released from target cells alone. Oth "percentage lysis" values are relative to ^{51}Cr released in the presence normal spleen cells.
† Syngeneic with C57Bl/6 ($H2^b$).
‡ Syngeneic with BALB/c ($H2^d$).
§ Syngeneic with DBA/2 ($H2^d$).

The immune response measured here seems to be ce mediated. A complement C′ inhibitor, carrageenan, wa included in the assay media at a concentration whic blocks C′ dependent antibody activity[4,6]. Also, r soluble toxins including C′ dependent antibody could b detected in cell-free media collected after both the 5 da sensitization period and the 3 h assay period (Table 1 These findings are in agreement with data obtained usi *in vivo* sensitized cells[4].

Humoral immune responses have been induced *in vit* against a variety of antigens. In some reports[5,7–10], the responses *in vitro* have been equivalent to those found *vivo* in terms of the appearance after exposure to antig and the height of the immune reaction. Successful *vitro* immunization has facilitated investigation of th afferent limb of the humoral immune response by the u of cell fractionation[11–16], selective irradiation[17] ar

Table 2. TESTS FOR TOXINS RELEASED FROM SENSITIZED BALB/c SPLEEN CELLS

Assay media source. Cell-free supernatant of:	C'*	Per cent lysis ± s.e.†
Spleen cells + EL-4‡ (5 days)	+	0·71 ± 0·52
	0	0·33 ± 0·24
Spleen cells + EL-4‡ (5 days), washed, and cells incubated 3 h 0 EL-4‡	+	0·63 ± 0·31
	0	0·40 ± 0·33
Spleen cells + EL-4‡ (5 days), washed, and cells incubated 3 h + EL-4‡	+	0·32 ± 0·43
	0	Not done

* Complement (fresh frozen guinea-pig serum).

† Expressed relative to ^{51}Cr released in presence of supernatant from non-sensitized spleen cells.

‡ Non-^{51}Cr-labelled EL-4.

addition of specific antibody[18] or tolerance inducing antigen[10]. Antimetabolites have been used in these systems to study the dependence of humoral antibody formation on cell division and biosynthesis of RNA and protein[19,20].

Similar knowledge about the details of cellular immune responses is lacking largely because appropriate systems were not available for investigation. Systems that will contribute to such studies have been reported[21,22] in which *in vitro* sensitized rat lymphocytes were capable of destroying mouse embryo target cells in tissue culture. Simmons and Manson[23] have also been able to sensitize mouse spleen cells *in vitro* with an allogeneic membrane preparation. The sensitized cells were able to interfere with the immune function of cells syngeneic with the sensitizing antigen. Recently Häyry and Defendi[24] have reported *in vitro* induction of mouse cellular immunity to allografts in which both the sensitization and effector phases were successfully monitored.

In this study, the *in vitro* induction of immunity against allogeneic histocompatibility antigens has been demonstrated by a rapid, quantitative system. The immune response is specifically cellular and the intensity of this response was equivalent to that found after *in vivo* sensitization.

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Mixed Lymphocyte Cultures and HL-A Identity in Unrelated Subjects

INVESTIGATIONS using highly selected lymphocytotoxic and complement-fixing platelet antibodies have made it possible to define a considerable number of antigens belonging to the HL-A system. These antisera are determined by genetic information belonging to a still unknown number of closely linked loci, subloci or mutational sites. Most of the known HL-A antigens seem to belong to two series, 'LA' and '4', each of which contains a number of mutually exclusive genes and corresponding antigens. An ever increasing number of antigens are being identified[1–5]. The various genes and corresponding antigens, which can be determined, are given in Table 1.

Table 1. FREQUENCIES OF THE '4' AND 'LA' GENES

Determinant	Gene frequencies Danes	Norwegians
HL-A1	0·1557	0·1334
HL-A2	0·3211	0·3780
Ba*	0·0427	0·0384
HL-A3	0·1497	0·1742
HL-A11	0·0524	0·0537
HL-A9	0·1091	0·1188
HL-A10	0 0423	0·0244
Li	0·1059	0·0724
"0"	0·0211	0·0067
HL-A5	0·0392	0·271
R*	0·0725	0·0804
SL	0·0586	0·0331
HL-A7	0·1466	0·1448
AA	0·0145	0·0055
HL-A8	0·1174	0·1058
HL-A12	0·1253	0·1444
HL-A13	0·0229	0·0122
BB	0·1180	0·1450
FJH	0·0500	0·0608
LND	0·0948	0·1043
"0"	0·1402	0·1366

A maximum of four different HL-A genotypes is possible in siblings, provided that crossing-over within the HL-A locus has not occurred. It is most likely therefore that siblings are truly identical with respect to HL-A, if they give the same typing results, even when all their HL-A antigens cannot be demonstrated. It has been shown unequivocally that cells from HL-A identical siblings are not stimulated in mixed lymphocyte cultures, by contrast with mixed lymphocyte cultures for donors not identical with respect to HL-A, where stimulation always occurs[6]. This implies that all antigens of importance for stimulation in mixed lymphocyte cultures must be determined by genetic information identical to or closely linked to that of the 'LA' and '4' subloci. If the HL-A system consisted only of the two subloci mentioned, any individual should have a maximum of four HL-A antigens, and the absence of stimulation in mixed lymphocyte cultures should be evident for unrelated cell donors having the same four antigens exactly as for HL-A identical siblings.

Mixed lymphocyte cultures for four pairs of unrelated cell donors identical with respect to four well defined HL-A antigens were tested, and we here present the results. A skin graft was, moreover, performed between members of one of the pairs. Pairs of individuals carrying identical HL-A antigens were sought using a previously described data retrieval system (KH: not marginal punch cards)[1] and looking only for subjects having four well-defined HL-A antigens (and genes): two 'LA' and two '4' antigens. This excluded the possibility of uncertainty arising from as yet undetectable antigens belonging to these two subloci. The control subjects were selected without their HL-A type being known but they were typed retrospectively.

HL-A typing was performed as previously described using cytotoxicity and complement fixation with lymphocytes and platelets as antigens, respectively[7,8]. Lymphocytes from the test subjects were typed both in Copenhagen and in Århus. In addition to lymphocytotoxic

sera of well defined specificity, a varying number of multispecific sera were also used. In some cases, typing of the families of the test subjects allowed us to deduce the HL-A genotypes.

Mixed lymphocyte cultures were prepared as previously described[9]. Cultures contained 1×10^6 responding lymphocytes and 1×10^6 stimulating lymphocytes, rendered incapable of participating in the response by pretreatment with mitomycin C (Sigma). Culture volumes were 2 ml. and the medium consisted of 'TC 199' (Glaxo) with 30 per cent 'Intradex' (Glaxo), supplemented with 30 per cent serum pool from all the cell donors included in the experiment. All cultures were harvested in duplicate. In one experiment, cultures were harvested at 24 h intervals from day 2 to day 6, whereas the others were harvested 90 h after the preparation of the cultures.

After incubation for 4 h with 0·2 μCi ^{14}C-thymidine (specific activity 56·2 μCi/mmole; Radiochemical Centre, Amersham), cells were collected on glass fibre filters and prepared for liquid scintillation counting as described previously[10]. All samples were counted in a Packard 'Tri-Carb' liquid scintillation counter, and the results were expressed as background c.p.m. per 1×10^6 responding lymphocytes of the initial cultures.

A full thickness skin graft was performed as described by Rapaport[11].

Table 2. SEX, AGE, BLOOD GROUPS AND HL-A TYPES

Expt. No.	Subject	Sex	Age	Blood group	HL-A type
1	T. A.	M	34	A	2, 5/3, 7
	A. L.	F	33	O	2, 3, 5, 7
	K. K. (control)	F	22	B	2, 3, 7, FJH
	A. A.	F	41	O	1, 8/3, 7
2	F. J.	M	47	O	1, 3, 7, 8
	L. K. (control)	F	37	B	1, 8/1, 4A2*
	R. L.	F	56	O	3, 7/2, R*
3	M. C.	M	37	A	3, 7/2, R*
	R. K. (control)	M	27	A	2, Li, 5, BB
	R. E.	F	26	O	2, 7, 9, BB
4	R. J.	F	28	O	2, 7, 9, BB
	K. O. (control)	M	34	AB	2, 3, 12, 407*

Four experiments were performed. The results of the HL-A and blood grouping are given in Table 2. It can be seen that the four pairs each carried identical antigens from the 'LA' and '4' series, whereas the control subjects were always different from the test subjects. It may be added that cells from each of the four pairs exhibited identical reaction patterns when tested with a varying number of multispecific antibodies.

Results of the mixed lymphocyte culture tests are given in Table 3. A and B refer to the two cell donors carrying the same four well defined HL-A antigens; K refers to unrelated cell donors; A + Bm refer to a mixture of A leucocytes as responding cells and mitomycin-treated lymphocytes from B as stimulating cells.

Fig. 1 illustrates an experiment (No. 1) in which the incorporation of thymidine was followed during the first 5–6 days of culture. Incorporation still increased after day 4, but we harvested routinely after 90 h because it has been shown previously that the most reliable quantitation is obtained by measuring the thymidine uptake early in the culture period[12]. In all cases mixtures of unrelated cell donors with identical 'LA' and '4' types caused significantly higher stimulation than the unstimulated controls.

Fig. 2 shows the ratio between mixed cultures and controls in these experiments as compared with four experiments according to the same scheme but using HL-A identical siblings as cell donors. Maximum stimulation in mixtures from HL-A identical siblings was only twice the control, whereas the stimulation in mixtures from 'LA' and '4' identical cell donors was 2·2–13 times the controls. It is evident from Table 2 that in all experiments except No. 4, the stimulation obtained between the test subjects was lower than in the mixtures with mitomycin-treated cells from the control subjects; the probability that this arises by chance is $P = 0{\cdot}035$; the lowest stimulation was in experiment No. 2, mixture Am + B. Skin grafting was performed from A to B and the skin survived for 18 days.

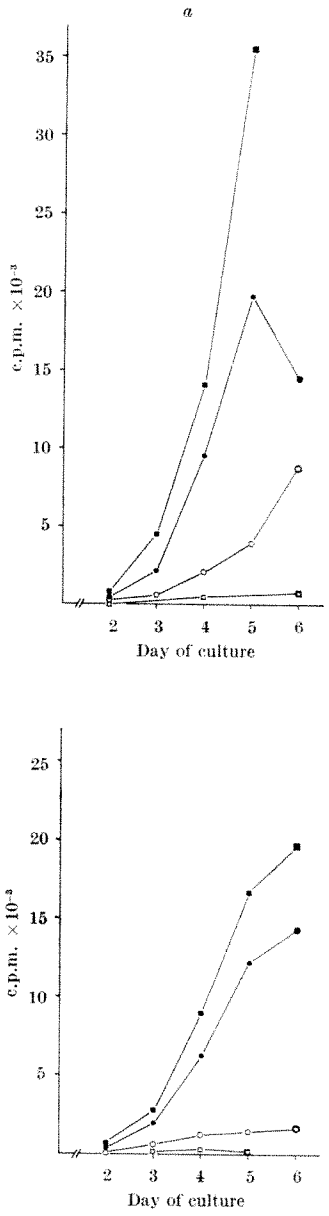


Fig. 1*a* and *b*. Experiment 1. A, L and K unrelated. A and L typing identical; HL-A2, 3, 5 and 7. *a*: ○, A + Lm; ●, A + Km; ■, K + Lm; □, A + Am. *b*: ○, L + Am; ●, L + Km; ■, K + Am; □, L + Lm.

Because each of the four pairs carried two different antigens from each of the series, 'LA' and '4', they could not possess hitherto unknown alleles from these two series. In other words, they carried pair-wise only the same antigens from the 'LA' and '4' series. The experiments showed clearly that the leucocytes from these pairs were able to stimulate each other in mixed lymphocyte culture tests although the stimulation was sometimes very low (Table 2, Fig. 1). This observation may be explained in three different ways: first, the stimulation may be attributed to the presence within the pairs of different but as yet unknown HL-A antigens controlled by genes on one or more loci or sites linked to the 'LA' and '4' subloci. Second, some of the antigens recognized in these subjects may be heterogeneous; that is, they may not be completely identical in different individuals. Third, possible genotypic differences in these phenotypically

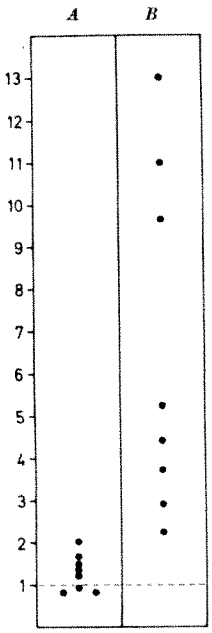


Fig. 2. Ratio between mixed cultures and controls. *A*, HL-A identical siblings; *B*, unrelated persons typing identical for four HL-A antigens.

identical pairs may be responsible for the stimulation. The stimulation observed in experiment 3, however, is incompatible with the third possibility, for RL and MC were genotypically identical. The second explanation implies that the antibodies used for typing are not monospecific, which does not seem likely for experiments 1 and 2 which only involved some of the most well defined HL-A antigens. Nevertheless, it seems worth noting that the demonstration of cross-reactivity between some HL-A antigens[13] has made it very difficult to define monospecific antibodies. We feel the first explanation—the existence of one or more mutational sites in addition to 'LA' and '4'—is the most likely. It is supported by the recent observation of an antibody reacting with an antigen which is apparently controlled by a gene not belonging to the 'LA' and '4' series[14].

Thus we have not succeeded, using 'LA' and '4' typing, in selecting unrelated pairs which did not stimulate each other in mixed lymphocyte culture. But Table 2 seems to demonstrate that the degree of selection was sufficient in all but one case to ensure a stimulation in mixed lymphocyte culture inferior to that elicited by cells from a randomly selected, unrelated individual. Fig. 1 illustrates that the degree of compatibility obtained between the test subjects in this experiment was much better than with the control subject.

Table 3. RESULTS OF THE MIXED LYMPHOCYTE CULTURE EXPERIMENTS

Expt. No.	1	2	3	4
A:	TA	AA	RL	RE
B:	AL	FJ	MC	RJ
K:	KK	LK	PK	KO
A + Bm	2,071	2,549	1,767	2,907
B + Am	1,206	924	715	2,557
A + Km	9,598	3,419	2,537	1,404
B + Km	6,280	2,312	4,162	3,714
K + Am	9,021	1,618	2,913	2,148
K + Bm	14,133	2,739	3,689	3,630
A + Am	473	266	331	219
B + Bm	325	411	246	236
K + Km	546	612	123	216
Am + Km	124	119	102	193
Bm + Km	182	82	67	63

In experiment 2, the AA cells only slightly stimulated the FJ cells, and this good compatibility was confirmed by the survival of an AA skin graft on FJ for 18 days. By comparison, skin grafts exchanged between ABO-compatible, unrelated individuals survive for $12{\cdot}1 \pm 1{\cdot}55$ (s.d.) and for $20{\cdot}0 \pm 4{\cdot}23$ (s.d.) days between HL-A identical siblings[15]. In experiment 4, however, the control cells stimulated the RE leucocytes less than the RJ cells, in spite of the fact that the control cells were incompatible for HL-A3, 12 and a new antigen from the '4' series, 407*, whereas no incompatibility could be demonstrated for the RJ cells. When repeated, this experiment revealed a higher stimulation in the mixtures A + Km and B + Km than in those of A and Bm and B + Am, respectively. The earlier results may have been subject to technical errors, but could also be explained by the presence in RJ cells of as yet unrecognized antigens capable of causing more stimulation than the HL-A3, 12 and 407* antigens. If this is so, and if the degree of stimulation in mixed lymphocyte culture parallels the strength of the incompatible HL-A antigens, these unknown antigens may be strong transplantation antigens. This is, however, supported neither by the rather close correlation between the match grade for 'LA' and '4' antigens and the results of kidney transplantations[2], nor by the observation that it seems to be difficult to produce cytotoxic antibodies against HL-A antigens not belonging to the 'LA' and '4' series. J. J. van Rood and V. P. Eijsvoogel have recently published the results of similar investigations[16].

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In Vitro Sensitization of Mouse Peritoneal Mast Cells with Reaginic Antibody

EXPERIMENTAL models for the study of anaphylaxis usually make use either of the whole animal or of isolated organs. Although such complex systems have many advantages in that they reproduce the clinical conditions, it is obvious that their complexity makes it difficult to analyse separately the various phases of the anaphylactic reaction. The use of isolated cell systems would be better suited to study, for instance, the interaction between cells, antibody and antigen.

Techniques using isolated cells have been recently developed for human leucocytes[1], rat[2] and mouse[3,4] peritoneal mast cells. Two classes of anaphylactic antibodies in the mouse have been described. One of them, designated γ_1, is a non-complement fixing 7S immunoglobulin[5]; the other is a thermolabile, reaginic type of antibody[6-9], probably similar to human IgE. Both antibodies act on the mast cell, but their mechanisms of action are different. Thus the reagin fixes firmly to the mast cells and cannot be washed out[7-9], while γ_1 does not fix to the cells and induces cell damage through the formation of a soluble complex with antigen[3,10,11].

In vitro passive sensitization with γ_1 antibody is readily achieved[3,10,11], but passive sensitization with reaginic antibody has been obtained only *in vivo*[8]. The possibility of performing *in vitro* fixation of reaginic antibody on the peritoneal mast cells of mouse is of the greatest interest for the study of the mechanism of sensitization. Using purified antibody preparations we have now succeeded in passively sensitizing isolated mast cells with reaginic antibody.

Antibody was produced in a strain of Swiss mice, random-bred for many years in a closed colony at the Institute Oswaldo Cruz, Rio de Janeiro, Brazil, and inbred in our laboratory. In order to obtain sizable quantities of antibody, ascitic fluid was induced[12] by two peritoneal injections of 0·3 ml. complete Freund adjuvant emulsified in saline a fortnight apart. After about 3 weeks, when the animals had abundant ascitic fluid, immunization was carried out by injecting subcutaneously 500 μg of highly conjugated dinitrophenylated (DNP) ovalbumin mixed with $Al(OH)_3$ gel[7-9], followed 2 to 3 weeks after by two weekly injections of DNP ovalbumin emulsified in complete Freund adjuvant. Ascitic fluid was collected several times from the mice after these treatments. The pooled ascitic fluid contained about 1 mg/ml. of total precipitating anti-DNP antibody. Specific purification of anti-DNP antibody was made in the cold, following essentially the method of Farah *et al.*[13], with minor modifications[14]. The antigen used for precipitation was DNP bovine serum albumin (DNP-BSA) and the monovalent hapten was DNP-OH. From 77 mg of antibody in the ascitic fluid, 34 mg (44 per cent) of purified antibody was recovered. The concentrations were calculated measuring the optical density at 2800 Å, assuming that o.d. per μg of protein is 0·00143. Immunoelectrophoretic analysis indicated the presence of γ_1 and γ_2 (ref. 5).

Anaphylactic activity of the purified antibody could be demonstrated by passive cutaneous anaphylaxis (PCA). When 10 μl. of serial dilutions of purified antibody was injected into the skin or ear of several recipients[9,15], positive PCA reactions were obtained at concentrations of 2·5 μg/ml. or 80 μg/ml., when challenge was made after 2 h on 10 days respectively. The PCA reaction after 10 days was negative if the antibody was previously heated at 56° C. These results indicated that both γ_1 and the reaginic antibodies were present in the preparation.

In vitro experiments were performed as described[7-10] with peritoneal cells obtained from normal Swiss mice, about 2 months old. The cells were washed three times in Barron-modified[16] Ringer solution, by centrifugation, and finally resuspended so that a concentration of 200,000 to 400,000 mast cells were present per ml. Sensitization with purified antibody was carried out at various temperatures and for different times, as indicated. The total volume of the antibody-cell mixtures was about 0·4 ml. The antibody preparations were dialysed against Ringer solution before they were added to the cells. After sensitization the cells were washed five times with 1 ml. Ringer medium, resuspended in 0·2 to 0·4 ml. of the same medium and incubated at 37° C for 15 min. 1 μg of specific antigen (DNP-BSA) in a volume of 10 μl. was then added, and incubation at 37° C was continued for a further 5 or 10 min. The degranulated and intact mast cells were counted after staining with toluidine blue. Histamine release was measured by bioassay, after centrifugation, in both supernatants and the sedimented cells[9].

Table 1. *In vitro* SENSITIZATION OF MAST CELLS WITH REAGINIC ANTIBOD

Experiment	Conditions of sensitization: Antibody concentration (μg/ml.)	Time	Temperature (°C)	Per cent of degranulated cells
1	625	19 h	4	63
	312	19 h	4	29
2	625	17 h	4	70
3	625	17 h	4	34
	625	10 min	4	2
4	625	1 h	20	52
	625	30 s	20	4
	625 heated*	1 h	20	2

After sensitization, and before challenge with the specific antigen (1 μ DNP-BSA), the cells were washed five times.

* Antibody previously heated at 56° C for 5 h.

The results obtained in various conditions and in different experiments with cell suspensions from different animals are presented in Table 1. It can be seen that sensitization of the cells, as shown by mast cell degranulation, was readily achieved at a concentration of total antibody of 625 μg/ml., after incubation for 1 h at 20° C. or overnight at 4° C. At a concentration of 312 μg/ml. of antibody, sensitization was reduced. Sensitization for very short periods of time (10 min at 4° C, or immediate centrifugation after addition of the antibody at room temperature) was ineffective; in experiment 2 of Table 1 histamine release was also measured and amounted to 43 per cent of the total histamine of the cells. Heating of the antibody at 56° C for 5 h completely abolished the sensitizing capacity. Controls performed with cells to which no antibody or no antigen was added, but otherwise treated in the same manner as the other experiments, gave minimal degranulation or histamine release (2 to 5 per cent). No sensitization was obtained with whole serum containing reaginic antibody even at higher concentration.

Experiments were performed to verify the action of the γ_1 antibody contained in the purified preparation by incubating the mast cell suspension for 10 min at 37° C without further washing. In such experiments, degranulation of 50 per cent of the mast cells was obtained at a concentration of 2·5 μg/ml. of antibody, and challenge with 1 μg/ml. of DNP-BSA. Heating of the antibody at 56° C for 5 h did not affect the sensitizing activity caused by the γ_1 antibody.

The results in Table 1 cannot be attributed to an incomplete washing of γ_1, but really result from the firm fixation of reaginic antibody on the cells. In effect, negative results were obtained when the times of incubation with antibody, before washing, were too short, or when the antibody was previously heated at 56° C.

The fact that sensitization was obtained *in vitro* only with purified antibody, and not with whole antiserum, suggests that non-specific γ-globulin of the reaginic class interferes by competition for a receptor site on the cells with the reaginic antibody. In contrast, the fact that the preparation of antibody contained a great amount of γ_1 antibody in relation to reaginic antibody, suggests that γ_1 does not interfere with the sensitization of the cell by reaginic antibody.

Diffusion factors are minimized in our *in vitro* system, and this could be a valuable tool for acquiring more knowledge about the cellular mechanisms of sensitization. Further experiments are in progress.

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Presence of HL.A Antigens on Human Reticulocytes as demonstrated by Electron Microscopy

THE presence of human histocompatibility antigens (HL.A) has been demonstrated on all the human nucleated cells so far studied, but unlike mouse H2 antigens they have not been found on erythrocytes. Harris *et al.*[1], using serological methods, have suggested that they might be present on human reticulocytes. By electron microscopy and an indirect Ferritin labelled antibody technique[2], we have studied these antigens at the membrane of bone marrow erythroblasts and erythrocytes. We have observed them on erythroblasts and reticulocytes and their disappearance on mature erythrocytes. Heparinized bone marrow was obtained by sternal aspiration from three subjects, two normal (A Rh^+ and O Rh^+) and one with mild aplastic anaemia (A Rh^+). The supernatant rich in leucocytes was collected after partial sedimentation of erythrocytes. Cells were washed three times in phosphate buffered saline *p*H 7·2 (PBS) containing 20 per cent foetal calf serum. Aliquots of 5×10^6 cells were incubated for 30 min at 37° C in a 1/3 dilution of an anti-HL.A antiserum, washed three times in PBS, and incubated for a further 30 min at 37° C in ferritin labelled sheep globulin antihuman IgG, prepared as previously described[3]. After three more washings, cells were fixed with glutaraldehyde postfixed in osmium, dehydrated in alcohol and embedded in Epon. Sections were stained with uranyl acetate and lead acetate[4] and examined using a Siemens E IA electron microscope with a magnification of 8,000 to 40,000 times. The anti-HL.A antiserum (provided by Professor J. Dausset) was a highly potent polyspecific anti-HL.A antiserum from a polytransfused patient. It reacted with the lymphocytes of 98 per cent of the French donors in cytotoxic tests up to a dilution of 1/500 to 1/1,000. The donor's blood, being group A, contained natural anti-B antibodies and no other detectable anti-erythrocyte agglutinins. In this respect it was tested against 12 isogroup donors using the immunoferritin technique and never reacted with erythrocytes.

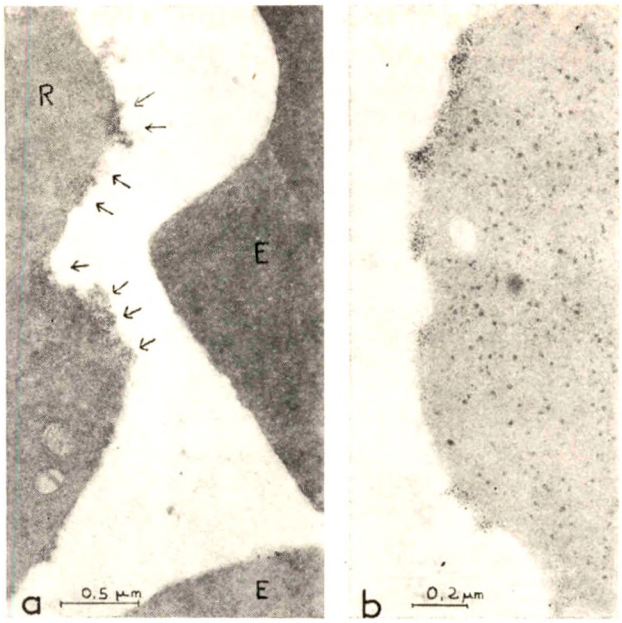


Fig. 1. *a*, Ferritin labelled reticulocyte (R) and unlabelled erythrocyte (E). ×35,000. *b*, Young reticulocyte with numerous "patches" of ferritin labelled areas. ×60,000.

We have already shown that HL.A antigenic sites are labelled as "patches" on the membrane of human lymphocytes[3]. These patches are 0·1 to 2 µm in length and separated by intervals devoid of any ferritin granules. The labelling observed on erythroblasts in the present experiments was similar, but the patches were less numerous and smaller than on the lymphocytes from the same subject. Bone marrow erythroblasts reacted whatever the stage of maturation, but labelling of the membrane was somewhat larger on the late erythroblasts than on the more immature cells. Specific HL.A labelling was never found on the membranes of mature erythrocytes with any of the anti-HL.A antiserum or cell donors. In the same experimental conditions, anti-blood group A or B antisera strongly labelled the erythrocytes bearing the corresponding antigens (D. S., F. M. K., M. G. N., J. P. L. and A. Senik, manuscript in preparation). The most striking observation is that all the examined reticulocytes, easily identified by the presence of polyribosomes and/or mitochondria, were found to be labelled with anti-HL.A antiserum (Fig. 1). No labelling was observed with ferritin conjugate when the cells were preincubated in normal serum from AB blood group donor, or in sera devoid of anti-HL.A antibodies.

These observations demonstrate that HL.A antigens are present on reticulocytes although they are not found on mature erythrocytes. Their disappearance from the latter is considered to be a pre-terminal event in the maturation of erythrocytes, which happens very late in the life of the reticulocyte, since no reticulocytes lacking HL.A antigens have yet been found in our preparations.

The disappearance of HL.A antigens from red blood cell surfaces might be due to the "masking" of these antigens at the membrane of mature erythrocytes. In fact, this is improbable because the immunization of human volunteers with erythrocytes failed to induce an anti-HL.A immune reaction detectable serologically or by skin grafting[5]. It is possible that HL.A antigens are eliminated from the cell membrane when important structural modifications of the membrane occur at the terminal stage of erythrocyte maturation, but it is more probable that the HL.A antigens at the cell surface are constantly renewed, as has been suggested for other macromolecules and

antigens[6,7]. The synthesis of HL.A antigens would persist in reticulocytes in which a high level of haemoglobin synthesis occurs. The duration of synthesis of the HL.A antigens would depend on the survival time of their messenger RNA and it would stop at the end of erythrocyte maturation with the disappearance of the polysomes and mitochondria. In this hypothesis HL.A antigens, in contrast with erythrocyte antigens, would be very short lived and quickly eluted or destroyed, disappearing very rapidly as soon as their synthesis stops. It is important to mention that the disappearance of HL.A antigens from the cell surface coincide with drastic changes in the morphological aspect and the general properties of red cell membrane. The relationships between changes in the structure and the antigenicity of the red cell membrane may suggest a model for the study of the role of HL.A antigens in cell membrane structure.

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Failure of Long-established Skin Homografts on ALS-treated Mice to induce Transplantation Tolerance

The ultimate immunological status achieved by treating animals with antilymphocyte serum (ALS) superficially resembles immunological tolerance. The induction of tolerance has been refuted as a likely mode of action of ALS[1], except in conjunction with thymectomy[2,3], but ALS can—like other immunosuppressive agents[4-8]—promote the induction of transplantation tolerance[9]. While testing five ALS preparations for their activity to prolong skin homograft survival, we noted that these antisera were quite potent, facilitating the prolonged survival of a large percentage of test skin homografts. We therefore wondered whether or not the presence of homologous skin on ALS-treated mice for a long time would induce tolerance to the transplantation antigens present in the test graft.

ALS was raised in 2–4 month old New Zealand white rabbits by injecting 200×10^6 CBA mouse lymph node cells (suspended in 0·5 ml. of physiological saline and mixed with 0·5 ml. of Freund's complete adjuvant) in all footpads. This was repeated 3 weeks later and the rabbits received a third dose of 100×10^6 CBA node cells in saline intravenously 1 week later. Rabbits were exsanguinated 1 week after the third injection. The sera were heat-inactivated (56° C for 30 min) and sterilized by filtration. CBA mice were used to test the activity of the ALS preparations. Mice received 0·5 ml. of serum subcutaneously 2 and 5 days after transplantation with full-thickness A skin grafted orthotopically on the right chest according to a standard method[10]. ALS-treated mice that bore healthy A skin tests grafts for 109 days (group I) or for 181 days (group II) were regrafted with full thickness skin from A mice and—for control purposes—with skin from C57Bl/6 mice on the left chest. ALS-treated mice that had rejected A test skin grafts some time before day 109 or 181, respectively, were similarly regrafted with A and C57Bl/6 skin, as was a group of normal CBA mice. To avoid bias, A and C57Bl/6 skin grafts were placed alternately in the upper and lower graft beds. Grafts were first inspected 9 days after the operation and then daily.

Table 1 summarizes the results. CBA mice of group I originally comprised thirty animals, used to test the potency of three ALS preparations. When regrafted 109 days after the primary A skin grafting, twenty-eight animals were available. The activities of two other preparations of ALS were tested using twenty CBA mice. Of these thirteen and fourteen could be regrafted satisfactorily in group II 181 days after primary A skin grafting. Although there was considerable variability in the survival times of both A and C57Bl/6 skin grafts, particularly in group II, mice bearing long-established A skin grafts clearly did not support the prolonged survival of a second A graft. The rejection of such grafts seemed to follow first-set kinetics irrespective of whether the original A test graft was still retained or had been rejected. With respect to time, rejection was not different from that of skin of the unrelated strain C57Bl/6, nor from that of A and C57Bl/6 skin grafted to normal CBA mice (controls). Thus, neither tolerance nor sensitization had occurred.

During rejection of a second A skin graft and of the simultaneously grafted C57Bl/6 skin transplant, the primary A test grafts showed no signs of rejection. Hair clipped on the occasion of regrafting grew again normally and the grafts resumed their pregrafting appearance. In fact, on termination of the experiment, ten of the sixteen animals in group I which were regrafted 109 days after being forced to accept an A skin graft under ALS treatment still bore viable grafts 297 days later (406 days total). One CBA mouse of this group rejected its A test graft 99 days later (208 days total), whereas in five mice A grafts suffered chronic contracture and were considered rejected 85 days later (194 days total, two mice), 71 days later (180 days total, two mice), and 57 days later (166 days total, one mouse). Of the seven animals in group II, four bore healthy skin grafts 329 days after regrafting (510 days total), and one CBA mouse died with a viable graft 297 days afterwards (478 days total). In the remaining two mice of this group the A test grafts

Table 1. SURVIVAL OF A AND C57Bl/6 SKIN GRAFTS ON ALS-TREATED CBA MICE WHICH HAD RETAINED OR REJECTED A SKIN TEST GRAFTS

Group	Skin grafting	State of A test graft at regrafting with A and C57BL/6 skin	No. of CBA mice	MST (days)	±95 per cent confidence limits	s.d.
I	A→CBA	Accepted for 109 days	16	9·3	±0·30	1·06
		Rejected before day 109	12	9·8	±0·50	1·09
	C57Bl/6→CBA	Accepted for 109 days	16	9·8	±0·50	1·12
		Rejected before day 109	12	10·2	±0·40	1·07
II	A→CBA	Accepted for 181 days	7	10·7	±1·59	1·22
		Rejected before day 181	7	9·4	±1·48	1·24
	C57Bl/6→CBA	Accepted for 181 days	5	10·4	±1·50	1·18
		Rejected before day 181	8	8·7	±1·26	1·25
Controls	A→CBA	—	17	10·4	±0·64	1·14
	C57Bl/6→CBA	—	17	9·8	±0·35	1·08

Table 2. FATE OF HAMSTER SKIN GRAFTS TRANSFERRED TO NORMAL OR HOMOGRAFT SENSITIZED HAMSTERS AFTER RESIDENCE FOR 150 DAYS ON F1 HYBRID HAMSTER HOSTS

Skin graft transferred from hybrid hamster host	No. of grafts	Immune status of new hamster host	Survival times of skin grafts (days)
LSH from (CB × LSH)F1	5	Normal LSH	5 × > 98
LSH from (CB × LSH)F1	15	LSH sensitized to CB	1 × > 143; 3 × > 98; 1 × 70; 2 × 20; 1 × 18; 1 × 16; 1 × 13; 5 × 7
CB from (CB × LSH)F1	6	CB sensitized to LSH	3 × > 143; 1 × 16; 2 × 10
MHA from (CB × MHA)F1	5	MHA sensitized to CB	3 × > 209; 2 × 96

underwent chronic contracture and were regarded as lost 122 and 52 days later (303 and 233 days total).

These results strongly suggest that A skin homografts accepted by CBA mice under the influence of ALS do not constitute a strong enough stimulus to confer transplantation tolerance, in spite of the fact that CBA hosts supposedly were exposed to A transplantation antigens for up to 181 days. The observation that rejection of a second A skin graft did not harm long-established A test grafts on the same animal requires an explanation. The only evidence of the A antigenicity of these original A test grafts was their crop of albino hair; there is no evidence as to whether or not the vascular endothelium of these grafts was of A or of CBA antigenicity.

An obvious procedure to test the antigenicity of these original A test grafts would be to transplant them back on normal A strain mice and to observe acceptance or rejection. This was not attempted, however, for two reasons: first, because the skin grafts persisting on ALS-treated mice seemed too small to allow satisfactory transplantation; the second, because earlier experiments with Syrian hamsters on a similar problem had yielded equivocal results. In these experiments, large skin grafts (about 2 × 2 cm) from parental strain hamsters were excised from appropriate female F1 hybrid recipients (Table 2) after 150 days. The edges of these grafts were trimmed by several millimetres to ensure the removal of any ingrown skin of host origin, and the grafts were transplanted either to normal parental strain male hamsters or to similar hamsters sensitized by skin homograft rejection to the antigens which might have been "picked up" by the grafts during their 150 days on appropriate F1 hybrid hosts. The fate of these grafts on their new hosts is shown in Table 2. The results with normal secondary hosts were clear-cut: replacement of vascular endothelial and fibroblast cells was not evident. When, on the other hand, sensitized secondary hosts were used, the results were less certain. Of twenty-six grafts so tested, 50 per cent were rejected within 20 days, and 50 per cent survived for 70 days or longer. Although this would indicate that at least in half of the grafts replacement of the vascular endothelium by that of the host had taken place during prolonged residence, as suggested by Billingham *et al.*[11], the reliability of these results is questionable. Similar difficulties were experienced with these grafts by Silvers *et al.*[12], that is, healing-in crisis of the rather thick and fibrotic grafts, and partial necrosis and contracture, which made transplantation and scoring somewhat hazardous. But it is difficult to ascribe all rejections to purely technical reasons. Clearly a more reliable means should be used to reveal the possessor of the vascular endothelium of long-accepted skin grafts. The use of fluorescent antibodies, as suggested by Medawar[13], should help to throw light on this most challenging problem of transplantation biology which has puzzled many investigators[14–18].

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Differential Rejection of Neurones and Neuroglia in Canine Cardiac Allografts

USING a free-grafting technique, Barnes[1] demonstrated that brain tissue is at least as antigenic as skin allografts in mice. After the grafting procedure the neuronal cells become necrotic in his experiments, whereas the associated neuroglia, being more resistant to ischaemic anoxia, survived. A second-set form of rejection was found on subsequent skin grafting. He concluded that in this type of brain allograft, antigenicity is dependent on viable glial tissue. Such free-grafting experiments however, give no indication of the reactions invoked by viable neurones. An answer to this problem is important in the consideration of integrated neural allografting as in whole nerve, heart and brain grafts, for example.

As part of a study of the effects of immunosuppressive agents on the survival of canine cardiac allografts, we have noted the presence of histologically normal neurones in ganglia in proximity to both the sino-atrial and atrioventricular nodes in transplanted hearts. We used the technique of cervical cardiac allografting described by Mann *et al.*[2], which involves establishment of circulation through the coronary system of the implanted heart by anastomosis of the common carotid artery and external jugular vein of the recipient animal to the aortic arch and pulmonary artery, respectively, of the implant. Unmatched mongrel male dogs were used as donor and recipient animals. The average time of cardiac ischaemia was 20 min, during which period the heart was maintained at 5–10° C by immersion in saline. The mean survival time of implants without immunosuppressive therapy was 8 days, whereas survivals of up to 37 days were achieved by immunosuppression with azathioprine and hydrocortisone. The diagnosis of terminal rejection was based on progressive ventricular failure and on gross

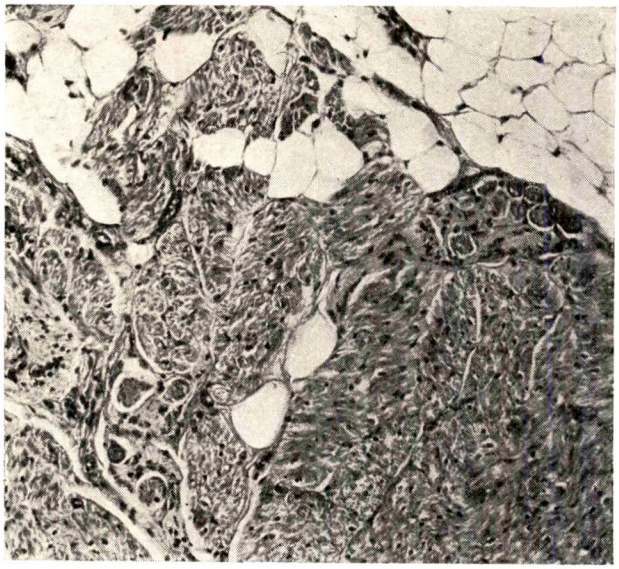


Fig. 1. Low power (×84) view of ganglia in recipient heart.

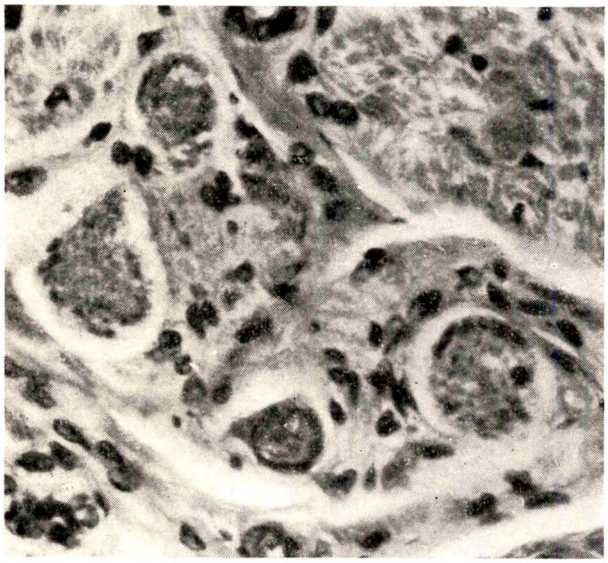


Fig. 2. High power (×330) view of ganglion in recipient heart.

electrocardiographic deterioration, but most hearts were still beating feebly when removed.

The implant was removed and the recipient animal killed, and appropriate blocks from both hearts were then fixed in 10 per cent buffered formalin. Routine 6 μm sections were stained with haematoxylin and eosin, periodic acid–Schiff, methyl-green pyronine and picro-Mallory stains. Sections from eight implant-recipient heart pairs, with an implant survival of at least 8 days, were examined. All implanted hearts demonstrated the typical features of cellular graft rejection[3], but sections from recipient hearts including the ganglia were essentially normal (Figs. 1 and 2). The cellular infiltrate, which consisted of lymphocytes, larger mononuclear cells and occasional neutrophils, was particularly conspicuous in and about neural ganglia, being much more intense than in the surrounding myocardium (Figs. 3 and 4). Nevertheless in all cases, the neurones were well preserved, whereas the supporting cells of the ganglia were destroyed. This condition of differential destruction prevailed not only in unmodified rejection but also in the ganglia of hearts which had survived for up to 37 days with immune suppression.

Our observations indicate that neural tissue in allo transplanted hearts is intimately involved in the rejectio process, so that as a whole it is at least as antigeni as cardiac muscle. The intensity of cellular infiltrat about the ganglia may imply that such tissue is mor strongly antigenic than cardiac muscle, but other factor such as the greater vascularity of the areas in whic these ganglia were situated must also be considered.

Of possibly greater significance is the observatio that neurones in the ganglia were spared, whereas th associated supporting tissue was not. The normal light microscopic appearance of neurones further suggest that they are only weakly antigenic and could be expecte to survive in the allografted heart. We have institute long term experiments to test this hypothesis and t elucidate the nature of the apparent host tolerance t neural cells.

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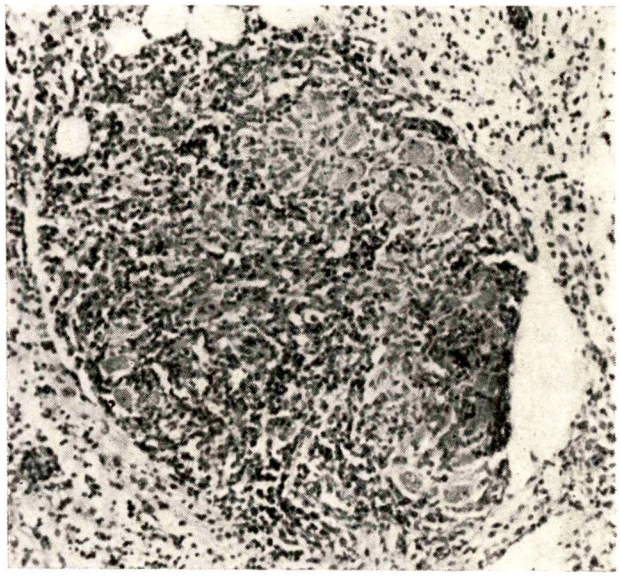


Fig. 3. Low power (×84) view of ganglion in an allotransplanted heart.

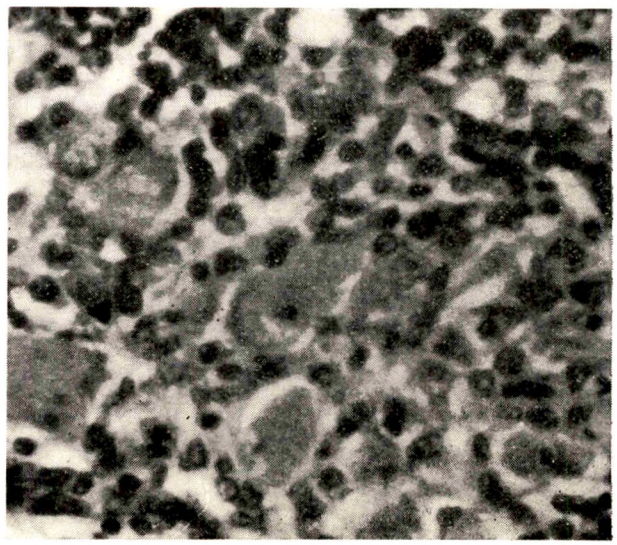


Fig. 4. High power (×330) view of ganglion in Fig. 3.

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Antitumour Effect of Lymphoid Cells activated by Interferon

INTERFERON induces a cytotoxic effect in normal mouse spleen cells against allogeneic tumour target cells in tissue culture[1,2]. We here present evidence that syngeneic lymphoid cells may be similarly activated by interferon and that syngeneic lymphoid cells plus interferon suppress the growth of tumour cells *in vivo*.

Interferon was prepared in L cells. Chick embryo allantoic fluid containing Newcastle disease virus (NDV) of initial activity 10^9 ID_{50}/ml. (320 haemagglutinating units per ml.) was irradiated with ultraviolet light until ineffectivity to chick embryo was completely lost. The fluid containing inactivated virus was diluted 1 : 20 in 'medium 199' and was then added to monolayer L cell culture. The culture medium was collected after incubation for 48–72 h and 6 M HCl was added to pH = 2. It was then kept at 4° C for 72 h and neutralized with 6 M NaOH to pH 7·5. To check the activity of the interferon the preparation was titrated on L cells. These preparations completely protected L cells from the > 100 $TCID_{50}$ (50 per cent tissue culture infectious doses) of encephalomyocarditis virus.

To check the toxicity of interferon against L cells each sample of interferon was titrated on L cells before use The dilutions used in the experiments were those which did not destroy L cells

The control culture fluid was prepared in the same way, irradiated allantoic fluid of normal embryos being added to L cells.

In the *in vitro* experiments, we used as target cells a line of mouse fibroblasts (L cells) which had been first obtained by Earle from tumour cells of mouse strain C3H (ref. 3). The seeded dose was 10^5 cells/ml. Each tube contained 1 ml. of the liquid. Spleens and lymph nodes from mouse strains BALB/c, C57Bl/6j, C3H, C3H(f)D and from F_1 hybrids between the first two strains were removed aseptically. These organs were disrupted to produce a cell suspension in medium 199. The suspensions were then filtered through two layers of gauze and washed three times with medium 199 by centrifuging at 600g for 10 min at 4° C.

The design of experiments *in vitro* has been described elsewhere[1,2]. Interferon and the control fluid were added to L cells together with lymphocytes. In all the experiments there were control tubes in which interferon and the control fluid were added to the L cells without any lymphocytes. The results of the experiments were obtained by counting the number of live L cells on the second or third day of incubation.

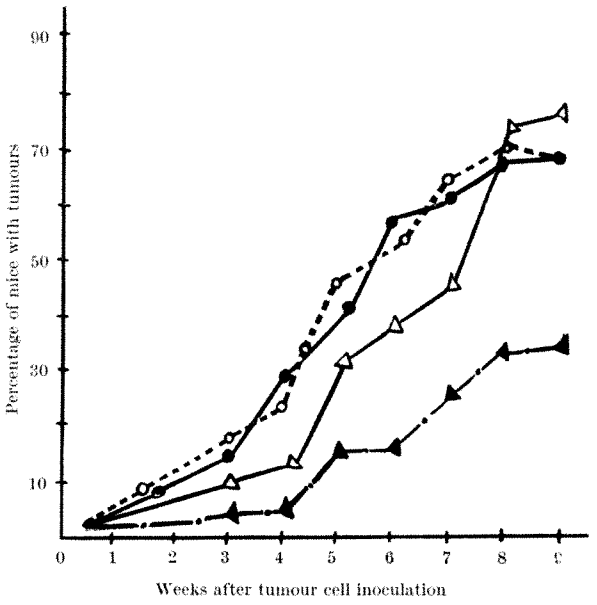


Fig. 1. Rate of tumour development. ○ - - - ○, Tumour cells (group 1); ●——●, tumour cells and lymphocytes (group 2); △——△, tumour cells and interferon (group 3); ▲——▲, tumour cells, lymphocytes and interferon (group 4).

Experiments *in vivo* were carried out with mouse strain C57Bl/6j (20–30 g). Tumour cells were obtained from syngeneic C57Bl/6j mice, with primary sarcomas induced by intramuscular inoculation of 9,10-dimethyl-α-benzanthracene in oil. Cell suspensions were prepared from tumours which were aseptically removed and then minced and filtered through two layers of gauze. The suspensions were washed by being centrifuged twice in Hanks's solution. The viability of tumour cells was checked by staining with eosin–trypan blue. Suspensions of spleen cells were prepared simultaneously with those of tumour cells as described for experiments *in vitro*. Tumour and spleen cell suspensions were mixed in appropriate ratios. Some of the mixed suspensions were diluted with medium 199, the others with interferon. The cell suspensions were maintained at freezing point. 1 ml. samples of the mixed suspensions (with or without interferon) were injected subcutaneously into mice without previous incubation.

The mice were organized into four groups, each group consisting of ten to fifteen mice. Controls were inoculated with tumour cells alone, tumour cells plus syngeneic lymphocytes or tumour cells plus interferon. The experimental group consisted of mice inoculated with tumour cells plus lymphocytes and interferon. Between 10^6–10^7 tumour cells were injected into each mouse and 50–100 times as many lymphocytes. Before tumours appeared mice were observed weekly; after tumours started growing, they were observed twice a week. The results *in vitro* were analysed by Student's t test, *in vivo* results by the χ^2 method.

As shown in Table 1 interferon alone caused inhibition of the growth of L cells in some experiments (Nos. 6–8). But in most experiments it did not inhibit the growth of cells and even stimulated it. Allogeneic spleen cells alone either failed to affect the growth of cells or destroyed them.

When interferon was added with allogeneic spleen and lymph node cells it always had a destructive effect on L cells. This effect cannot be interpreted as a result of the toxicity of interferon added to that of the lymphocytes: in all the experiments but one, neither a single component nor both of them used separately destroyed L cells, but when used together they exerted a marked cytotoxic effect. Syngeneic lymphocytes tested in the

Table 1. THE EFFECT OF INTERFERON ON THE INTERACTION BETWEEN ALLOGENEIC AND SYNGENEIC LYMPHOCYTES AND L CELLS

Experiment	Strain of mice donors of lymphocytes*	Average number of live L cells (×1000) after 2–3 days incubation with: Medium 199	Control fluid	Interferon	Dilution†	Lymphocytes in Medium 199	Control fluid	Interferon
1	BALB/c	—	110±3	141±6	Crude	—	118±8	20±3
2	BALB/c	151±10	172±22	238±11	Crude	124±11	135±13	73±10
3	BALB/c	102±6	—	128±11	Crude	33±5	—	17±2
4	BALB/c	159±26	—	156±16	1/200	82±6	—	58±4
5	BALB/c	190±22	—	97±9	Crude	184±13	—	56±9
6a	BALB/c				Crude	131±15	—	33±5
b	C57Bl/6j	174±15	98±7	85±6	Crude	134±13	95±14	36±4
c	F_1(BALB/c ×C57Bl/6j)				Crude	131±13	—	20±4
7	BALB/c	219±6	213±9	131±3	1/5	233±5	209±18	113±5
8	BALB/c	202±24	184±9	174±11	1/10	218±13	229±10	120±9
	Mean±s.e.	171±14	155±24·5	143±17		141±21	157±26	55±12
9	BALB/c	160±18	—	82±14	Crude	174±25	—	33±7
	C3H				Crude	108±15	—	42±11
10	BALB/c	101±10	130±20	138±10	Crude	58±4	65±10	45±6
	C3H				Crude	85±13	71±16	47±5

* Spleens were used in experiments 1–6, 9–10; lymph nodes in experiments 7–8.
† In experiments 1–3, 10 and 5, 9, five different samples of our own preparation of interferon were used. Their activities, expressed in antilogarithms, show the differences in the titres of encephalomyocarditis virus (in $TCID_{50}$) in non-treated cultures and those treated with interferon. The activities of interferon in our samples were respectively: 100; 1,000; 10,000; 100 and 1,000. In experiment 4 we used Difco interferon (activity 500 units/ml.) kindly provided by Dr T. C. Merigan.

Table 2. EFFECT OF INTERFERON PLUS LYMPHOCYTES* ON THE DEVELOPMENT OF TRANSPLANTED TUMOURS IN MICE

Group	Inoculated material	Total No. of animals	Number (per cent) of animals with tumours	χ^2 (between groups 1, 2, 3 and group 4)	$P <$
		Number (per cent) of developing tumours			
1	Tumour cells	44	31 (70)	11·56	0·002
2	Tumour cells + lymphocytes	40	25 (62)	6·9	0·01
3	Tumour cells + interferon	37	27 (73)	12·65	0·002
4	Tumour cells + lymphocytes + interferon	44	15 (34)	—	—

* 5×10^1–10^2 lymphocytes per tumour cell.

same series of experiments (Table 1) also caused destruction of L cells under the influence of interferon. Control fluid alone as a rule did not affect the growth of tumour cells. Neither did it induce a cytotoxic effect of lymphocytes. The difference between the number of live cells in the experimental group (lymphocytes plus interferon) and their number, even in that control group where it was the least, was always statistically significant: $P < 0{\cdot}05$.

Fig. 1 and Table 2 summarize the results of three experiments *in vivo*. Interferon alone did not affect either the rate or the incidence of tumour development. Neither did syngeneic lymphocytes when used alone. By contrast, interferon inoculated together with syngeneic lymphocytes reduced the number of tumours and delayed their development. The suppression of tumour growth was statistically significant.

The mechanism of the effect of interferon on lymphoid cells remains obscure but our results suggest that it activates lymphoid cells and makes them able to suppress the growth of tumour cells. Recently the suppression of tumour growth in animals inoculated with interferon inducers has been described[5,6]. Such suppression is probably related to the activation of lymphocytes by interferon. The suggestion is also supported by the finding that allotransplantation reaction is increased (personal communication of H. B. Levy) by the double-stranded polyribonucleotide poly I·poly C. It is of interest that in Levy's experiments, administration of double-stranded RNA suppressed the development of leukaemias to a much lower degree. This may be attributed to defective lymphocytes which failed to be activated by interferon.

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Foetal Adrenal Necrosis induced by 7-Hydroxymethyl-12-Methylbenz(a)-anthracene and its Prevention

THE polycyclic hydrocarbon 7,12-dimethylbenz(a)-anthracene (DMBA), a potent carcinogen, has been used extensively to study the induction of mammary tumours in rats[1–3]. Among its other biological effects, DMBA produces severe necrosis of the inner zones of the adrenal cortex in the mature rat[4]. The immature rat (< 30 days of age), however, is not susceptible to the adrenocorticolytic effects unless it is pretreated with ACTH (ref. 5). In the mature rat 7-hydroxymethyl-12-methylbenz(a)-anthracene (7-OHM-12-MBA), a derivative of DMBA, has a more powerful adrenocorticolytic action than the parent hydrocarbon[6], but it also has no effect on the adrenal gland of the immature rat[7]. Pretreatment with β-diethylaminoethyldiphenyl-*n*-propyl acetate (SKF 525-A), a potent inhibitor of hepatic microsomal drug-metabolizing enzymes, protects the adrenal glands of the mature rat from the damaging effects of both DMBA and 7-OHM-12-MBA (ref. 8).

Recently we described the embryopathic actions of DMBA and 7-OHM-12-MBA in the Sprague-Dawley rat[9]. We now report that at day 17 of pregnancy the adrenal glands of the rat foetus are susceptible to the adrenocorticolytic effects of 7-OHM-12-MBA and, further, that these can be prevented by pretreatment with SKF 525-A.

Pregnancy was timed and confirmed as described before[9]. The foetal adrenal glands were obtained from the litters of Sprague-Dawley rats treated on day 17 of pregnancy with a single intravenous dose of 7-OHM-12-MBA (2·5 mg/100 g maternal body weight) in an oil emulsion. Controls received an equivalent volume of oil emulsion which contained no hydrocarbon. The mothers were killed on day 20 of pregnancy; their adrenal glands and at least five pairs of foetal adrenal glands from each litter were fixed in 4 per cent neutral buffered formaldehyde. Paraffin sections were stained with haematoxylin and eosin.

In all cases following treatment with 7-OHM-12-MBA on day 17 of pregnancy, the foetal adrenal glands were

ble 1. RESULTS OF PRETREATMENT WITH SKF 525-A (2 mg/100 g MATERNAL DY WEIGHT) ON FOETAL ADRENAL DAMAGE FOLLOWING 7-OHM-12-MBA EATMENT (2·5 mg/100 g MATERNAL BODY WEIGHT) AT DAY 17 OF PREGNANCY

Pretreatment	Treatment	No litters (No. of pairs of adrenals assessed)	No. of pairs of adrenals showing severe necrosis
—	7-OHM-12-MBA	4 (30)	30/30
—	Control emulsion	2 (15)	0/15
SKF 525-A	7-OHM-12-MBA	4 (21)	0/21*
Saline	7-OHM-12-MBA	3 (20)	20/20
SKF 525-A	—	3 (25)	0/25

* Eight pairs showed histological evidence of scattered single cell necrosis in e central zone.

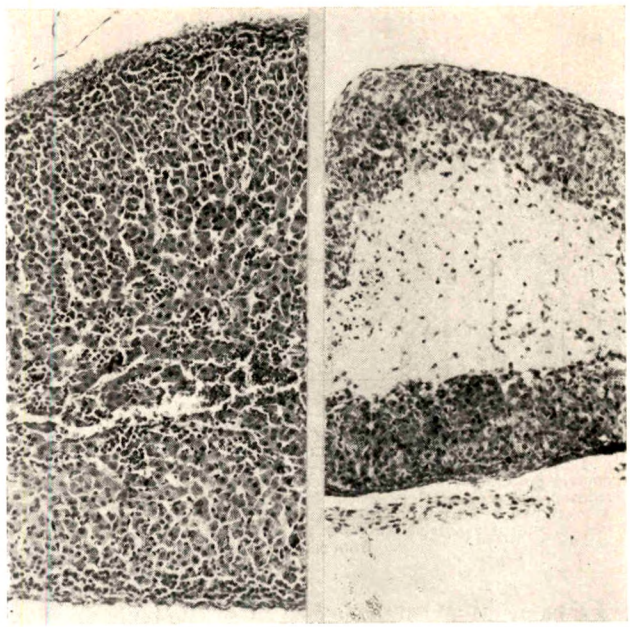
duced in size and they all showed severe necrosis of he central zones (Table 1). The severity varied a little a degree not only between litters but also within the ame litter: in most glands the necrosis was diffuse Fig. 1), but in a few, though severe, it was focal in charac-er. The necrosis involved the inner part of the cortex nd the presumptive medullary zone, which in the 20 day oetus is composed of irregular cords of adrenocortical ells surrounding the clusters of sympathetic medullary-ell precursors; the outer part of the zona fasciculata ontained single scattered necrotic cells, but the zona lomerulosa was undamaged. None of the adrenal glands rom the control groups treated with the oil emulsion howed any evidence of necrosis. The adrenal glands of he mothers all showed extensive severe cortical necrosis.

The results of pretreating with SKF 525-A before hallenge with 7-OHM-12-MBA at day 17 of pregnancy re shown in Table 1. SKF 525-A, dissolved in saline, vas given intraperitoneally (2 mg/100 g maternal body veight) 30 min before 7-OHM-12-MBA. Control animals eceived an intraperitoneal injection of an equivalent olume of saline or SKF 525-A. The mothers were killed n day 20 of pregnancy. Pretreatment with SKF 525-A effectively protected the adrenal glands of the foetuses nd severe necrosis was not found in any case. In approxi-mately 40 per cent of the "protected" glands, however, here was histological evidence of slight damage in the central zones—nuclear pyknosis or necrosis of scattered single adrenocortical cells. The adrenal glands of the mothers were fully protected by SKF 525-A pretreatment.

It is interesting that the adrenal glands of the 17 day foetal rat should be susceptible to 7-OHM-12-MBA whereas those of the postnatal immature animal are not. Because a metabolite of 7-OHM-12-MBA is probably the ultimate adrenocorticolytic agent in the mature rat[10], and because the liver of the immature rat differs significantly in its microsomal hydroxylating activities from that of the mature animal[11], it seems likely that the foetal adrenal necrosis is due to the transplacental passage of an active adrenocorticolytic metabolite from the mother. This view is supported by the fact that SKF 525-A protects both the foetal and the maternal adrenal glands from 7-OHM-12-MBA.

It may also be significant that the foetal adrenal gland undergoes rapid growth and development from day 16 of pregnancy until birth under the influence of foetal ACTH (ref. 10), while adrenocortical functions diminish for a time in the early postnatal period[13]. The failure to induce adrenal necrosis with DMBA in the immature rat has been correlated with a diminution in the corticosterone content of the gland as compared with the mature animal: pretreatment with ACTH converts the refractory gland to a susceptible state[5], and it may be that active corticosterone biosynthesis is necessary for the adrenocorticolytic action of both DMBA and 7-OHM-12-MBA. If this is indeed the case it would seem from our experiments that the outer part of the zona fasciculata in the 17 day foetus differs functionally from the inner part of the cortex.

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a *b*

Fig. 1. *a*, Adrenal gland of 20 day control foetus. The cortex shows early zonation and in the presumptive medullary zone there are irregular clusters of sympathetic cells. *b*, Adrenal gland of 20 day foetus treated with 7-OHM-12-MBA on day 17 of pregnancy. Note reduction in size and massive necrosis of the central zones. (Haematoxylin and eosin, ×60.)

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Influence of Hydrocortisone and Glucagon on Liver Tyrosine Transaminase and on Brain Tyrosine, Norepinephrine and Serotonin

CURRENT hypotheses concerning biochemical origins of endogenous depression centre around serotonin (5HT)[1] and norepinephrine[2–4] changes in the brain. Recently, attempts have been made to relate disturbances in biogenic amine metabolism in the brain to metabolic factors outside the brain. Green and Curzon found[5] that administration of hydrocortisone resulted in a decrease of 5HT in the brain (by way of an increase in tryptophan pyrro-

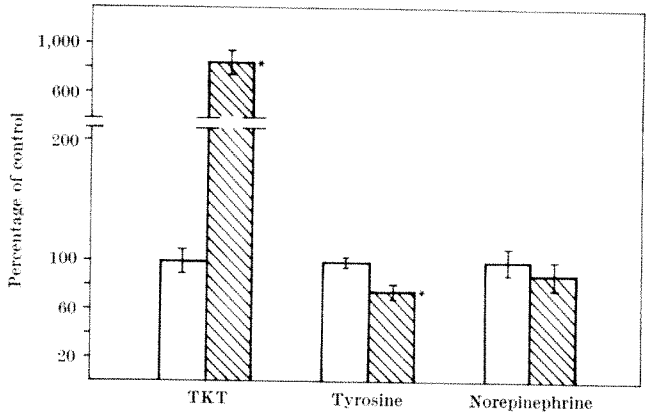


Fig. 1. Influence of glucagon and hydrocortisone on TKT in liver, tyrosine in plasma and norepinephrine in the whole brain. Enzyme activity of TKT was measured in percentage of control. The mean values for the control animals (100 per cent) are: tyrosine 10·8 μg/ml.; norepinephrine, 0·49 μg/g. Each group consisted of nineteen to twenty-two rats. Vertical bars indicate standard deviation of the mean. White columns, control animals; hatched columns, treated animals. * $P < 0.01$ differs from control.

lase, the principal catabolic enzyme of tryptophan). This evidence gives strong support to the 5HT hypothesis of endogenous depression[5,6]. Takahashi *et al.* noted[7] that after oral application of a tyrosine load there is a significant increase in the level of plasma tyrosine in maniacs and depressives as compared with control persons[7]. This increase is attributed to a reduced activity of α-ketoglutarate transaminase (TKT) (TKT is the principal catabolic enzyme of tyrosine) in patients with endogenous depression. Finally, patients with endogenous depression show a lower level of plasma tyrosine, phenylalanine, and tryptophan than normal subjects[8]. The reduction in the level of these amino-acids presumably leads to a disturbance in amine levels in the brain.

The influence of peripheral factors on amine metabolism in the brain is largely unknown. The possibility that one of the principal enzymes in tyrosine metabolism, TKT, has an influence on tyrosine levels and amine metabolism in the brain has not been investigated, but a recent study of brain TKT did suggest that this enzyme is important[9].

Chirogos *et al.*[10] have examined the possibility that high levels of plasma tyrosine influence tyrosine levels in the brain after the i.p. injection of tyrosine, and have shown that, after 1 h, the tyrosine levels in the plasma and brain were equal. We have attempted to establish whether a low level of plasma tyrosine is accompanied by a low level of brain tyrosine and, if so, if it affects the level of norepinephrine in the brain. TKT activity can be increased by hydrocortisone[11] and by glucagon[12,13], and it is also influenced by norepinephrine. High levels of norepinephrine suppress the TKT rhythm[14] and low levels of norepinephrine increase TKT activity in the liver[15], while lowering it in the brain[9]. Because hydrocortisone, by itself or together with glucagon, increases the level of tryptophane pyrrolase[12], which according to Green and Curzon[5] should lead to a reduction in 5HT levels in the brain, we attempted a replication of their findings.

The TKT activity in the livers of male Sprague–Dawley rats (200–300 g) was estimated according to Diamondstone[16]. Plasma and brain tyrosine were measured by the method of Waalkes and Udenfriend[17], and norepinephrine and 5HT in the whole brain were estimated according to Maickel *et al.*[18]. The animals were kept in normal lighting. Hydrocortisonacetate (2·5 mg/100 g i.p. 5 h before decapitation) and glucagon (0·5 mg/100 g i.p. 5 and 3 h before decapitation) were injected between 10.00 a.m. and 11.00 a.m. The rats were starved for the last 8 h before decapitation although tap water was freely available. The control animals received equal volumes of 0·9 per cent salt solution injected at the same times.

TKT was increased by glucagon and hydrocortisone approximately 835 per cent (see Fig. 1) in comparison with the control animals (100 per cent). The corresponding plasma tyrosine level was reduced by 24·5 per cent as compared with that of the control animals.

The reduced levels of plasma tyrosine can be attributed to the increased TKT activity[9,19,20]. The significant decrease in the levels of brain tyrosine is accompanied by a proportional decrease in plasma tyrosine (Table 1). Levitt *et al.*[21] have shown the effect of varying the tyrosine concentration in the perfusion fluid on the rate of norepinephrine formation in the heart. Because our lowered tyrosine levels are in the border area of full saturation of the tyrosine hydroxylase it was possible that there was reduced formation of norepinephrine.

Table 1. TYROSINE IN THE PLASMA AND BRAIN OF RAT

	Plasma	Brain
Control	10·6 ± 0·42	15·5 ± 0·64
Treated with hydrocortisone	8·3 ± 0·32*	10·9 ± 0·91*

Each group consisted of twelve rats. Results are expressed as μg/ml and/or μg/g ± s.d. of the mean. After injection of hydrocortisone + glucagon (five rats) comparable results were found. * $P < 0.01$ differs from control.

Our experimental animals showed a slight decrease (11·4 per cent) in the level of norepinephrine in comparison with the controls, but the difference is not significant (see Fig. 1), and there was no difference when the TKT was induced by about 550 per cent by giving hydrocortisone and glucagon independently. Moreover, in the heart, the increased TKT caused by glucagon and hydrocortisone (twenty-two rats) had no influence on the norepinephrine. After injections of glucagon and hydrocortisone, the TKT rises sharply so it can be assumed that normal daily changes in TKT level have no influence on norepinephrine levels in the brain.

The tryptophan pyrrolase level should also be increased by hydrocortisone and glucagon[12], but we found only a slight (5 per cent) and insignificant decrease in 5HT in the brains of our experimental animals (fifteen rats) compared with that in the control animals. With hydrocortisone alone, we also did not find any decrease in 5HT: one group of animals (ten rats) received a dose of hydrocortisone identical to that given by Green and Curzon (0·5 mg/100 g i.p.). As in Green and Curzon's study these animals were killed 6 h after injection. Another group (fifteen rats) received a dose five times as high (2·5 mg/100 g i.p., 5 h before decapitation), but again we did not find a decrease in the 5HT level in response to hydrocortisone (acetate) injections.

Our results show that a decrease in level of peripheral plasma tyrosine through an increase of TKT is accompanied by a parallel lowering of tyrosine level in the brain. The lowering of tyrosine levels in the brain does not result in a decrease of brain norepinephrine. Furthermore, we were unable to demonstrate a decrease of 5HT in the brain after application of hydrocortisone.

To summarize, we were not able to affect the levels of norepinephrine and 5HT in the brain by increasing the concentration of TKT and tryptophan pyrrolase in the liver. It is therefore unlikely that the decrease in the levels of biogenic amine in the brain, which is assumed to occur in endogenous depression, can be attributed to increased TKT or tryptophane pyrrolase production by the liver.

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Synergistic Action of Sodium and Angiotensin on Brain Mechanisms controlling Water and Salt Balance

INFUSIONS of hypertonic NaCl into the third brain ventricle of goats have been found to cause drinking[1], release of antidiuretic hormone (ADH)[2] and natriuresis[3]. Thirst may also be elicited in the rat by intravenous infusions[4] and intrahypothalamic injections[5] of angiotensin. The effects of angiotensin and hypertonic saline on drinking are additive when both substances are administered intravenously to nephrectomized rats[4]. For these reasons it seemed of interest to study how infusions into the third ventricle of angiotensin alone, or in combination with moderately hypertonic NaCl, would affect central mechanisms of importance in the regulation of water and salt metabolism.

Slow (10 μl./min) infusions into the cerebrospinal fluid of the third ventricle were made for 30 min periods in five goats, and for repeated 5 min periods in two of these animals. The infusion technique has been described earlier[1]. The goats were either in normal water balance or were hydrated by giving 100 ml. of water/kg body weight by stomach tube into the rumen 90 min before the infusions were started. Angiotensin (Hypertensin, Ciba) was dissolved either in slightly hypotonic (0·12 M) NaCl, or in hypertonic (0·33 M) NaCl, and was infused at a rate of 1–2 ng/kg/min. For comparison infusions of 0·33 M NaCl without angiotensin were also made.

When the goats were in normal water balance, 30 min infusions of 0·33 M NaCl or of angiotensin dissolved in hypotonic saline caused cumulative drinking of a total of 1–2 l. of water. These infusions also resulted in a two to five-fold increase in renal sodium excretion. The infusions of angiotensin together with hypertonic NaCl induced much more conspicuous polydipsia and more pronounced natriuresis. The animals drank 4 to 6 l. of water and renal Na^+ excretion rose to levels about 1 mequiv/min (= ten times control excretion).

During hydration, 30 min infusions of 0·33 M NaCl or of angiotensin dissolved in hypotonic saline caused little or no drinking. A moderate inhibition of the water diuresis became apparent towards the end of the infusion period and lasted for about 40 min. Renal sodium excretion became moderately elevated (two to three times control level). Also in the hydrated goat, however, the combination of angiotensin and hypertonic NaCl acted as a powerful thirst stimulus, and water had to be withheld in order to prevent fatal overhydration. Conspicuous natriuresis developed during the 30 min infusions (peak level >ten times above control Na^+ excretion). The natriuresis was succeeded by a long-lasting (2 h), profound inhibition of the water diuresis with high urine osmolality.

In two of the goats 5 min intraventricular infusions of 0·33 M NaCl, of angiotensin dissolved in hypotonic saline, and of angiotensin together with 0·33 M NaCl were made alternately during hydration. The brief infusions of 0·33 M NaCl and of angiotensin dissolved in hypotonic saline did not inhibit the water diuresis of the animals. Five minute infusions of angiotensin dissolved in 0·33 M NaCl, however, effectively inhibited the water diuresis for 40 to 60 min. During this antidiuresis urine osmolality rose four to five times, implying that the inhibition was due to release of ADH.

The observation that injections of minute amounts of hypertonic NaCl into the anterior hypothalamus may elicit polydipsia[6] has led to the suggestion that a hypothalamic "osmoreceptor" mechanism in Verney's[7] sense regulates not only the release of ADH but also the urge to drink. Infusions of hypertonic glucose or saccharose into the third ventricle, however, apparently do not elicit thirst or release ADH in the goat[1,8]. This indicates that an elevated Na^+ concentration is a more efficient stimulus to the thirst eliciting and ADH releasing mechanisms than is a rise in extracellular osmotic pressure as such. The present study shows that all the effects on water and salt metabolism that are obtained in the goat by infusions of hypertonic NaCl into the third ventricle may be reproduced to a certain extent by similar infusions of angiotensin. It is also obvious that angiotensin markedly potentiates the effects of hypertonic NaCl infused into the ventricle. A possible explanation might be that angiotensin mimics the effect of an elevated extracellular Na^+ concentration by changing (increasing ?) the transport of Na^+ into brain cells which are involved in the regulation of water and salt metabolism. This would mean that the intracellular, rather than the extracellular, Na^+ concentration may determine the activity of these neurones.

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Antagonism of Pressure and Anaesthesia

THE increase in ion permeability of 4 per cent phosphatidic acid–96 per cent phosphatidyl choline liposomes when exposed to liquid anaesthetics has been reported[1,2], and it was deduced that this increase was caused by increased freedom of movement of the lipid molecules, especially at the aqueous-lipid interface.

In 1950, Johnson and Flagler[3] reported that the spontaneous swimming motion of tadpoles disappeared when

they were anaesthetized with various liquid anaesthetics or when unanaesthetized tadpoles were exposed to hydrostatic pressures of 200–350 atmospheres. When anaesthetized tadpoles were exposed to hydrostatic pressures of 150–350 atmospheres, however, spontaneous swimming motions reappeared.

One possible hypothesis consistent with these facts is that in the anaesthetized state some membrane in the central nervous system of the tadpole is in an expanded state corresponding to the increased freedom of molecular motion in liposomes treated with anaesthetic. One would then expect the application of pressure to compress the membrane back towards the unanaesthetized, unexpanded state. At the same time the concentration or activity of the anaesthetic within the membrane should remain approximately constant, the changes observed being attributed to molecular "packing" effects.

To test this hypothesis we have exposed both newts and liposomes to anaesthetics and pressure. In liposomes we find that the increase in permeability to K^+ and Rb^+ brought about by the anaesthetics butanol, ether and nitrogen in doses just sufficient to abolish the righting reflex of newts can be counterbalanced by an increase in pressure of about 150 atmospheres. If higher doses of anaesthetic are used then higher pressures are required to restore the permeability to the unanaesthetized, atmospheric pressure level. Pressure was applied with the non-anaesthetic gas helium. Application of helium pressure alone led to a fall in ion permeability.

Similarly we have studied the ability of a newt (*Triturus eristatus*) to right itself[4] under the influence of anaesthetics and pressure. Butanol, ether and nitrogen at the doses used on liposomes abolished the righting reflexes. Application of pressure either hydrostatically or with helium restored the righting reflex at around 150 atmospheres. Application of pressure alone led to progressive loss of the righting reflexes above 150 atmospheres.

Both sets of experiments are broadly consistent with our hypothesis, and the remarkable parallelism between the newt and the liposome suggests a mechanism that is consistent in each case with the Meyer–Overton hypothesis which correlates anaesthetic potency with lipid solubility.

The evidence obtained by studying the nuclear magnetic resonance of anaesthetics (for example, benzyl alcohol) on erythrocyte, synaptosome, myelin and vagus nerve membranes[5,6] shows that the anaesthetic causes some increase in freedom of movement of all these membranes, and it is possible that the primary mechanism of action of the anaesthetic in all cases is an increase in the volume of the lipid part of the membrane, equivalent to an increase in disorder or freedom of movement of the lipid molecules. Such an effect might also cause conformational changes in proteins intimately associated with lipid.

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Cell Potassium by ^{39}K Spin Echo Nuclea Magnetic Resonance

ALTHOUGH there is recent evidence[1–4] that cell potassiu is complexed, it is still generally believed that intercellul K^+ is in free solution. Nuclear magnetic resonan (NMR) has already provided direct evidence that anoth cellular cation (Na^+) is complexed[5–8,20,21]. When the sa approach was used to study cell ^{39}K, the first measuremen barely detected an NMR signal from saturated aqueo KCl with the same instrumentation which measured N at a concentration of 0·005 M. NMR sensitivity was limite primarily by the magnetic fields available from conve tional electromagnets (13,000 gauss). The key to succe sf NMR measurement of ^{39}K was the opportunity for t use, for a few days, of a superconductive magnet t generate a magnetic field of approximately 50,300 gaus Besides increased sensitivity resulting from a high resonant frequency, the superconductive magnet provide additional improvement in signal-to-noise ratio fro prolonged averaging made possible by the high stabilit of the magnetic field. A further improvement in exper mental technique was the use of pulsed NMR, whic meant that relaxation time could be measured directly thus eliminating some of the ambiguity inherent in th interpretation of steady state NMR data. A spin-ech NMR spectrometer (model PS60–AW, Nuclear Magneti Resonance Specialties, Inc.) operating at 10^7 Hz was used with a low noise preamplifier, and RC filtering of approx imately 50 per cent or less of T_2. Before each measure ment, the magnetic field was adjusted for NMR resonanc using the beat pattern of a saturated solution of K_2CO (12 M K^+). In spite of the improvements in instrumenta tion, the measurement of K^+ in a living system would stil not have been possible but for the use of a bacterium (*H. halobium*) with an extraordinarily high concentration o K^+ (4–5 M) (ref. 9 and unpublished results of M. and B Ginzburg).

At the NMR resonance of ^{39}K, a 1·0 M KCl solution subjected to a single 90° pulse showed a free induction decay (Fig. 1, lower), which conformed to a single exponential (Fig. 1, upper). Because the observed free induction decay is considerably shorter than would have been produced by the inhomogeneity of the field of our magnet

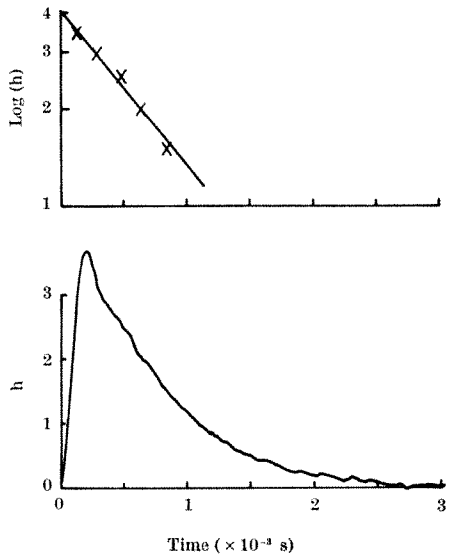


Fig. 1. Free induction decay of 1 N K^+. The lower curve shows free induction decay of 2 ml. of aqueous 1 N KCl solution at approximately 28° C. The voltage output of the sample (h) in arbitrary units is plotted against time after a single 90° pulse, which was repeated every 15×10^{-3} s. Data shown here are the mean of 400 repetitions of the experiment. RC filtering of $0{\cdot}52 \times 10^{-3}$ s was used. The upper curve is a semilog plot of the same data. Linearity indicates a single exponential decay, for which T_2 was estimated to be $0{\cdot}9 \times 10^{-3}$ s.

($1:10^5$ DSV), and because the shape of free induction decay produced by magnetic inhomogeneity should be Gaussian rather than simple exponential, we believe that the free induction decay in Fig. 1 is a measure of transverse NMR relaxation time (T_2). A similar interpretation has been used in other systems[12]. Three measurements of free induction decays of ^{39}K in aqueous 1·0 M KCl yielded a mean value of $0{\cdot}8 \times 10^{-3}$ s for T_2. As far as we know, this is the first published measurement of T_2 of ^{39}K in any state (liquid, solid, or biological). Only two steady state NMR studies of ^{39}K are known to us[10,11].

The concentration dependence of T_2 of aqueous ^{39}K was studied briefly. Mean values of T_2 for 1 M KCl, 4 M KCl, and 12 M K^+ (K_2CO_3) were 0·8, 0·9, and $1{\cdot}15 \times 10^{-3}$ s respectively. These data suggest that T_2 increases with increasing K^+ concentration, in contrast to Na^+, which behaves in the opposite way[14]. The difference may relate to the opposite effects of these two cations on the structuring of H_2O (ref. 15).

The NMR behaviour of K^+ in packed, wet ion exchange resin ('Dowex 50') is shown by the data in Table 1. T_2 observed for the K^+ signal in the wet resin is almost equal to that in aqueous solution. But the initial height of the free induction decay of K^+ in wet resin is only 41 per cent of the height expected for an aqueous solution of the same K^+ concentration as in the resin, indicating that 59 per cent of resin K^+ is invisible to this NMR technique (Table 1). It is reasonable to suppose that the invisible fraction represents K^+ complexed by the resin at charged sites with large electric field gradients, which interact with the quadrupole electric moment of ^{39}K to produce a T_2 which is significantly shorter than the instrumental filtering time constant of $0{\cdot}25 \times 10^{-3}$ s.

Table 1. NMR BEHAVIOUR OF K^+ IN PACKED WET ION EXCHANGE RESIN

	T_2	h_0
1·67 M K^+ on 'Dowex 50' resin (measured)	0·55	7·0
Aqueous 1·0 M KCl (measured)	0·60	10·1
Aqueous 1·67 M K^+ (calculated)		18·2

T_2 transverse NMR relaxation time in units of 10^{-3} s; h_0, initial height of the free induction decay, which was determined by extrapolation of the linear semilog plot back to $t=0$. The same arbitrary units of h_0 are used for both resin and control. Because h_0 should be directly proportional to concentration of K^+ (ref. 12), a 1·67 M KCl solution should have $h_0=18{\cdot}2$, which compares with $h_0=7{\cdot}0$ observed for the 1·67 M K^+ which was measured by flame photometry[13] in the ion exchange resin sample. The resin and the 1·0 M KCl both were 2 ml. in volume, and were measured in identical conditions, which include 2,048 repetitions averaged, RC filtering of $0{\cdot}25 \times 10^{-3}$ s, and a repetition rate of one experiment per 56×10^{-3} s. The cation exchange resin, 'Dowex 50-X4' (100–200 mesh), was converted to the potassium form by washing the resin on a Buchner funnel with 1 N KOH followed by 10 mM KPO_4 buffer (*p*H 8·0) and then packed by the same technique of centrifugation which was used for the bacteria. A sample (2 ml.) of this packed wet resin was used for NMR analysis.

Table 2. NMR BEHAVIOUR OF K^+ IN PACKED BACTERIA (*H. halobium*)

Sample	T_2 Data	Mean
Bacteria (sample 1)	0·37	0·37
Bacteria (sample 2)	0·40, 0·26	0·33
Aqueous 1·0 M KCl	1·25, 0·50	0·87

T_2 is transverse NMR relaxation time in units of 10^{-3} s. The bacterial samples and 1·0 M KCl were 2 ml. in volume, and were measured in identical conditions, which include RC filtering of $0{\cdot}3 \times 10^{-3}$ s, and a repetition rate of one experiment per 15×10^{-3} s. The number of measurements averaged varied from 1,250 to 4,000. The bacteria were grown approximately by the method of Kushner[19], and were packed into 2 ml. pellets by centrifugation at 100,000*g* for 30 min. K^+ concentrations of $2{\cdot}89 \pm 0{\cdot}13$ M and $3{\cdot}10 \pm 0{\cdot}10$ M per unit volume of packed bacteria were measured for samples 1 and 2 by techniques described before[13].

From K^+ in packed bacteria, we obtained a free induction decay, which was single exponential in shape (Fig. 2), from which T_2 was measured (Table 2). Because these values of T_2 for K^+ in bacteria (mean = $0{\cdot}35 \times 10^{-3}$ s) are almost as short as the RC filter time constant of the spectrometer ($0{\cdot}30 \times 10^{-3}$ s), it is possible that the real value of T_2 for K^+ in bacteria is considerably shorter than we recorded. The short T_2 of K^+ in bacteria compared with aqueous K^+ (Table 2) implies that electric field gradients near K^+ are higher in bacteria than in free aqueous solution, which suggests that cell K^+ is complexed by fixed charges[1–4,9], and/or is solvated in semicrystalline cell water (refs. 12, 16–18 and our unpublished results).

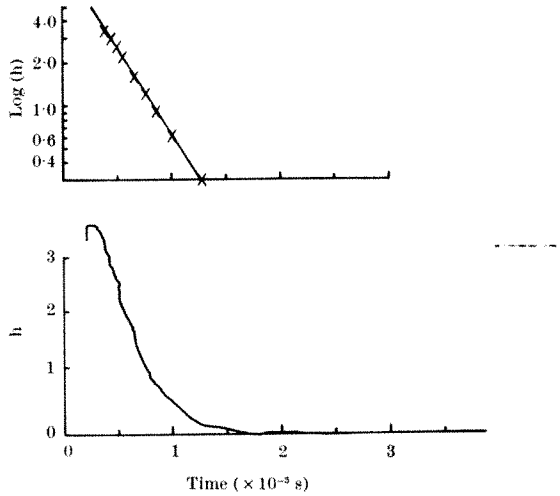


Fig. 2. Free induction decay of K^+ in bacteria. The lower curve shows free induction decay of K^+ in 2 ml. of packed bacteria (*H. halobium*) at 28°–33° C. The voltage output of the sample (h) in arbitrary units is plotted against time after a single 90° pulse. Data shown here are the average of 4,000 repetitions of the experiment at a rate of one repetition per 15×10^{-3} s. RC filtering of $0{\cdot}3 \times 10^{-3}$ s was used. The upper curve is a semilog plot of the same data. Linearity indicates a single exponential decay, for which T_2 was estimated to be $0{\cdot}37 \times 10^{-3}$ s.

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Structural Implications of Banding in Unstained Collagen

THE three currently accepted three dimensional models for the structure of the collagen fibril predict alternating regions of high and low density along the length of the fibril, with a repeat length (D) of about 650 Å (Fig. 1*a*)[1–3]. As in the previous two dimensional "modified quarter-stagger" model[4,5], the denser regions (A bands) are about 0·4 D in length, the less dense or "hole" regions (B bands) are about 0·6 D, and the tropocollagen molecular length is 4·4 D. The electron micrographs of negatively stained native and reconstituted collagen, on which the models are largely based, vividly display large banded features which have been interpreted as due to the higher permeability of the B bands to the dense metal stain[6,7]. Although electron micrographs of unstained collagen fibrils could be expected to reflect the intrinsic longitudinal density distribution, very few studies have included such micrographs, and these were not detailed[8,9]. Consequently, we have re-examined unstained collagen fibrils by a simple technique. The observed density patterns seem to conflict with the above models and with the prevailing interpretation of negative staining in collagen.

Collagen fibrils were separated from the fresh Achilles tendons and tail collagen of 6 month old white rats by 10 kHz sound waves in distilled, deionized water. The supernatant was transferred to collodion-coated copper grids and dried. Negatively stained preparations were made with sodium phosphotungstate (Na-PTA) at *p*H 7·4 according to the technique of Cox *et al.*[10].

Micrographs were made on a modified RCA EMU 3-D electron microscope at 100 kV.

Fig. 1*b* shows an unstained tendon collagen fibril. Noteworthy are the wider dark bands with at least two asymmetrically located light lines in each, and the narrower light bands with two very light lines at their extremities. The widths of the dark and light bands are approximately 0·58 D and 0·42 D respectively; where D, the repeat length, is about 655 Å. For comparison, Fig. 1*c* shows a typical negatively stained fibril of rat tail collagen. The similarities in relative band lengths, relative densities and fine structure lead to the identification of the A and B bands in the unstained specimens exactly as in the negatively stained fibrils[6]. The same unstained banding pattern has been observed in beef tendon fibrils after demineralization by acid.

This assignment is confirmed in Fig. 2 which shows a fibril that was negatively stained for only a portion of its length. The continuity and phase of the banding pattern across the border region are maintained, in spite of the reduction in contrast.

Although the proposed models for the collagen fibril predict 0·6 D regions (B bands) of lower density than the A bands, the current study of unstained fibrils shows the reverse to be true. Our interpretation is based on the fact that, in the absence of coherent scattering, the relative intensities in the electron image are simply a function of the mass/unit-area of the specimen and are relatively independent of the scattering species[11]. As the profiles show no significant difference in thickness from one band to the next, one can suppose that the unstained pattern (Fig. 1*b*) truly represents the longitudinal density distribution in the fibril. The Na-PTA stain, furthermore, serves merely to emphasize the natural band densities rather than to reverse them.

It is possible to reconcile the three dimensional models with the unstained density distribution in at least two ways. First, if the length of the tropocollagen molecule were 4·6 D (about 3000 Å) instead of 4·4 D and the hole regions were 0·4 D, the densities would conform to the observed pattern. There then would be five 0·6 D dense regions and four 0·4 D "hole" regions per equivalent molecular length. Alternatively, a dense material can be supposed normally to inhabit the 0·6 D hole regions and be replaced by or bind Na-PTA during the staining. Quantitative determinations of the mass

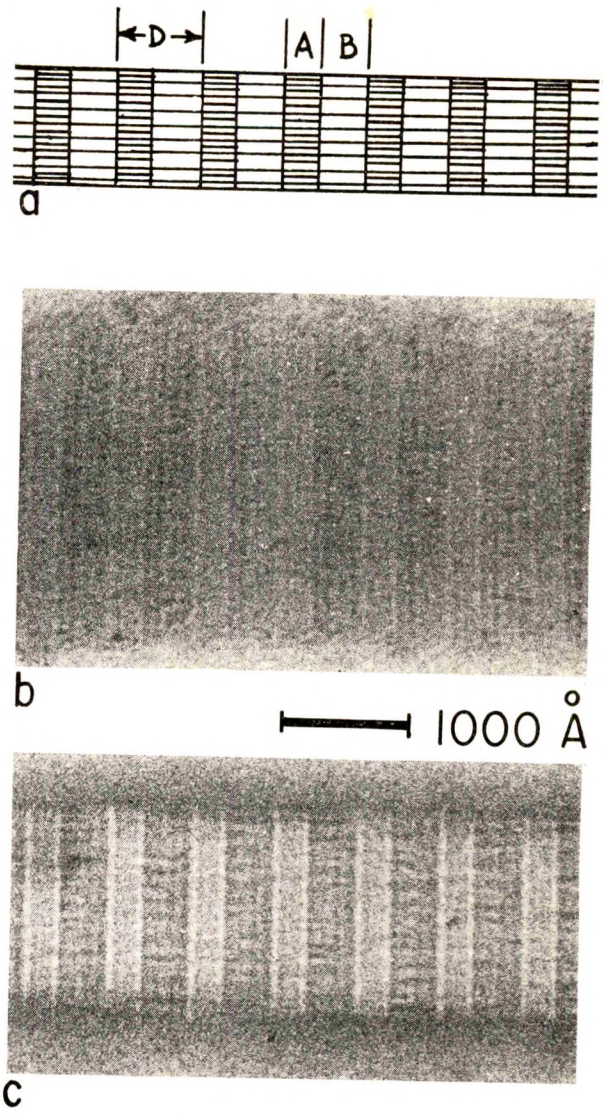


Fig. 1. *a*, Simplified qualitative representation of the density pattern predicted by the currently accepted models for the collagen fibril. *b*, Electron micrograph of an unstained collagen fibril from a rat Achilles tendon. Note the larger dense "B bands" and lighter "A bands", contrary to the predictions of the current models. *c*, Rat tail collagen fibril "negatively" stained with Na-PTA (also typical of negatively stained Achilles tendon fibrils). The correspondence of the two banding patterns suggests that a reversal in contrast does not occur with the stain, except at the margins of the fibril.

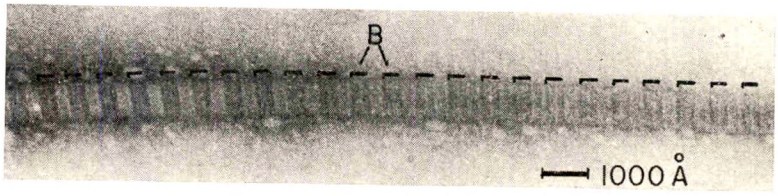


Fig. 2. A portion of a tendon fibril that was "negatively" stained along the left hand half and unstained on the right. This was accomplished by exposing half of a prepared unstained grid to the Na-PTA solution and choosing a fibril that crossed between the stained and unstained regions. The continuity and phase of the banding pattern across the micrograph are emphasized by the brackets adjacent to the B bands.

density along the unstained native fibril are being undertaken and should serve to distinguish these possibilities and refine a better model.

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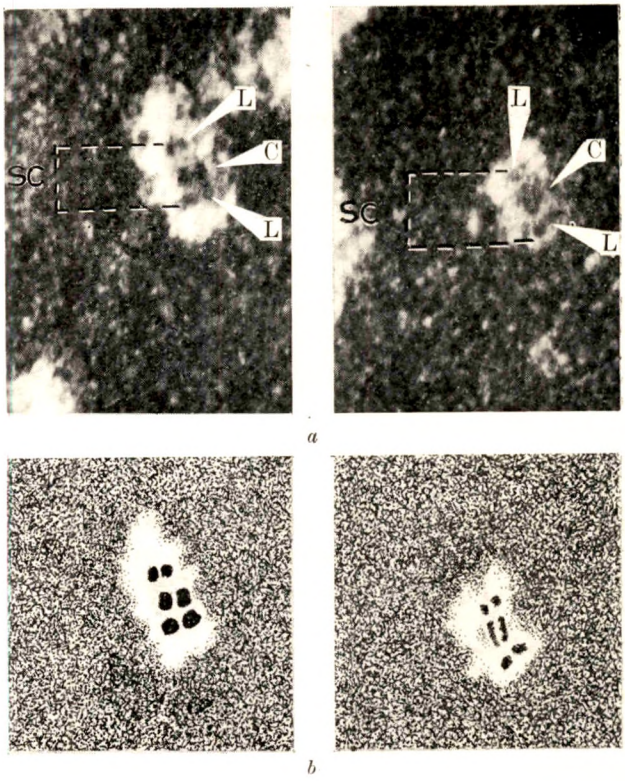


Fig. 1. *a*, Transverse sections through synaptonemal complex of synizetic chromosomes showing arrangement of different elements. L, Lateral element; C, central element. *b*, Line drawings of *a* (both ×48,750).

Axial Elements in Plant Meiotic Chromosomes

THE importance of the synaptonemal complex (SC) has been confirmed by its regular occurrence in sexually reproducing plants and animals. In spite of the regular presence of elements of the SC in chiasmatic as well as in achiasmatic[1] meiosis, in haploids, diploids and triploids[2] its precise function is uncertain. It is a matter of conjecture that the SC serves alternately or simultaneously as a mechanism for synapsis and/or recombination. It is almost certain, however, that the SCs are not essential to recombination itself (that is they are not present in prokaryotes and do not accompany somatic crossing over[3]) but are required for the more regular and extensive exchange at meiosis.

Anthers of the diploid lily (variety Enchantment Orange) were fixed with glutaraldehyde followed by OsO_4, essentially as described by Ledbetter and Porter[4]. Thin sections were cut on a Porter Blum microtome, stained with uranyl acetate and lead citrate and viewed with a Siemens Elmiskop I at 80 kV. Transverse sections through the SC of fifty-eight nuclei at meiotic prophase with a synizetic knot of the chromosomes[5] have revealed the bipartite nature of the lateral as well as of the central elements (Fig. 1). The typical arrangement is that of a pair of bipartite lateral elements separated by a bipartite central element. The diameter and arrangements of individual elements observed in transverse sections are compatible with those observed in the same experimental material through longitudinal sections (Fig. 2).

After premeiotic DNA replication, the two axial elements of the sister chromatids lie side by side. In most of the material studied earlier, each individual chromosome seems to consist of one chromatid. This is because of the compact nature of their association due to the presence of protein rich substances. In haploids of *Petunia* and *Antirrhinum* at leptonema, I have found axial elements which are bipartite in agreement with earlier reports[3,6,7].

After replication, and because of the characteristically compact organization of axial elements, segregation of chromatids may not take place in individual sister chromatids until the SC is organized[8,9]. This agrees with the long established finding that four-strand crossing over takes place at pachynema. At pachynema (synizesis[10]), "tetrads of lateral elements"[3] have been observed from single SC (Fig. 1). The cores in bivalents of crayfish spermatocytes were reported to consist of five strands[11]. In cross-section there were two strands on each side of a central element. Presumably the complex represented the two chromatids joined by a fifth element which occurred at the interface between the homologous chromosomes.

This work was carried out at the Max–Planck–Institut für Züchtungsforschung, Cologne, with the financial assist-

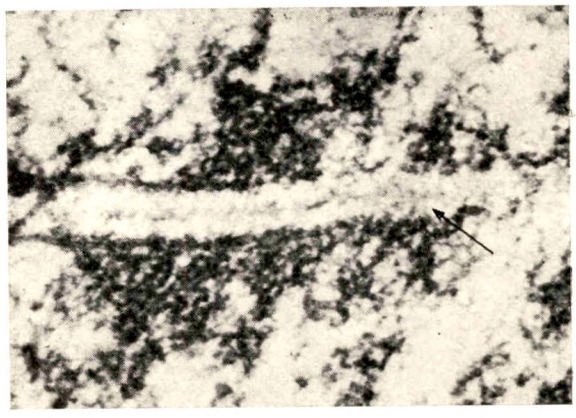


Fig. 2. Longitudinal section through synaptonemal complex of synizetic chromosomes showing dense microfibrils (arrowed) (×45,000).

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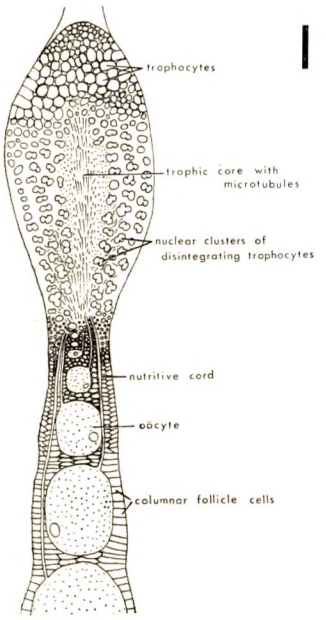
Extensive System of Microtubules in the Ovariole of *Dysdercus fasciatus* Signoret (Heteroptera: Pyrrhocoridae)

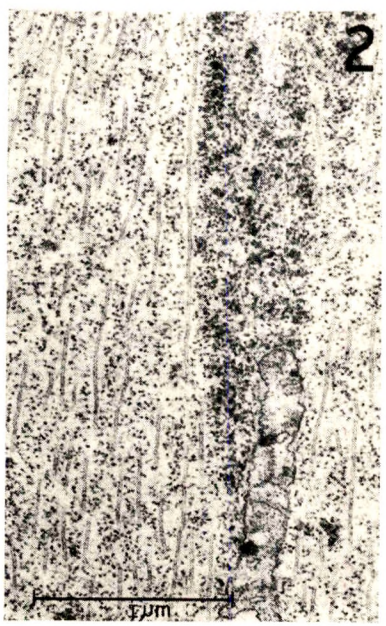
MICROTUBULES are especially interesting cell organelles because important intracellular functions are continually being attributed to them which can often be related to their patterns of distribution. These proteinaceous tubules, 180–300 Å in diameter, occur in plant[1] and animal cells[2], mostly in small numbers. Using material fixed with glutaraldehyde and stained with uranyl acetate and lead citrate, I have found that in the ovarian trophic core and nutritive cords of the bug *Dysdercus fasciatus* (Fig. 1) microtubules occur in similar high densities (Figs. 2, 3 and 4) to those in some nerve axons[3] and specialized structures of Protozoa[4]. In a longitudinal section of the trophic core, they cover an unusually large area (up to 350 × 80 μm). Their external diameter is 270 Å.

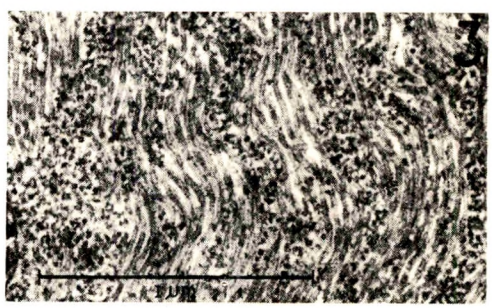
The microtubules in the trophic core and nutritive cord run longitudinally and roughly parallel (Fig. 2). In the nutritive cord and centre of the trophic core, they are straight in longitudinal section, but elsewhere in the trophic core, they form flow-like patterns around the trophocytes (Fig. 3). The microtubule density is roughly constant throughout the trophic core and nutritive cord, but slightly less in the latter, for the tubules are roughly 1000 Å apart in both tissues, but 3000 Å apart in the nutritive cord (Fig. 4). Ribosomes are always seen between microtubules. The tubules form bundles in the posterior part of the trophic core and in the nutritive cord (Fig. 3), and between these bundles are ribosomes, glycogen granules and mitochondria (Figs. 2 and 3). The two most important roles of microtubules are thought to be in guiding cytoplasmic movement[5] and in intracellular structural support (unpublished results of J. R. Gibbins, L. G. Tilney and K. R. Porter).

In *Dysdercus*, the nutritive cord may be 3 mm long. It is surrounded by columnar follicle cells which become squamous as the oocytes grow. I suggest that because of this change of shape and oocyte growth, a longitudinal tension develops which could constrict the nutritive cord. It therefore needs support and in this need is comparable with long, thin structures such as axopodia, cilia and flagella which protrude into the surrounding environment. Here support is provided by geometrically arranged microtubules and in cilia and flagella the 9 + 2 arrangement also plays an important part in movement. The nutritive cord is also comparable with cells under permanent stress, such as certain plant cells and precuticularized insect epidermal cells which do not protrude and therefore do not need such a rigid support. The microtubules found in these cells may also serve a supporting function[6,7]. I suggest that in the nutritive cord of *Dysdercus* the microtubules represent an intermediate situation. They support the nutritive cord but have a more random arrangement than in protruding structures or motile systems, a greater density than insect or plant epidermal cells, and they cover a much greater area.

The oocytes differentiate in series, the largest and oldest being most distal. The distance from an oocyte to the germarium therefore increases during development and the nutritive cord must lengthen accordingly. During adult life, this growth can be by a factor of × 15 (from 0·2–3·0 mm). Throughout its length, the diameter of the cord (30 μm) is approximately constant, as is the density of microtubules. This means that the tubules must either lengthen individually (which seems most likely) or multi-







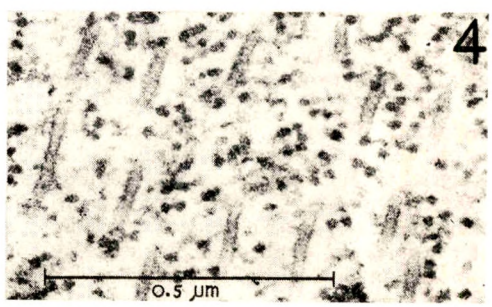


Fig. 1. Diagrammatic representation of the anterior part of the ovariole (not to scale). Fig. 2. Microtubules, ribosomes, glycogen granules and mitochondria in the trophic core. Fig. 3. Microtubules in simple flow-like patterns between trophocytes. Fig. 4. Showing size and spacing of microtubules.

ply. They may therefore serve both as general support for the cord and as important elements in its morphogenesis.

The trophic core is a large, syncytial area which, like the nutritive cord, needs internal support, to prevent invasion or obliteration by disintegrating posterior trophocytes.

In the absence of resisting structures such as microtubules, these cells could wander, for they are not attached to a basement membrane, or interconnected by desmosomes. Further, in telotrophic ovarioles, nutritive material passes from the germarium to the oocytes[8]. Without some internal support disintegrating cells might be swept inwards with this cytoplasmic movement.

In a longitudinal section of the trophic core, microtubules form three zones. They are cut in transverse section at the edge of the core, longitudinally in the centre and obliquely in the intermediate region. It is not yet clear whether these are distinct populations or one system of tubules which bend, but these observations nevertheless confirm that the tubules are the principal strengthening material within the tissue.

Anterior to the trophic core is a ligament which suspends the ovariole in the abdominal haemolymph. During oocyte development this ligament is subject to longitudinal stress. In the trophic core, the central zone of microtubules may help the ligament to sustain this tension while the peripheral zones may function in maintaining the cylindrical shape of the core.

Microtubules are also thought to be involved in protoplasmic streaming and it seems likely that the microtubules of *Dysdercus* could serve this function in addition to support. Indeed, this might be their most important function, for in the trophic core and nutritive cord, the microtubular bundles could act as boundaries of channels through which nutritive substances pass to oocytes. They could thus play a very large part in oocyte development and the importance of not allowing the trophic core to be invaded by trophocytes would then become clear.

MacGregor and Stebbings[9] report a massive microtubule system in *Notonecta* ovaries and it therefore seems likely that extensive microtubule systems will soon be recognized as an important characteristic, both of telotrophic ovarioles and cytoplasmic masses in general.

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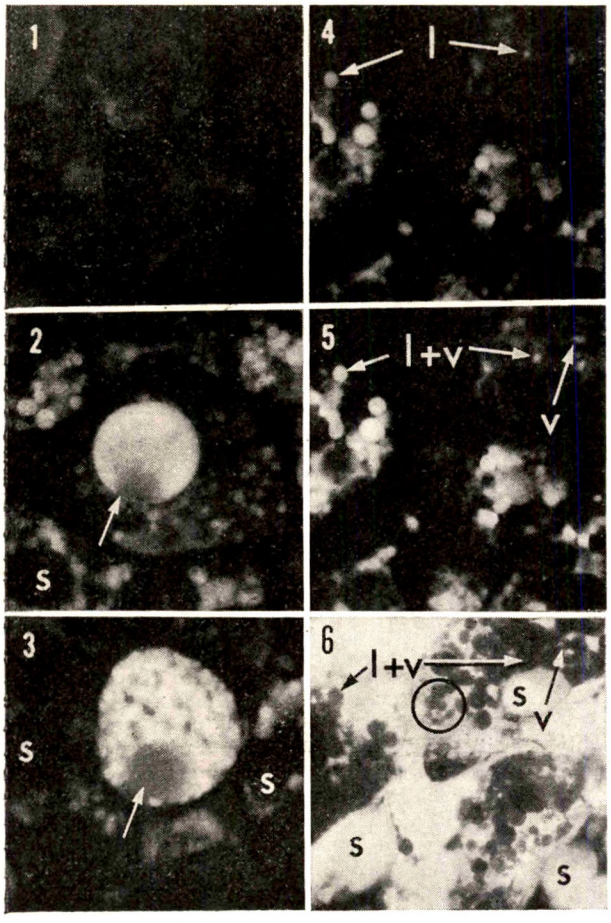
Localization of Legumin and Vicilin in Bean Cotyledon Cells using Fluorescent Antibodies

SUBCELLULAR fractions of bulked protein bodies isolated from the storage tissues of seeds contain several or many types of protein[1–3], but in general it is not known whether this diversity occurs within an individual protein body, or whether the different proteins are deposited in separate compartments during seed maturation. Immunofluorescence techniques have enabled us to resolve this question in respect of the subcellular distribution of legumin and vicilin, two important globulins stored in the cotyledons of many legumes[4–6].

Fluorescent antibodies were prepared, using as antigen mixed legumin and vicilin extracted from immature seeds of *Vicia faba* L. variety Sharpe's Conqueror, and purified by repeated ammonium sulphate prepicitation, dialysis and molecular sieve chromatography. Rabbits were hyperimmunized by subcutaneous injections, and homologous antibodies were adsorbed directly from antisera onto columns of *V. faba* globulins cross-linked by treatment with glutaraldehyde[7]. Eluates[7] were concentrated, and labelled with fluorescein isothiocyanate (FITC)[8] to provide a mixed conjugate from which FITC-anti-legumin and FITC-anti-vicilin could be isolated. Further purification was required because anti-legumin has been reported to cross-react with vicilin and anti-vicilin with legumin[5,9], a result which either confirms that the two antigens have amino-acid sequences[6] and thus some antigenic determinants in common, or suggests that the antigen preparations used were impure. Like other workers using isoelectric precipitation[4–6,10], we found difficulty in preparing pure samples of vicilin and legumin. But by centrifuging samples of the FITC-mixed conjugate which had been treated with either excess legumin or excess vicilin preparations, supernatants containing only antibodies that were demonstrably incapable of cross-reacting were obtained. Double diffusion tests confirmed that although the conjugates had sustained reduction in titre they consisted only of highly specific FITC-anti-vicilin or FITC-anti-legumin. The mixed or these monospecific conjugates were applied singly or sequentially, during 1–7 h periods at 37° C, to approximately 10 μm transverse sections of bean cotyledons, cut in a cryostat and representing various developmental stages. The cotyledon tissues had been fixed in 2·5 per cent glutaraldehyde and subsequently stored in cold buffer containing hibitane. No non-specific fluorescence could be detected in control sections pretreated with unlabelled antisera (Fig. 1).

Fluorescence microscopy indicated that the synthesis of vicilin precedes that of legumin in the developing cotyledons. Cells located in and close to the vascular strands, or in a paradermal zone underlying the abaxial epidermal transfer cells[11], become labelled earlier in seed maturation than most others. At the subcellular level, legumin and vicilin are clearly located within protein bodies (Figs. 2–6). The identification of the protein bodies and the presence in them of storage globulins have also been checked by electron microscopy using ferritin-labelled antibodies. Some protein bodies were not completely filled with the antigens (Fig. 2), and the distribution of fluorescence often paralleled the distribution of the marginal deposits of electron-dense material considered to be protein and seen in ultrathin sections of developing protein bodies of *V. faba*[12]. Enhancement of fluorescence after sequential staining with FITC-anti-legumin and FITC-anti-vicilin (in either order) showed that most reactive protein bodies contained both globulins (Figs. 4 and 5). A very few, perhaps transient developmental stages, were found to contain vicilin but no detectable legumin (Fig. 5).

Cells of immature cotyledons contain in addition protein bodies which apparently lack both legumin and vicilin (Figs. 3–6) and which cannot be induced to fluoresce even after freezing and thawing or treatment with acetone or Triton-X100. Sequential staining with mercuric-bromo phenol blue[13] or acid fuchsin at low *p*H[14] (Fig. 6) reveals that proteins are nevertheless present in them. They are found at most stages of development, including the early period when vicilin is sparse and legumin is but a trace component. Because some legumes begin to accumulate albumins before their globulins[15,16], these non-fluorescent protein bodies may represent a type that specializes in the storage of albumins; alternatively



Figs. 1–3. Fluorescence photomicrographs of portions of *V. faba* cotyledons fixed at 63 days from anthesis. Original photographs on high speed Ektachrome using Zeiss UG 1 exciter filter and −65 and 41 barrier filters. (1) Control (see text)—no green fluorescence. (2) and (C) Reaction with FITC-labelled mixed anti-legumina and anti-vicilin. Both of the arrowed protein bodies are large enough to have been cut open during sectioning; one (2) evidently contains antigen(s), the other (3) does not. Both figures include numerous smaller, labelled protein bodies.

Figs. 4–6. Sequential staining of the same field of view with: (4) FITC-anti-legumin; (5) FITC-anti-vicilin; (6) acid fuchsin (bright field microscopy). The enhancement of fluorescence as between (4) and (5) is shown by most protein bodies (for example 1 compared with 1+v) and indicates the presence of both legumin and vicilin. It is much more obvious in the original colour photographs. v Represents a protein body containing no detectable legumin. Other protein bodies (such as those encircled in 6) do not react with either antibody. s, Starch grain. (Figs. 1, 4, 5 and 6, ×350; Figs. 2 and 3, ×200.)

they might be albumin-containing precursors, destined to receive legumin and vicilin at a later stage. It is intended to clarify this point through the use of labelled anti-albumin antisera, and it should be possible to determine whether the cohabitation now established for legumin and vicilin extends further to include albumins.

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Are Endogenous Gibberellin-like Substances involved in Floral Induction

The concentration of endogenous gibberellin-like substances has been reported to increase in cold-requiring plants during exposure to a low temperature[1–3]. Synthesis of gibberellin is also known to be blocked by certain growth retardants, some of which inhibit flowering[3–6]. Thus there is increasing evidence for an involvement of gibberellin in floral induction. In studies with broccoli (*Brassica oleracea* variety *italica*), it was found that low temperature enhanced flowering and that (2-chloroethyl)trimethylammonium chloride (CCC) did not retard floral induction, but that 1,1-dimethylaminosuccinamic acid (B995) did[7,8]. Although both these compounds are considered to be growth retardants, B995 has not been shown to affect the endogenous concentration of gibberellin. It was therefore important to determine the effects of CCC and B995 on this concentration in broccoli in relation to floral induction.

Broccoli plants were treated twice with CCC as a soil drench at a concentration of 0·5 g/plant to give a total of 1 g/plant. B995 was applied as a spray in a concentration of 2,500 p.p.m. These retardants were applied 35 and 46 days after seeding in a 24°–27° C growth chamber (1,800 foot candles for 16 h daily). On the day after the last treatment with these retardants plants were transferred to a 5° C chamber (350 foot candles for 16 h daily) for 3 weeks. Control plants were grown continuously at 24°–27° C. After the time at 5° C treated and control plants were collected and frozen to await analysis for gibberellin. Nine plants which had received each treatment followed by subjection to cold were returned to a growth chamber at 24°–27° C for a further week. These and control plants were then examined with respect to growth and flowering.

Plant tissue to be analysed for gibberellin-like substance was homogenized in 90 per cent aqueous methanol (1 : 5 w/v). After extraction at −20° C for 24 h the homogenate was filtered and extraction was repeated on the residue. The residue was then extracted with half the volume of methanol used in the first extraction. Combined methanol extracts were flash evaporated until only the aqueous phase remained. The evaporating flask was rinsed several times with water and 5 per cent $NaHCO_3$. This aqueous solution was centrifuged to remove precipitated substances. The supernatant was partitioned five times with equal volumes of chloroform to remove pigments and lipids, and the aqueous phase was adjusted to *p*H 3·0 and partitioned three times with ethyl acetate. Combined ethyl acetate fractions were evaporated to dryness.

The gibberellins in the ethyl acetate residues were chromatographed on silica gel partition columns by the method of Powell and Tautvydas[9]. The lettuce hypocotyl bioassay was used to detect gibberellin-like substances[10]. Each fraction from the column of silica gel was bioassayed in triplicate, and all samples were run in duplicate.

None of the plants grown at 24°–27° C initiated floral primordia. At 5° C all those treated with CCC flowered

and 88 per cent of control plants flowered. Only 33 per cent of plants treated with B995, however, were induced to flower at 5° C.

Fig. 1*A* shows that plants grown continuously at 24°–27° C contained little gibberellin-like substances. The content of these substances seems to be increased by treatment at 5° C (Fig. 1*B*). There are also zones of activity present in plants kept in the cold which are not readily observable at 24°–27° C. This suggests a qualitative change in gibberellin-like substances when plants were subjected to cold temperatures.

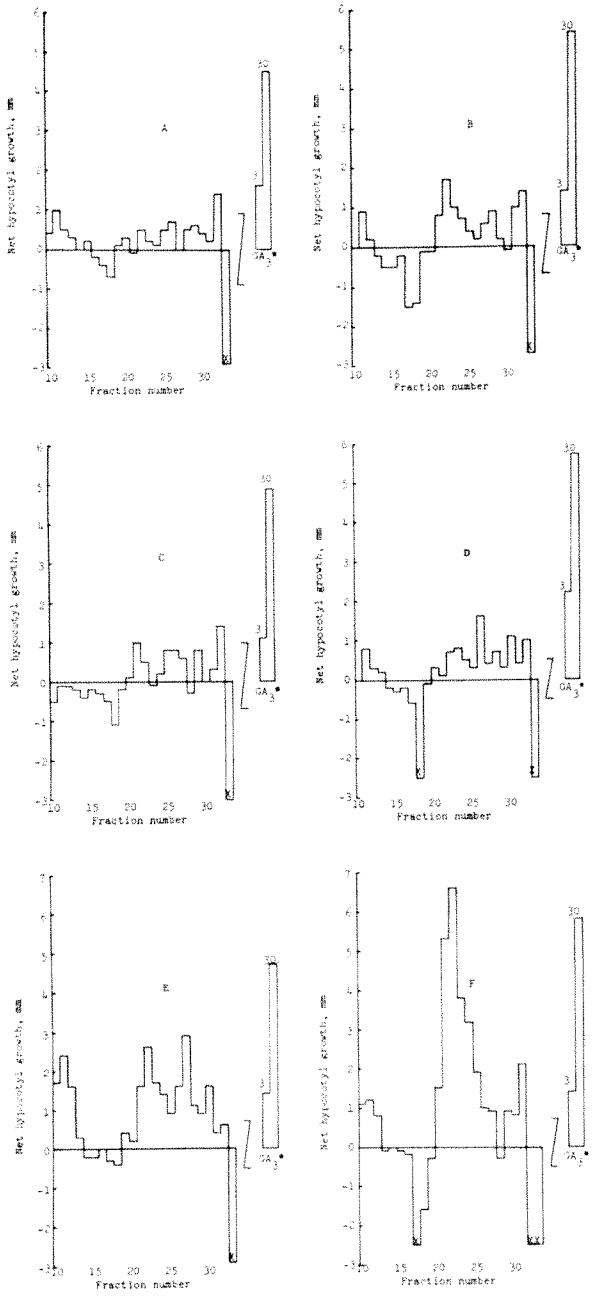


Fig. 1. Methanol extractable gibberellin-like substances in Watham 29 broccoli. Plants were (*A*) grown continuously at 24°–27° C; (*B*) exposed to 5° C for 3 weeks; (*C*) treated with CCC and grown continuously at 24°–27° C; (*D*) treated with CCC and exposed to 5° C for 3 weeks; (*E*) treated with B995 and grown continuously at 24°–27° C, and (*F*) treated with B995 and exposed to 5° C for 3 weeks. Gibberellin-like substances were detected by lettuce hypocotyl bioassay after column chromatography. Weight of tissue extracted was: (*A*) 297 g; (*B*) 352 g; (*C*) 397 g; (*D*) 345 g; (*E*) 284 g; (*F*) 324 g. X, Germination inhibited; Z, spread of controls. * ng of gibberellic acid/tube.

The influence of CCC on endogenous gibberellin-like substances is shown in Fig. 1*C* and *D*. The concentration in plants grown continuously at 24°–27° C is low (Fig. 1*C*), and there seems to be no increase at the low temperatures (Fig. 1*D*). The concentration of endogenous gibberellin-like substances seems to be reduced slightly by CCC when the weight of tissue extracted and activity present are compared with those of control plants. The number of gibberellin-like substances present in plants treated with CCC at 24°–27° C (Fig. 1*C*) is comparable with those present in untreated plants at 5° C (Fig. 1*B*) except for activity in fraction 10. This suggests that qualitative changes did not occur during cold treatment but rather that the concentration present at 24°–27° C was too small for detection.

The most marked effect on endogenous gibberellin-like substances—an increase at both temperatures—was observed after treatment with B995 (Fig. 1*E* and *F*). Plants grown continuously at 24°–27° C and treated with B995 (Fig. 1*E*) had four prominent peaks of activity. But only three peaks were present at 5° C (Fig. 1*F*). The very large peak from fraction 20–26 (Fig. 1*F*) could have been a combination of the two peaks present at 24°–27° C (Fig. 1*E*). There was a large increase in gibberellin-like substances in plants treated with B995 and subjected to low temperature.

These results suggest that gibberellin-like substances as revealed by this bioassay are probably not involved in floral induction of broccoli. The apparent increase in these substances at 5° C may be a result of decreased growth. It is also clear that B995 does not block the synthesis of gibberellin-like substances in this tissue, which is in agreement with earlier reports[5,6]. B995 therefore must inhibit floral induction through its effects on other processes or as a consequence of there being too high a concentration of gibberellin. Our findings are somewhat paradoxical, for the action of B995 is often reversed by exogenous gibberellin. CCC may have had a slight inhibitory effect on the synthesis of gibberellin-like substances, but not on floral initiation. Thus the use of these two compounds should provide more information about the physiology of flowering.

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Competitive Adsorption of Phosphate with Polygalacturonate and other Organic Anions on Kaolinite and Oxide Surfaces

Some plants are known to remove phosphate readily from soils low in phosphate. It is likely that root excretions and microorganisms greatly modify the chemical environment in the rhizosphere and this may result in the more

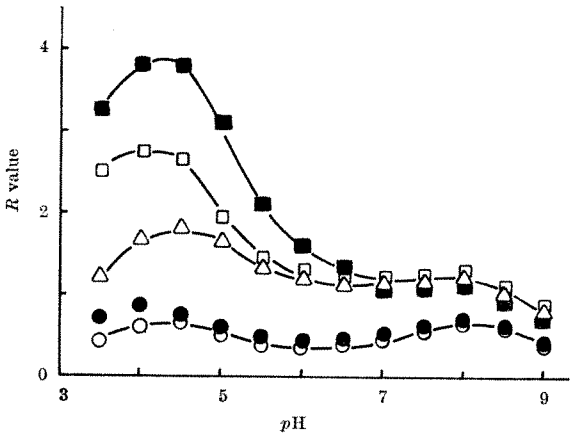


Fig. 1. The relationship between pH and the competitive ability (R) of polygalacturonate (PGA) and galacturonate to desorb phosphate from kaolinite. Initial P, $6{\cdot}45 \times 10^{-6}$ M; ○, 0·0001 per cent PGA; △, 0·001 per cent PGA; □, 0·01 per cent PGA; ■, 0·1 per cent PGA; ●, 0·1 per cent GA.

ready displacement of the adsorbed phosphate from soil colloids. Organic compounds found in the root exudates of several plants, reviewed by Rovira[1], include many organic acids known to form strong complexes with metal ions. Examination of the root–soil interface under the electron microscope has also revealed a slimy gel between the root and the soil colloidal particles[2,3], which has been called "mucigel" and is considered to consist of pectic substances, chiefly partially methylated polygalacturonic acids.

We have investigated the effect of organic compounds known to be exuded by plant roots on the adsorption of phosphate by kaolinite, gibbsite and goethite. Kaolinite (0·5 g in 200 ml.) or oxide (0·05 g in 25 ml.) was added to a solution containing either phosphate alone or phosphate and organic anion in combination in 0·01 M KCl, and equilibrated for 24 h at $21 \pm 1°$ C. The various organic compounds tested are shown in Table 1. A synthetic pectic acid–polygalacturonic acid (molecular weight approximately 25,000) was used to examine the possible effect of mucigel. The influence of these organic compounds on the adsorption of phosphate by kaolinite and oxide surfaces was assessed by R which is defined by

$$R = \frac{\text{P adsorbed alone} - \text{P adsorbed in the presence of the organic anion}}{\text{P adsorbed in the presence of the organic anion}}$$

This relationship shows that if no decrease in phosphate adsorption occurs, R is zero and when the organic ion completely stops the adsorption of phosphate, R is infinity.

Table 1. COMPETITIVE ABILITY (R_{max}) OF VARIOUS ORGANIC ANIONS ON CLAY AND OXIDE SURFACES

Anion	pK_1	pK_2	Kaolinite		Gibbsite		Goethite	
			R_{max}	pH at R_{max}	R_{max}	pH at R_{max}	R_{max}	pH at R_{max}
Citrate	3·13	4·76	4·3	4·8	6·00	5·25	0·23	4·25
Tartrate	3·03	4·37	1·5	4·3	0·9	5·25	0·1	4·00
Oxalate	1·25	4·28	7·0	4·0	2·6	5·75	0·1	4·00
Malate	3·46	5·05	1·2	4·5	1·0	5·00	0·1	4·00
Succinate	4·21	5·64	0·2	No max	0·2	5·5	—	—
Malonate	2·85	5·7	1·9	4·5	0·6	5·75	—	—
Lactate	3·86	—	0·2	No max	0·2	No max	—	—
Acetate	4·76	—	0·2	No max	0·2	No max	—	—
PGA (0·1 %)*	3–5		4·0	4·25	1·4	4·0	0·4	4·00
Ga (0·1)†	3·48	—	0·5	4·5	0·8	7·0	—	—

The concentration of KCl was 10^{-2} M. The initial organic anion concentration was $1{\cdot}59 \times 10^{-5}$ M; initial P, $6{\cdot}45 \times 10^{-6}$ M; kaolinite, 0·5 g/200 ml.; oxide, 0·05 g/25 ml.

* The concentration of polygalacturonate was 4×10^{-5} M because molecular weight is approximately 25,000.

† The concentration of α-D-galacturonate was 5×10^{-3} M.

The results (Table 1) show that citrate and oxalate are very effective in decreasing the adsorption of phosphate by kaolinite and gibbsite. On the other hand, malonate, tartrate and malate are moderately effective, while acetate, succinate and lactate hardly decrease phosphate adsorption. Other experiments, not reported here, indicate that the amino-acids α-L-alanine, L-threonine, DL-aspartic acid and L-lysine, found in plant root exudates, also failed to decrease phosphate adsorption on any of the three absorbants.

A significant result is the marked effectiveness of polygalacturonate in decreasing phosphate adsorption (Table 1 and Fig. 1) particularly on kaolinite. It is also interesting that the monomer galacturonate hardly decreased adsorption of phosphate (Fig. 1 and Table 1). A related observation is that the polymer has been found to complex Ca^{2+} whereas the monomer does not[4].

Two other features of these results are worth mentioning although no satisfactory explanations can be given. The first is the observation that for any organic anion R is greatest at a pH which corresponds to the second pK value of the organic acid. For polygalacturonic acid R is maximum at what could be its second pK value. The second is that values of R for the organic anions on goethite are very small compared with the values obtained on kaolinite or gibbsite, but even here polygalacturonate is the most effective anion (Table 1).

The organic anions which are effective in decreasing the adsorption of phosphate by the three adsorbents are those capable of forming chelates containing five and/or six membered rings with metal ions[6–8]. Polygalacturonate, which was as effective, if not more so, than the other chelating agents used, is also known to form stable chelates with metal ions[9,10]. Phosphate is considered to be "specifically" adsorbed on kaolinite and oxides by the formation of a surface complex through ligand exchange of phosphate with H_2O molecules and/or OH^- coordinated with Fe^{3+} (goethite) or Al^{3+} (kaolinite and gibbsite) ions in the surface[11,13]. Detailed studies of the adsorption of citrate have shown that citrate is also specifically adsorbed by these adsorbants[5,14]. Thus, when phosphate and an organic anion are present together, the decrease in phosphate adsorption by an adsorbant must arise from the specific adsorption of the organic anion resulting from competition between phosphate and the organic anion for adsorption sites. Whether an organic anion is capable of decreasing phosphate adsorption or not would be determined by the relative stabilities of the Fe (or Al)–organic anion complex and the Fe (or Al)–phosphate complex.

The marked effectiveness of polygalacturonate in decreasing phosphate adsorption by the three adsorbants indicates that plants may possess a very efficient way of desorbing phosphate from soil colloids at the root/soil interface by utilizing the large concentration of pectic substances in the mucigel around their roots.

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Field Evidence for Polarized Light Sensitivity in the Fish *Zenarchopterus*

POLARIZED light perception has never been unequivocally proven in vertebrates except man. An unusually alert human observer can see by inspection the plane of polarization (*e*-vector) in linearly polarized light by virtue of Haidinger's brushes, a faint blue and yellow image of intraocular origin produced by the selective absorption of polarized light by the yellow macular pigment of the fovea. Attempts to demonstrate polarized light perception in other vertebrates have, however, usually failed (for example in birds[1,2]).

Nevertheless, there are actually three previous reports of polarized light responses by teleost fishes[3–5], but none of these was adequately followed up to provide needed additional data or reasonable explanations for certain ambiguous or anomalous experimental results. We now report consistent evidence that the hemiramphid teleost *Zenarchopterus*, a west Pacific half-beak, can have its directional preferences affected by linearly polarized light.

Using SCUBA small specimens of *Zenarchopterus* (*Z. dispar* (Cuvier and Valenciennes) and *Z. buffoni* (Cuvier and Valenciennes)) were placed in a covered flat cylindrical dish and positioned at the focus of an automatic Robot camera directed vertically upward. The azimuth orientation of the fish was photographed every 5 or 10 s. Set on a tripod in 3–5 m of clear calm water the apparatus was screened so that except for a lens aperture the fish could not see the equipment, the observers or landmarks on the bottom (Fig. 1). The upper half of the fish's visual field, however, comprised the surrounding water, its surface and, within the critical angle, the Sun and sky. The behaviour of the fish confined under these conditions was usually calm but responsive.

Table 1*. ORIENTED BEHAVIOUR OF HALF-BEAKS

	No polarizer	With polarizer 0°	45°	90°	135°	WP totals	Row totals
Experiment 1 (August 7)		—	—	—	—	235°	—
30 min	(55)	(23)	(24)	(25)	(0)	(72)	(127)
Experiment 2 (August 11)	—	—	—	132°	—	—	—
62 min	(72)	(40)	(48)	(51)	(0)	(139)	(211)
Experiment 3 (August 12)	123°	147*	96°	138°	106°	123°	—
73 min	(133)	(77)	(74)	(75)	(75)	(301)	(434)
Column totals	120°	167°	87°	139°	106°	—	—
	(260)	(140)	(146)	(151)	(75)	(512)	(772)

* In each category the upper figure is the mean vector of the fishes' heading; all are significant at the 1 per cent level or better. The lower figure (in parentheses) for each category is the number of heading measurements.

Three experiments were carried out on different individual half-beaks (Table 1). Their oriented behaviour was measured under natural illumination (including sky and water polarization[6]) and under conditions where the polarization pattern was altered by placing a linear dichroic polarizer over the experimental vessel, with its plane of polarization at 0°, 45° and 90° relative to the Sun's bearing in the first two experiments and also at 135° for the third.

Sequences of about twenty-five exposures were made every 10 s in experiments 1 and 2 and every 5 s for experiment 3 for each of the four to five experimental conditions tested. In the longer experiments these were cyclically repeated. Because of initial operational difficulties and different timing intervals the number of observations and duration of each experiment were different (Table 1). Thus more than half of our measurements came from experiment 3.

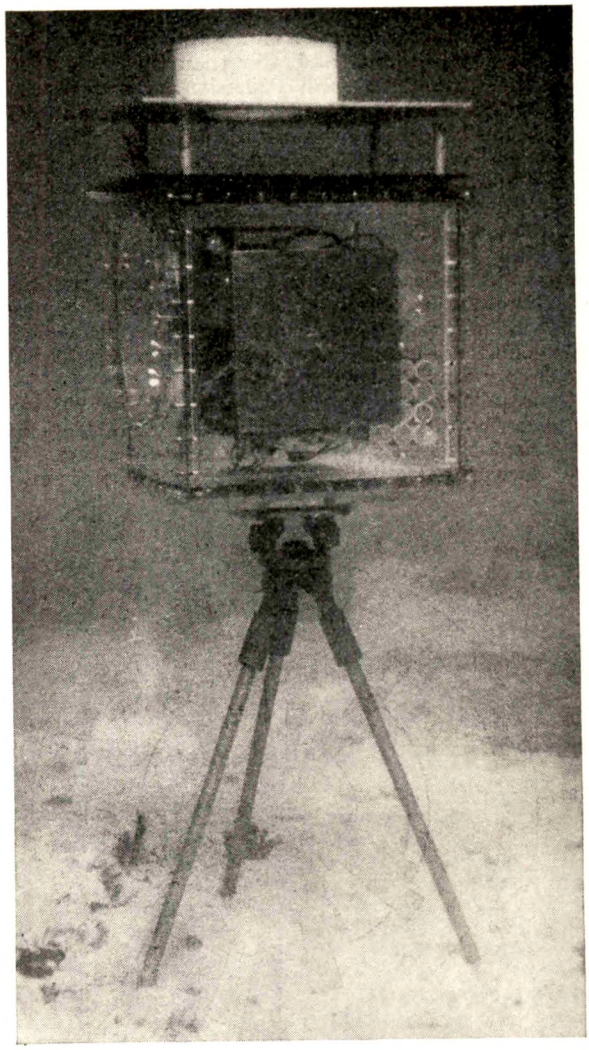


Fig. 1. Underwater experimental set-up. The fish under study (not seen in the photograph) is in a closed transparent vessel inside the white cylindrical screen above. Natural submarine illumination is visible to the animal in the upper hemisphere of its visual field. In most tests this was modified by placing over the white screen a polarizer with its *e*-vector in different specific azimuth orientations. An automatic camera enclosed in the large lucite box recorded the fish's heading at 5 or 10 s intervals.

Each heading, defined as the direction of the anterior end of the fish's longitudinal axis relative to the Sun's bearing, was determined from the film negative with the aid of a projection enlarger. Measured clockwise from 0°, the angles were grouped over 10° sectors centred around 0°, 10° and so on to 350°. Appropriate statistics were used for analysing their circular distribution[7] as well as chi-square techniques where useful. Mean vectors were calculated, their significance being tested with z and that of differences between them with F.

Only in experiment 3 and the pooled total data were mean vectors significant at the 1 per cent level obtained for all conditions tested. These mean vectors, the n's on which they are based and the conditions tested are presented in Table 1. The circular distributions for the total data are plotted in Fig. 2.

In experiment 3 all five conditions tested (Table 1) yielded mean vectors significant at the 1 per cent level or better. Natural illumination evoked menotactic orientation with a mean vector at 123°; this is not significantly

different from a direction of 138°, the mean for the 90 vector test. Vector directions for both 0° and 45° (but not that for 135°) differed at better than the $P < 0{\cdot}05$ level from that under natural illumination. Comparisons between mean directions for the four *e*-vector orientations tested reveal an interesting dichotomy. The mean directional responses to polarization planes differing by 90° (0°–90°, 45°–135°) are indistinguishable, yet those provoked by *e*-vectors differing by 45° (0°–45°, 45°–90°, 90°–135°, 0°–135°) are sharply distinct.

Pooling of all field measurements yields four mean vectors all indicating significantly different orientation behaviour at better than the 1 per cent level. Comparison of the differences between the six possible pairs among the four conditions tested shows that five are significant at the 2·5 per cent level or better.

Thus polarization planes at 0° or 45° or 90° evoke significantly different orientation patterns. The fishes also orient differently with 0° *e*-vector or 45° *e*-vector than they do with natural illumination alone. The directional pattern with 90° *e*-vector (Fig. 2*D*) is, however, like that with just natural illumination (Fig. 2*A*). This implies behaviour which is affected by the polarization of natural illumination because the *e* vector is perpendicular to the Sun's vertical in both the sky and the sea[8,9]. Furthermore the same orientation with 90° polarization and natural illumination indicates that the other different mean vectors with and without the polarizer do not result just from intensity reduction by the dichroic filter.

Although we cannot yet assess the relative importance of the Sun's position, sky and water polarization or other possible factors in the menotactic orientation of *Zenarchopterus*, our data do show that linearly polarized light significantly affects this behaviour. Furthermore, the fact that the 90° position of the polarizer would reinforce the degree of sky and water polarization, but not change its pattern in the great circle coincident with the Sun's bearing, supports the hypothesis that the fishes' usual azimuth orientation is a function of the Sun's bearing as well as the polarization pattern. This is further sustained by

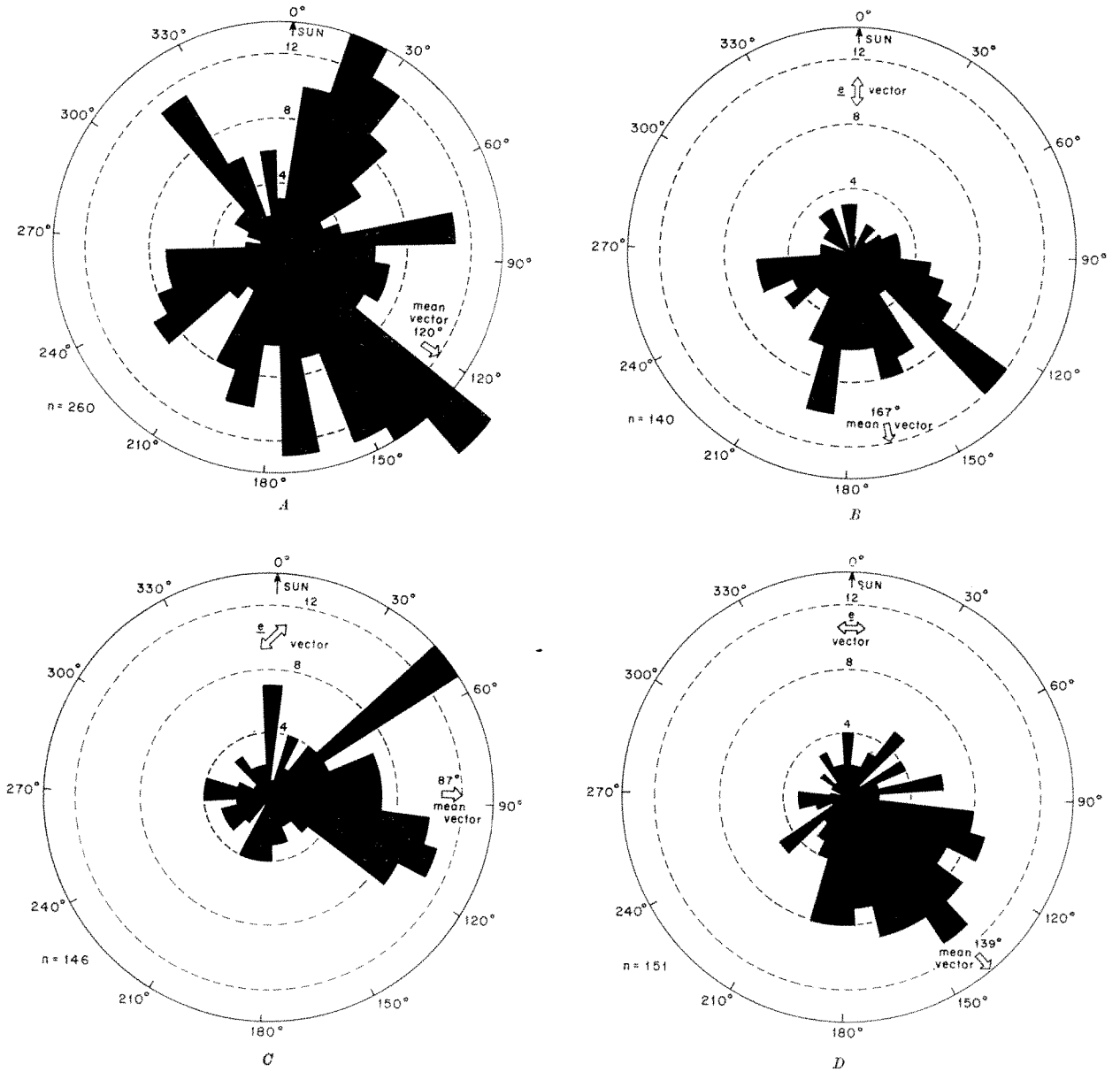


Fig. 2. Pooled results of underwater experiments on azimuth orientation of *Zenarchopterus*. *A*, Under natural illumination; *B*, same but with added polarizer having its *e*-vector at 0° (relative to the Sun's bearing); *C*, same but with *e*-vector at 45°; *D*, same but with *e*-vector at 90°. The radial coordinate represents number of observations grouped in 10° intervals around the circle. Mean vectors for each condition are shown by the single-headed broad arrows.

laboratory experiments on *Zenarchopterus* to be reported elsewhere; they demonstrate polarotactic behaviour patterns quite distinct from the menotactic orientation observed in the field.

To understand the interaction of such polarotactic and menotactic orientation[9], field experiments must be carried out at various times of day with different geographical Sun's bearings. Pre-sunrise and post-sunset experiments might isolate the influence of natural *e*-vector patterns from the direct effect of the Sun's rays, but further study would be required of the anomalous submarine polarization patterns known to be present at dusk[8,10,11].

The question of the mechanism of *e*-vector perception in a teleost is intriguing. It has been suggested that extraocular dichroic structures might provide the necessary analyser (personal communication from T. Kuroki, 1968), yet the fresh skin and skull over the midbrain region of *Zenarchopterus* where there is a translucent "window" do not show significant dichroism which might modulate a pineal or neighbouring midbrain photoreceptor.

Fishes, of course, lack rhabdoms whose specific fine structure relative to the orientation of visual pigment chromophores underlies polarized light sensitivity in arthropods and cephalopods[9,12–17]; nor do they have the macula lutea thought to be responsible for Haidinger's brushes in man.

At the suggestion of Dr Gary Bernard of the Yale Ophthalmology Department we are investigating the possibility that twin cones, which are of unknown function but occur in the retinas of a number of teleosts[18,19], might provide an analyser mechanism. Although the fine structure of their receptor membrane is quite different from that of a rhabdom their ordered arrangement in rectangular or oblique patterns in certain fishes[20] is strikingly reminiscent of the regular mosaic of rhabdomeres and rhabdoms of arthropod compound eyes[21]. Thus far light microscopic sections of *Zenarchopterus* retina prepared in collaboration with Mrs Mabelita Campbell have shown that twin cones are prominently present in regular array. It remains to be shown whether they may provide any basis for *e*-vector discrimination. Experiments to test this are in progress with Dr Haruo Hashimoto.

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Retention of a Conditioned Avoidance Response after Metamorphosis in Mealworms

METAMORPHOSIS of holometabolous insects involves extensive reorganization of their nervous systems[1,2]. In spite of such changes, behavioural modifications can be demonstrated at the adult level after manipulations in the larval stage[3–5]. For example, the adult reactions of *Nemeritis* and *Drosophila* to chemical cues have been altered by exposure to these cues during the larval stage[3,4]. One limitation of these experiments, as Thorpe points out, is that the behavioural modification cannot be assessed directly in the larval stage. Using a more formal conditioning procedure, Borrell du Vernay[5] found that *Tenebrio* trained to discriminate between surfaces and thus avoid shock in the larval stage showed subsequent savings in relearning the discrimination as adults. Unfortunately, this study did not include controls for such variables as sensitization to shock and familiarization with the test situation.

We report here an unambiguous demonstration of retention though metamorphosis. The first of our two experiments showed that either larval or adult *Tenebrio molitor* L. could learn a passive avoidance task; the second demonstrated that responses learned by larvae persisted in adults.

The training chamber in both experiments was a uniformly illuminated lucite box 5 × 7·5 cm in size. The floor was divided into two parts: one with a lucite surface and the other with a copper surface. Copper wires, 7 mm apart, were suspended 1 mm above the copper surface. When an insect bridged the space between the wires and the underlying copper surface, it received shock until it returned to the lucite surface. Insects, selected randomly from stock culture, were isolated for at least 7 days and then individually introduced on to the lucite (safe) surface. The interval between introduction to the lucite surface and the first movement to the copper surface was termed the response latency; an increase in response latency during successive days of training was a measure of learning. Each daily session ended when an insect remained on either surface for 30 min. Fig. 1 summarizes the results from our first experiment. On day 1, for both larvae and adults, the copper surface was not electrified, thereby providing a measure of the preferences of untrained insects. The latency of the first response (from lucite to copper) did not exceed 200 s for larvae or 40 s for adults—all animals had a pretraining preference for the copper surface; the adults and larvae spent 89 and 89·5 per cent respectively of the total time in the chamber on the copper.

On subsequent training days (days 2–6 for larvae and days 2–7 for adults) the copper surface was electrified. As Fig. 1 shows, the latency of the first response increased significantly during training. This increase, however, need not represent only passive avoidance conditioning, but it could be attributed in part to habituation effects or to the fact that the animal sampled shock without actually crossing to the copper surface. During the next 2 days we investigated these alternatives.

On the day after training, no shock was given, but both larvae and adults remained on the lucite surface for the full 30 min (Fig. 1): apparently sampling did not account for the increase in response latency observed during training. Each animal was then left in the apparatus until it had explored both surfaces and remained on

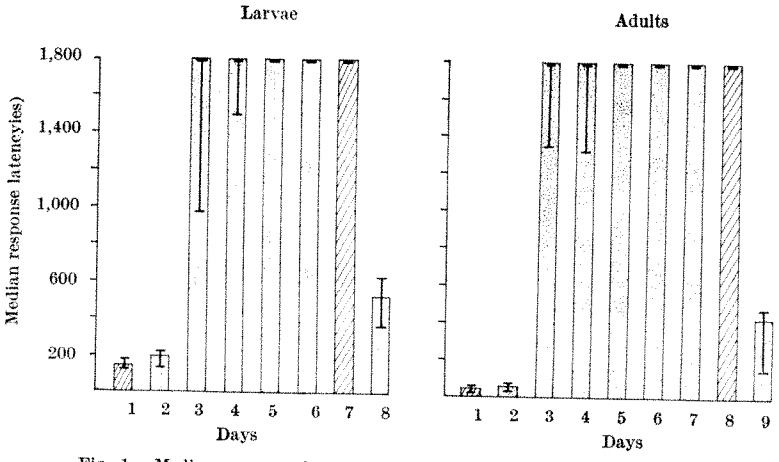


Fig. 1. Median response latency for the first movement of larval and adult *Tenebrio* from lucite to copper surface. Interquartile intervals are indicated by brackets. Ten animals comprised each group. Stippled columns, copper surface electrified; hatched columns, copper surface not electrified.

one of them for 60 min. On the next day, the copper surface was electrified again, and response latencies decreased sharply (Fig. 1). Because the pattern of preference built up during training had been reversed by 1 day without shock, habituation to the lucite surface could not possibly explain the increase in response latencies during training. It is most probable that increased latency reflects true conditioning.

Our second experiment involved the same training procedure, but each animal was trained as a larva and tested as an adult. Control groups were used to determine the degree to which conditioning, habituation, sensitization and/or familiarization in the training sessions with larvae contributed to the behavioural modifications following metamorphosis. One experimental and two control groups were used. The experimental group (trained) consisted of larvae that were trained for at least 10 days, allowed to pupate and tested for retention 3 days after the adult moult. One control group (no treatment) consisted of larvae that were allowed to explore the testing apparatus in the absence of shock, allowed to pupate and tested as adults. The other control group (non-contingent shock) consisted of larvae that were exposed to non-contingent shock in the training chamber, allowed to pupate and then tested as adults. In the non-contingent shock procedure, larvae were exposed to shock-on and shock-off for approximately the same periods of time as were larvae in the trained group, but a lucite barrier prevented movement; they were transferred from one surface to the other by hand.

Retention in adult beetles was measured by two criteria: (1) duration of first period on the safe surface (avoidance latency), and (2) number of errors before the beetle remained on the safe surface for 30 min (trials to learn). On both indices (Table 1), the trained group performed significantly better than either of the control groups: relative to the controls, the trained group showed longer avoidance latencies ($P < 0.01$) and fewer trials to learn ($P < 0.01$). (The Mann–Whitney U two-tailed test was used.)

Persistence of behavioural modification through a moult is not a new idea, as indicated by previous studies[3–6]. Our present study has shown that neither sensitization nor habituation explains retention following a moult. It is more significant that persistence of a specific conditioned response survives the extensive reorganization of the central nervous system during the pupal stage. Implicit in these data, and in need of further investigation, is the probability that storage of information occurs in specific portions of the central nervous system which retain their integrity through the pupal and imaginal moults.

In either larva or adult, learning to avoid aversive situations is of obvious advantage to the individual insect; certainly such behavioural plasticity would be favoured by natural selection. In this kind of learning, the general exploratory tendencies of the insect are inhibited by a set of learned clues. The fact that the inhibition persists not only within a single instar but also across the pupal stage suggests that each adult will tend to remain in the area which was most favourable for its own larval growth. Thus adult habitat preferences are biased by larval experience. This "place learning" is a conservative phenomenon, reinforcing the isolation of those local populations which are concentrated within the more favourable niches of a heterogeneous environment. Place learning could play a part parallel to and/or complementary with the preimaginal conditioning reported by Thorpe and Jones[3].

Table 1. MEDIAN AND INTERQUARTILE RANGE OF AVOIDANCE LATENCY (S) AND TRIALS TO LEARN IN ADULT *Tenebrio* PREVIOUSLY TRAINED IN THE LARVAL STAGE

Group	*n*	Avoidance latency		Trials to learn	
No treatment	25	41 (33–49)		3 (2–4)	
			$P < 0.01$		$P < 0.01$
Conditioned	25	1,800 (1,800–1,800)		0 (0–0)	
			$P < 0.01$		$P < 0.01$
Non-contingent	31	83 (15–204)		3 (2–4)	

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Note added in proof. Shortly after we submitted this manuscript, an article on learning in *Tenebrio* by Borsellino and his co-workers[7] came to our attention. In contrast to our passive avoidance procedure, the Borsellino study used maze learning; it was satisfying to find that retention over metamorphosis occurred with both types of conditioning.

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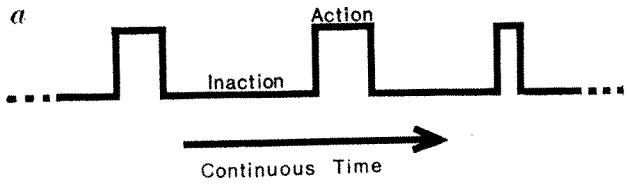
Rhythmic Patterns in Human Interaction

Now that the importance of rhythmicity in biological behavioural functions has been recognized, the traditional distinctions between psychology and physiology have had to be reappraised. For example, the regular alternation between mania and depression in manic-depressive psychosis suggests the possibility of underlying rhythmic phsyiological mechanisms[1]. Similarly, the temporal structure of social interaction has been investigated and regular patterns have been found[2]. Chapple[3] and others[4] have suggested a loose analogy between these patterns and biological rhythms. Nobody, however, has analysed the temporal structure of interaction with the perspective and statistical techniques of chronobiologists.

Rhythmic patterns of behaviour seemed to emerge in the course of a study of interactions among the patients in sheltered workshops. (These workshops are intended to provide non-competitive employment for physically and mentally handicapped people who do not need to be in hospital permanently, with the aim of helping them to develop the physical and social skills necessary for economic independence.) After the study, one subject was chosen for an intensive chronobiological investigation. So far, frequency analysis has given consistent evidence for high frequency rhythms in interaction; the cycles had periods of approximately 3·0, 6·0 and 12·8 min, which can be regarded as harmonics.

The subject, selected from a group of sixteen, was a woman of 26, mentally retarded and suffering from phenylketonuria (PKU). She had a strong tendency to engage in social behaviour in the absence of any obvious partner or object to which her actions could be related; this tendency I refer to as "interactional auto-action", with the intention of describing only the form of behaviour and not its content. Eighty per cent of the subject's social contact activity was recorded as auto-action and, of the remaining 20 per cent, more than half was initiated by the subject. In other words, the subject herself initiated more than 90 per cent of her recorded social contact time—that is, the time during which she was engaged in auto-action or dyadic interaction. This subject's interaction, especially her auto-action, with its apparently predominant independence resulting in effective behavioural isolation, thus seemed to offer an excellent opportunity to search for an endogenous, free-running rhythm in the absence of external synchronizers.

The behaviour of the subject was recorded by an observer[5] using a remote-recorder which provided a precise record of the temporal patterns of interaction. The data, sampled at a frequency of 100 points/min, were generated as a square wave representing the two conditions of action and inaction (see Fig. 1). The total data sample consisted of numerous "observations"—an observation being the period of time during which the pertinent behaviour of the subject was recorded. The distribution of these sessions fell into two groups (Fig. 2). The 1967 data consisted of fifteen observations of about 15 min, gathered at randomly determined times during the working day; and in 1968, during intensive investigation, twenty-nine 30 min observations were recorded as frequently as possible. The second group allowed for the successive repetition of any existing temporal patterns and for the investigation of longer periodicities. The subject was thus under observation for more than 1,100 min (or 110,000 data points), an adequate sample with which to look for high frequency temporal patterns without any restrictions imposed by infrequent sampling or small sample size.



b

(1) Action	(2) Inaction			(3) Cumulative Time
00007		32	028	02099
	00003	23	029	02102
00010		32	030	02112
		21	031	02185
		12	032	02295
		21	033	02313
	00321	14	034	02433
		43	035	02442
00029		32	036	02462
		21	037	02472
	00252	14	038	02714
		43	039	02717
00004		32	040	02718
		21	041	02727

Continuous Time

Fig. 1. Representation of the raw data. During an observation the subject was observed in her natural setting in a sheltered workshop and, with the aid of a remote interaction chronograph, was continuously recorded either as interacting (action) or not interacting (inaction). This method yielded a continuous square wave (*a*, schematic and not to scale) of the binary on-off condition in terms of a tone on magnetic tape. The data were then converted to digital form (*b*) by an analogue-to-digital converter sampling at the rate of 100 points/min. *b*, A sample of the computer output giving the numerical values of the square wave in hundredths of a minute. Columns 1 and 2 represent action and inaction, respectively. Column 3 gives the cumulative running time from the beginning of the observation. Thus the second action given, which lasted for 0·10 min, started at 21·02 min into the observation and ended at 21·12 min. The unlabelled columns are of no significance.

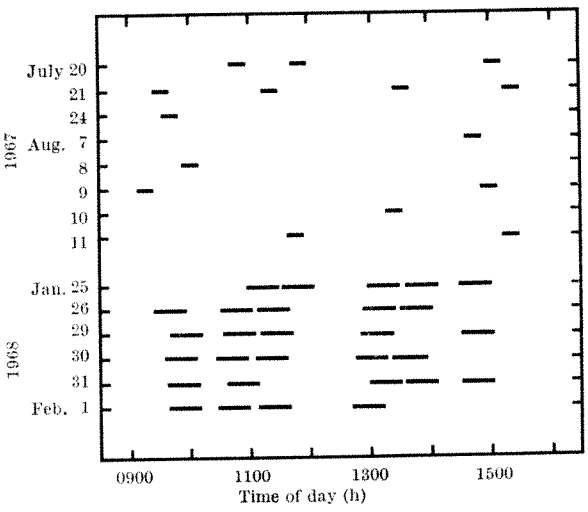


Fig. 2. The distribution of observations. Each observation, represented by a solid bar, constitutes a longitudinal sampling of interactional activity at the rate of 100 points/min. The time of sampling in 1967 was randomly determined; observations were gathered as frequently as possible in 1968.

Two different methods of frequency analysis were used to resolve the hypothesized high frequency signal component in the data. The first was a modification of the periodogram procedure described by Enright[6]. Rather than the root mean square amplitude, the natural logarithm of the smoothed variance values was used as the ordinate value. The smoothing, a simple frequency filtering technique, was achieved with a simple running mean and served to reduce ultra high frequency variation. Because the logarithmic function was used, multiplicative rather than absolute differences were represented on the ordinate scale; this made it possible to compare observations independent of mean levels of activity. The raw data were blocked into equal intervals of 15 s and the duration of interactional activity in each interval was computed. The resultant series of equally spaced sums of "contact duration" was taken as the input data for spectral analysis by the periodogram. The number of switches in the square wave was also computed for each interval and used for a separate periodogram analysis.

In this modified form the periodogram technique scanned all the individual observations for rhythmic components with periods of between 2·0 and 7·5 min; this range represents the spectral window of the analysis. When two sequential observations were separated by only a few minutes of "non-recorded" time, they were joined together to form a record of about 60 min in which the non-recorded time was considered as blank data. In these analyses the window was extended to include periods of up to 15·0 min.

The second method of frequency analysis used fast Fourier transforms as the basis for constructing a power spectrum. Again the raw data were blocked to provide a series of equally spaced sums as input data. The

Table 1. PERIODS OF DOMINANT RHYTHMIC COMPONENTS

Observation number	Contact duration			Contact switches	
	Periodogram		FFT	Periodogram	
	Single (min)	Joint (min)	Single (min)	Single (min)	Joint (min)
3	6·0–6·25	**		6·0–6·25	**
4	6·75	**		6·75	**
6	6·5	**			**
7	4·5	**	4·6		**
10		**	9·1		**
11	4·75–5·0	**			**
13		**			**
16	5·75–6·0			5·25	**
17		11·25		5·75	11·25
18		11·25–			
19		11·5			
21		**	3·0		
22			4·6		**
23		13·0			13·0–
24		13·25–	5·4–6·4		13·5
25		13·5			13·5
26		**			
27		13·25–	6·4		**
28		13·5	3·2		
29		**	3·2		
31		**	3·0		**
32	6·0		6·0		**
33	6·75–7·0	13·5		6·0	
34		13·5–		6·75	13·5
35	6·25	13·75	2·7		
36		**	3·6	6·0–6·5	
40		**		6·75	**
42			6·0		**
43	5·5		3·2		
Average Period ($\bar{\tau}$):	$\bar{\tau}_5 = 5·9 \pm 0·7$	$\bar{\tau}_{12} = 12·8 \pm 1·1$	2·5 $\bar{\tau}_3 = 3·0 \pm 0·3$ $\bar{\tau}_6 = 6·1 \pm 1·5$	$\bar{\tau}_6 = 6·2 \pm 0·5$	$\bar{\tau}_{12} = 12·9 \pm 1·1$

The results of the periodogram and fast Fourier transform power spectrum (FFT) analysis were computer plotted as a function of period length (in minutes per cycle). The values given represent signal peak positions whenever dominant peaks were present. Many of the joint-periodogram plots showed strong sub-peaks at approximately six minutes. A blank column entry signifies that no signal component was clearly in evidence; asterisks indicate that the particular analysis was not applied to the given observation. Unfortunately the statistical analyses that are available to investigate the significance of these peaks all involve unjustified assumptions, but this problem is common to all chronobiological techniques of spectral analysis. The averages and s.d. given are based on the values of the peak positions in each column.

power spectrum was used to examine only the contact duration data of single observations. The results of this and the periodogram analysis are presented in Table 1.

Table 1 shows that about 70 per cent of the data revealed a temporal periodicity with at least one of the analytical techniques. Considering the complexity of the variables under investigation and the problem of noise interference, this result is very encouraging. More important, however, is the consistency of the periodic components. Based on the individual observations, most peaks in activity correspond to a period of $6·0 \pm 1·0$ or $3·0 \pm 0·3$ min. Similarly, the "joint-periodograms" have yielded values which suggest a rhythmic component with a period of approximately $12·8 \pm 1·1$ min. Because all these results can be considered integral multiples or harmonics of one another, there seems to be a persistent rhythmic component underlying most of the observations. The findings thus suggest that there is a non-random variation in interaction expressed as a rhythmic manifestation of greater and lesser activity. The period of the rhythm seems to be about 3 min or an integral multiple of 3 min. The analyses also indicate a corresponding periodicity in the switching rate between action and inaction in the interactional activity.

Some of the observations, however, showed no periodicity. Because Aschoff[7] has suggested that a truly endogenous periodicity might depend on the functional state of the organism, it is not unreasonable to expect a variation in the manifestation of the periodic component corresponding to the physiological states of the person. In this respect it is interesting that the August 1967 data (observations 9 to 15), which yielded the poorest results in terms of the presence and consistency of periodic components, showed a significantly greater mean level of activity than either July or January ($P = 0·005$). This finding suggests that the mean level of activity may affect the appearance of the rhythmic component. Whether this influence is related to a variation in signal amplitude or the interference of an increased noise level is unknown.

The basis of the rhythm is not known. It may derive from a more general activity rhythm, an underlying cognitive or neural component[8,9], an excitability rhythm corresponding to blood CO_2 and respiration[10,11], or some unknown factor or factors. In any case, however, the similarity of the phenomenon to biological functions suggests that interactional activity may have strong foundations in biology; indeed, the distinction between physiology and behaviour at this level becomes artificial. As such the rhythm may provide the basis for the notion of an "interactional rhythm", an idea which has been used by interactionists with varying precision and definition[3,4]. In the context of rehabilitation, such a manifest rhythm would have a profound impact on a person's social relations, where it might lead to a reduced flexibility in interactional style[12]. An awareness of such a pattern would be of great importance in understanding and effectively dealing with behaviour of this kind.

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Book Reviews

SEARCHING FOR PRIORITIES

Biology and the Future of Man

Edited by Philip Handler. Pp. xxiv+936. (Oxford University: New York and London, July 1970.) $12.50; 105*s*.

THE future of man is becoming a fashionable topic these days, even in quite respectable circles. What could be more respectable than the Committee on Science and Public Policy of the US National Academy of Sciences, which has now found it appropriate to give the subject somewhat more than a passing glance—indeed, at a rough estimate, about 400,000 words worth? Not even the most ardent futurologist (always excepting Mr H. Kahn) could really be expected to keep the theme going for that number of pages, and this book does not. The title, in fact, seems almost an afterthought, for the book arose from a decision by the NAS to embark on a series of surveys of the state of the art in particular scientific disciplines, the intention being to discover what the material strengths and weaknesses in basic reasearch were in particular areas. It was the life sciences' turn in 1966, and the bulk of this book is, in effect, a sort of gigantic memorandum, written by a panel of nearly two hundred distinguished biologists; it is obviously the sort of tome which should be found on the shelves of the secretary of any funding agency.

How could such a vast panel function? The usual mode of such operations is to hand each section or chapter out to an expert, to accept what he produces with a few minor editorial amendments, and bulk the whole together with a few well chosen editorial link-words. Not so here. An interesting attempt was made to be more integrated. After each section was prepared, the entire survey committee seems to have met in a [sic] "grueling one week session" with each report "mercilessly exposed to . . . searching, sometimes scathing, criticisms". Such a procedure can certainly work well, but demands a degree of rapport and common purpose among the authors. One is tempted to indiscreet speculation as to just what Drs René Dubos, Theodosius Dobzhansky and Joshua Lederberg could have found to say to Dr Stephen Aldrich, of the Central Intelligence Agency, listed as a "special consultant to the study".

But the method should be judged by its results, and for me, as a finished product, *Biology and the Future of Man* fails. It is obviously deliberately selective, not a textbook but still an attempt to provide an integrated statement of just what the biological revolution of the last decade has been about. It is aimed, presumably, at the intelligent lay grant-giver (and, of course, his public), though it seems improbable that he should ever want to read it all. It runs the gauntlet through molecular and cell biology, biochemistry, developmental biology, physiology, genetics, evolution and ecology to medical practice, environmental health and agriculture, taking in *en route* such fashionable topics as the origin of life and (a full 10 per cent of the entire book) neurobiology and behaviour. Inevitably, the level of the reviews varies, as if the authors are not all talking to the same audience. Bits of the basic biochemistry are on a downright chatty, first year undergraduate level (". . . in 1953, the British biochemist Fred Sanger first demonstrated that the hormone insulin is not a random assembly of amino-acid residues . . ."). Portions of the neurobiology are on the level of a review article in a specialist journal. Terms are introduced without definition or explanation; very complex problems and concepts are tossed around in a manner surely incomprehensible to anybody but the expert. And what expert would be interested in a review that contains neither references nor attributions for work discussed, nor even an index?

These failings would be more excusable if the authors had really addressed themselves either to the theme of the book's title or, less grandiosely but more manageably, perhaps to the question of where research in their own particular area is going. But this, which should surely be the justification of the exercise, eludes them. Some, but not all, do conclude their reviews with an attempt to summarize the chief ongoing research questions, but it is the overview which is so surprisingly lacking: the self-critical approach which should do what the book ostensibly sets out to do, and provide some guidelines for the science policy-maker. If there are conclusions or recommendations, and it would be wrong to say there are none, they are rarely incisive or fundamental enough to ask "what are we doing this research for?". Rather, they assume the Everest-complex. The research is there, and ongoing; should we make the climb by the north or south face?

The overview, and the legitimation for the title of the book, comes in its last 40 pages—an essay, predominantly the work of the editor, which has the book's title as its own. For Handler, at least, there is a clear sense of biological priorities. The most pressing problem (always excluding war) is population and its growth; from excess population spring environmental pollution, overcrowding in the cities, violence, noise and stress. The overriding need is for better contraceptive techniques and governmental population policies. In this area lies the prime research responsibility of the biologist, and in that of improving agriculture. Much further down in the list of biological prospects and problems come those headline catchers of transplants and genetic engineering, a sensible and humane extrapolation of the conventional wisdom. One may not agree with Handler's priorities, but at least he knows what he is after. It might have been better had he written the whole book himself, without all these grand biologists (and the CIA man) to help and advise.

STEVEN P. R. ROSE

CELL BIOLOGY REVIEWS

International Review of Cytology

Edited by G. H. Bourne and J. F. Danielli. Vol. 27. Pp. xviii+407. (Academic: New York and London, February 1970.) 196*s*.

International Review of Cytology

Edited by G. H. Bourne and J. F. Danielli. Vol. 28. Pp. xviii+387. (Academic: New York and London, March 1970.) 196*s*.

Advances in Cell Biology

Edited by David M. Prescott, Lester Goldstein and Edwin McConkey. Vol. 1. Pp. ix+337. (North-Holland: Amsterdam; Appleton-Century-Crofts: New York, 1970.) Hfl. 72; 168*s*; $20.20.

HERE are three books containing articles that cover a wide range of cell biology; two are the latest volumes of a well established series founded twenty years ago, the other is the first-born of a new series. Because the aims of both series are similar from the readers' point of view it is worth comparing how they tackle the problem of presenting cytology to the specialist reader.

International Review of Cytology is simply a collection of articles from all corners of the cytologists' globe and there is no attempt to place articles on related topics together. Not that this in itself is a criticism, but it is a small inconvenience to the reader wanting to use the volumes as a source of information on a particular topic. For instance, in volume 27 there is an article on wound healing in higher plants, while in volume 28 Jacobs (with the exemplary presentation we have come to expect of this author) discusses regeneration of seive tubes in plants. Because the two topics are obviously related why could not the two articles have been juxtaposed, or even combined into a single review of regeneration? Similarly, Taylor's article on the possible mode of evolution of chloroplasts could have been profitably placed alongside the article concerning evolution of mitochondria that appeared in a volume in this series last year. I feel the editors could prevent each volume from becoming just a hodge-podge of reviews by a more foresighted and integrative policy in assembling material for each volume, so turning the series into a more streamlined source of information.

The first volume of the new series *Advances in Cell Biology* has a more uniform content. Five out of its seven chapters deal with various aspects of structure and function of the nucleus. Replication of bacterial and eukaryotic chromosomes is fully discussed by Kuempel and Prescott respectively, while Goldstein and Zetterberg each contribute a chapter on protein metabolism in nuclei. A chapter by Brinkley and Stubblefield on the centromere and centriole contains interesting speculations on how these two bodies work in dividing the chromosomes in cell division. Indeed, a speculative approach in each review chapter is one of the aims of the editors, but only within the framework of a comprehensive survey and synthesis of the literature on the topic under discussion. And with these aims in mind each author has prepared a contribution that should wear well.

On the other hand, the editors of *International Review* allow their authors more latitude in the presentation of their articles. Besides the general workaday review, these volumes contain three other categories of review: the historical, the "self" review and the "hypothetical" review. The history comes from Steward who traces work over the past fifty years on the uptake of salts into plant cells, and from Raven writing about work on the cortex of *Limnea* eggs. The "self" review is by Benazzi-Lentati who describes the studies by herself and her husband into gametogenesis in planarians. This is a useful review because many of their original papers are in Italian, but sadly the editors have neglected to make the author's English more readable. The third type of review is by King who selects certain facts to construct an hypothesis on the process of meitotic crossing-over. There seem to be a number of aspects of meiosis which the hypothesis will not account for, or it contradicts, and for this reason must be judged to be more of an essay in model building. Nevertheless, *International Review* remains an asset on library bookshelves and I imagine *Advances in Cell Biology* will become an asset too. It will be interesting to see how this latter series fares in the future.

PETER BARLOW

IMMUNOLOGY WORKSHOP

Histocompatibility Testing 1970

Edited by Paul I. Terasaki. (Report of an International Workshop held at UCLA and Palm Springs, California, January 20–26, 1970; and the Fourth International Histocompatibility Conference at Los Angeles, January 17–29, 1970.) Pp. 658. (Munksgaard: Copenhagen; Williams and Wilkins: Baltimore, 1970.) 148.50 D.kr.

THIS book is quite different from most of the rash of volumes resulting from meetings and symposia. The usual form is a disconnected assembly of semi-review chapters that were hastily put on tape on the flight to (or from) the meeting; new data are carefully excluded by the unwilling contributor for fear nobody will see them, but contrary to the pleas of the organizers. By contrast this volume is a compendium of all the latest information on the subject. Inevitably it does not describe a coherent story but is arranged in well classified parts: results of the workshop, genetics, serology, histocompatibility, chemistry, maternal-foetal incompatibility, mixed cultures, and methodology.

The reason for the quality of the book is historical; it is the fourth in a series (each being about 200 pages longer than the volume that preceded it), each resulting from a practical workshop and conference where the most up to date information is given and the most advanced ideas discussed. Because almost all those directly involved actually attend or are represented, the coverage is essentially complete.

Important points that emerged from the workshop were: confirmation of recombinants between the two HL-A segregant series, giving a cross-over frequency of the order of 1 per cent; additions to the list of internationally agreed specificity nomenclature; definition of "long" and "short" specificity of antisera (only the short being classifiable as monospecific); inability to attain monospecificity by absorption because of cross-reactivity within each segregant series.

The volume continues the record of an astonishingly rapid advance of the subject, mediated by the workshop meetings. The 1970 book is relatively wide ranging, and because future workshops are likely to be restricted to much smaller aspects of the subject, it is likely to remain a reference volume for some time.

Some basic ideas are still being questioned, for example, division into two segregant series, although this view has the majority in support. A third segregant series is mooted but is not finding much favour generally; to establish such a situation becomes extremely difficult in view of the serological cross-reactivity now demonstrated.

Possible association of HL-A phenotype with disease and of different haplotype patterns in different ethnic groups is discussed, and some differences in cell/tissue distribution could be a new important feature. On the central theme of practical tissue typing the methodology section has several contributions on micro-platelet complement fixation and other methods may also challenge the standard cytotoxicity techniques now in use.

All those involved in the transplantation field in its widest sense will find something important in the book, which makes a companion volume to the proceedings of the transplantation congresses. Outsiders would find the inevitable language problem for which there is no answer in sight.

D. A. L. DAVIES

METABOLISM OF INSECTICIDES

Biochemical Toxicology of Insecticides

Edited by R. D. O'Brien and Izuro Yamamoto (Proceedings of the Fifth US–Japan Cooperative Science Program held in Tokyo, June 1969.) Pp. vii+218. (Academic: New York and London, June 1970.) 79*s*; $8.50.

INTERNATIONAL conferences provide a unique opportunity for specialists in a restricted field to meet and to exchange ideas. Frequently, however, the brief abstracts issued to delegates before the conference provide the only record of the proceedings, to the detriment of those unable to attend. The Tokyo conference of 1969, by its terms of reference, precluded the attendance of experts from many nations, and it is therefore particularly appropriate that seventeen of the contributions have now been published, together with tables, figures, experimental techniques and references.

Most of the contributions possess high scientific merit and all are of interest to the advanced student or research worker. Several articles deal with the metabolism of organophosphorous compounds, carbamate insecticides, rotenone and pyrethroids, particular emphasis being placed on the role of microsomal systems and of the supernatant fraction remaining after the removal of microsomes. Other papers are concerned with the structure, action and metabolism of synergists; one of these, by Kuwatsuka, deals with the way methylene dioxyphenyl synergists are themselves attacked by microsomes.

Several papers, including some I have referred to, discuss such cognate topics as structure-activity relations of insecticides, the causes of selective toxicity and the distribution of insecticides within the tissues of various insects and higher animals. In one otherwise very interesting article on autoradiographic studies of benzene hexachloride isomers, it is unfortunate that the Japanese authors were apparently not able to use their technique to pursue the fate of the δ-isomer (which penetrates insects more rapidly than the toxic γ-isomer), as well as following the distribution of ^{14}C-labelled β and γ-isomers. Yamamoto reviews problems associated with the activity of pyrethroids; Hollingworth provides evidence that O-demethylation is a principal route by which di-methyl organophosphates are degraded. Dahm, in a lucid contribution, suggests that the cleavage of the aryl phosphate bond of parathion (a seemingly hydrolytic process) is a microsomal oxidase reaction, dependent upon NADPH.

It is a pity that the lustrous nature of so much of the scientific work has been somewhat tarnished by non-uniformity of presentation and by rather perfunctory proof-reading. Summaries are sometimes at the beginning of papers and sometimes at the end. In some contributions the tables and figures are conveniently placed in the text and in others they follow the references. Perhaps only the grammatical purist will be troubled by the vacillation of "data" from the singular to the plural (for example, pp. 4, 7, 35, 81), and by constant misuse of the definite article in the Japanese papers. In at least a dozen places, however, grammatical or printing errors lead to a lack of clarity in the scientific exposition (for example, pp. 3, 26, 73, 120, 201), for the reader has no option but to guess the nature of the error. Nevertheless, this book contains much excellent material and is highly recommended to all who have an interest in the metabolism, structure-toxicity relations and physico-chemical characteristics of insecticides.

K. A. Hassall

BODY REGULATOR

The Autonomic Nervous System

An Introduction to Basic and Clinical Concepts. By Otto Appenzeller. Pp. xi + 238. (North-Holland: Amsterdam and London; Elsevier: New York, 1970.) 105*s*.

The autonomic nervous system, sometimes known as the vegetative nervous system, is concerned with homeostatic regulation of bodily functions such as blood pressure and temperature. Books about the system are few and the literature available to students on its dysfunction in disease states is very limited. Lord Brain, in the last personal edition of his classic text in neurology, *Diseases of the Nervous System*, devoted only six of the volume's 879 pages to its disorders. This may suggest to many medical practitioners that autonomic disorders are of little significance, for Brain is studied by almost all post-graduate students of general medicine and by many undergraduates. During the past decade, however, the importance of hypothermia and orthostatic hypotension as complications of many diseases, has been recognized. Thus the current studies of L-dopa in the treatment of Parkinsonism have made neurologists increasingly aware that autonomic dysfunction forms a part of the disease complex of this and many other disorders. Dr Otto Appenzeller has contributed much to the study of autonomic disorders and it is therefore appropriate that he should produce a book on this enormous subject, much of its clinical background being developed from his own wide experience.

This book provides an excellent basis for anatomical reading in the subject but it has concentrated less than might be expected on the physiology of the system. It is a pity, too, that some of the illustrations upon this aspect, such as tracings to indicate blood flow changes, are poor. The anatomical side is strengthened by many observations in comparative anatomy, and it is this aspect of the book which deserves attention, rather than the sections on physiology or clinical problems. To take a specific example, such is the progress in the subject, that the book is already inadequate in its consideration of Parkinson's disease.

I must admit to one further difficulty. I have always looked on the autonomic nervous system as the efferent system of nerves comprising the parasympathetic and sympathetic nervous systems. Dr Appenzeller seems to have a wider view of the meaning of the word "autonomic". This might be correct from the *Oxford English Dictionary* definition of the word, meaning "self regulating", but his usage conflicts with J. N. Langley's original definition at the end of the nineteenth century. He has therefore included chapters on sleep, circadian rhythms and respiration which most neurologists would feel to be inappropriate in this text. Nevertheless the book brings together much useful information which will make it a valuable library source.

R. H. Johnson

FIRST EXPERIENCES

Early Learning in Man and Animal

By W. Sluckin. (Advances in Psychology, Vol. 2.) Pp. 123. (Allen and Unwin: London, August 1970.) 35*s*.

Differences between people are in part related to differences in the experiences they have encountered. Experience affects development in part through a process or group of processes commonly described as learning. Both because a *tabula rasa* may be peculiarly impressionable, and because what is learnt first may affect what is learnt later, learning early in development may be of peculiar importance. But at what stage is the young organism first capable of learning? What are the natures of the learning processes which then occur? Do they occur more readily then than later? How do they affect later development? These are some of the questions which Professor Sluckin explores in this students' guide. For his material he draws on recent studies of non-human species—chicks, puppies and monkeys in particular—as well as man; and he ranges from classical conditioning through imprinting and exposure learning to the development of language and personality. He succeeds in compressing great breadth of knowledge into a slim volume, giving brief introductions to viewpoints as divergent as those of Lorenz and Schneirla, Chomsky and Skinner, Freud and N. E. Miller. His approach is catholic, and the student may sometimes wish for clearer signposts to indicate just where the work of each theoretician is, and is not, fertile.

In a field of study which could too easily become a jungle of ill-defined terms, Sluckin is hardheaded about concepts, though his discussions of imprinting in man and of imitation would have been more incisive had he maintained his own distinctions. The reader will be introduced to a wide range of studies, and the bibliography will show him how to explore further.

R. A. Hinde

TERMITE BIOLOGY

Biology of Termites

Edited by K. Krishna and Francis M. Weesner. Vol. 2. Pp. xiv+643. (Academic: London and New York, May 1970.) 299*s*.

THIS is the second of two volumes of a composite work by some twenty authors. Broadly speaking, volume one was physiological and this volume inclines a little more towards ecology. The first chapter, however, deals with the role of symbiotic organisms in digestion. There is a very full picture of the variety of relation, from the truly symbiotic relation between lower termites and their unique flagellate genera to the less well defined situation in the Termitidae. There is a valuable review of the physiology of the mutualistic relationship, though the, perhaps unavoidable, exclusion of the similar relationship in the cockroach *Cryptocercus* is sometimes frustrating. Mukerji's account of termite embryology is also an excellent and well illustrated treatment of this under-worked field.

Noirot attempts with some success a more exhaustive and reasoned treatment of the nests of termites. Although relatively little is known of the behaviour by which these varied and often very characteristic nests are produced, Noirot's experiments on nest repair suggest some most interesting contrasts within the order. Krishna's chapter on taxonomy and phylogeny introduces the regional section of this book, with chapters on each of the zoogeographic regions and special chapters on Madagascar and on Hawaii and the Oceanic Islands. It was in this section that one might have hoped to find more about the ecology of termites, a subject which was hardly treated in the first volume. Many of these chapters, however, are in the form of a catalogue, genus by genus. Bouillon in his long chapter on the Ethiopian region does not attempt to deal with all the African forms. Instead he tries to give a general picture of the biology of termites in a tropical climate, illustrating, for example, energetics and spatial distribution with selected examples. Harris, dealing with the much smaller Palaearctic fauna, also succeeds in combining a general treatment with a partly ecological account of the life of termites in what is not generally a very favourable zone. The remaining chapters make heavy reading, moving at their worst from genus to genus with the times of mating flights and nest situation of each. The author of the final chapter summarizes it when he says "the principles of termite control have undergone few significant changes in several decades". He also advises that in sounding timber for termite damage the hammer "must be used carefully or not at all in the examination of finished wood".

The two volume work will become an indispensable reference work, but it is in some ways disappointing because of the unenterprising way it has been conceived. Nobody would suggest that species-by-species accounts are not valuable, but they can be very dull. Perhaps the reader who wants to know all about a particular species would be well enough served by the good index.

J. H. SUDD

SEX IN INSECTS

The Physiology of Insect Reproduction

By Frantz Engelmann. (International Series of Monographs in Pure and Applied Biology. Division: Zoology, Vol. 44.) Pp. ix+307. (Pergamon: Oxford and New York, July 1970.) 140*s*; $18.75.

THERE is a gigantic literature concerning the reproduction of insects. In this book F. Engelmann provides an exhaustive survey of physiological aspects ranging from sex determination and differentiation, the processes of mating and insemination, factors regulating fecundity, and the role of hormones in male and female, to the control of oviposition. All parts are covered comprehensively with almost every statement documented and with no words wasted on unresolvable hypotheses. The result is not a book for light and easy reading, but is one that will prove invaluable to those who really wish to learn what is known about a given topic and are prepared to read slowly. One naturally turns to those topics which are being most actively studied at the present time—pheromones in mating, or hormones and egg maturation—to find these complex subjects admirably covered.

It has long been one of the dogmas of insect physiology that hormones are not involved in sex differentiation. This belief was refuted a few years ago by Naisse who showed that in the glow-worm *Lampyris* the apical cells of the testis produce a masculinizing hormone, so that, whereas the ovaries develop normally in the neutral environment of the female, they are converted into testes if transplanted to the male at the appropriate time in development. As Engelmann points out, it may be that this phenomenon is more common in insects than is believed, and that in mosquitoes of the subgenus *Ochlerotatus*, which suffer feminization when the larvae are exposed to unduly warm conditions, it could be that an androgenic hormone in the male is inactivated at high temperature.

Besides the standard processes of insect reproduction, the many strange anomalies are also described: parthenogenesis, which is very frequent, heterogony or the alternation of sexual and parthenogenetic generations, viviparity in its many forms, functional hermaphroditism as it occurs in the notorious cottony cushion scale *Icerya*, traumatic insemination through the body wall as in some of the cimicids, and paedogenesis, that strange form of parthenogenesis where, sometimes for many generations (as in the fungus gnat *Miastor*), the oocytes in the larval ovaries grow into young larvae which consume the contents of their mother larva and then break out through the skin. The final section deals briefly with insect societies and the caste system in which reproduction is limited to the queen and her consort, who may produce many millions of offspring during their term of office.

The book has a glossary, a reference list with nearly 2,000 titles, and a subject index with the names of the insects discussed.

V. B. WIGGLESWORTH

FISH CULTURE METHODS

Traité de Pisciculture

By Marcel Huet, with the assistance of J. A. Timmermans. Fourth edition. Pp. xxiv+718. (Editions Ch. de Wyngaert: Brussels, 1970.) $19.00.

THE fourth edition of Professor Huet's treatise on fish culture has been revised, and also expanded to twice the length of the previous edition, and now appears as a substantial clothbound volume.

The original chapters on the construction and layout, the natural history, and the natural productivity of fishponds are little changed; but the account of the management and improvements of ponds has been enlarged to include more methods of weed control, and of influencing the chemical and physical factors concerned with increasing crops of fish.

The account of salmoniculture is unchanged in length, but that on carp culture has been much expanded and now includes not only the common carp, still the mainstay of European fish farming, but also brief accounts of other European and acclimatized Asiatic carps, and of the increasing and profitable culture of small cyprinids as live-bait fish for sale to anglers.

New chapters deal with the culture of percomorphid fishes (pike, pikeperch, walleye, black bass, tilapias), and silurids (the European *Wels*, catfish in America—a fast-growing industry—and *Clarias*, or mudfish, in the Eastern tropics). Under the heading of "Special Fish Cultures", Huet describes the culture of the eel in Europe and Japan, and fish culture in ricefields and brackish water. A new chapter gives a brief survey of fish culture by regions; and there are new chapters also on the diseases and enemies of fish, on artificial foods, which are rapidly gaining in importance, and on the harvesting, transport, and care of live fish. Methods of culture of 124 species of fish are given, of which cyprinids comprise twenty-eight species and salmonids twenty-two species.

More attention is given to the culture of fish to restock waters depopulated by engineering works, by pollution, or by excessive fishing. Detailed accounts are given of the culture of pike, coregonids, shads, grayling, and sturgeons.

An attractive feature of all editions has always been the splendid illustrations, which have been increased to 503 in this edition. But I must again regret the lack of an index, more than ever desirable in this much enlarged volume.

C. F. HICKLING

ROCK FABRICS

An Introduction to the Study of Fabrics of Geological Bodies

By Bruno Sander. Translated by F. C. Phillips and G. Windsor. Pp. xvii+641. (Pergamon: Oxford, London and New York, June 1970.) 240*s*; $32.

ALL geologists with more than a superficial knowledge of structural petrology will be aware of the fundamental contributions of the late Professor Sander to the development of this subject. For most British and American readers acquaintance with his ideas has been uneven and derived mostly at second hand, because his original papers and books were written in a difficult German style. This imposing volume is a translation of two textbooks published in the period 1948 to 1950 that set down Sander's views on rock fabrics based largely on his own work. They were intended to illustrate the scope and methods of fabric studies to those intending to apply them to their own problems, and to introduce them to non-geologists who might be able to adapt them to their own problems. The approach led through a highly philosophical introduction to a very practical treatment that included full accounts of field and laboratory techniques, as well as numerous fully worked examples. This translation was made in response to many requests for publication of an earlier version made by one of the translators (F. C. P.), and was undertaken at the express wish of Professor Sander.

The book is in two parts; the first introduces the theory and deals with large scale features down to the hand specimen, while the second is concerned with laboratory investigations and the interpretation of grain fabrics. It represents a very valuable addition to the somewhat sparse literature on this subject in English; the second part, in particular, presents a much more detailed account of the techniques and analysis of grain fabrics than has hitherto been available. In the interests of clarity some parts have been translated rather freely, but no attempt has been made to bring the work up to date or to make substantial changes to the original rather ponderous philosophical style. An obvious weakness lies in the age of the original text, but the translators were clearly aware of this and have added a useful bibliography of supplementary references up to 1967. The work is handsomely bound and many of the diagrams have been specially redrawn.

The translators are to be congratulated on the completion of an exceptionally difficult task, for this is an excellent production which will be a standard reference for research workers. Although the book will undoubtedly appeal to those who are already familiar with the subject, it cannot be so recommended to beginners. The translators would earn further acclaim if they could now see their way to producing a condensed account based on this material to meet their needs.

I. D. MUIR

RADIATION MEASUREMENT

Applied Radiation Protection and Control

By J. J. Fitzgerald. Vol. 1: Pp. xii+1–540. 250*s*; $30. Vol. 2: Pp. 541–1018. 175*s*; $21. (Gordon and Breach: New York and London, June 1970.)

RADIATION protection embraces many disciplines and, in consequence, it must be difficult for one man to write what the preface to this book calls a "Handbook of Radiation Protection and Control Data". It is certainly not possible for one reviewer to give adequate critical treatment to all twelve chapters (in these volumes), but a study of those which fall within my special competence is not encouraging as far as the others are concerned.

Many of the illustrations are taken from textbooks of the 1950s, US Atomic Energy Commission laboratory reports of the 1950s, or are derived from measurements made by the author in the 1950s. Much detailed information relates to apparatus which was available in the United States eight or more years ago, and, in general, the book is rather dated. Reference is made to publications of the US National Committee on Radiation Protection up to 1966 (although little use is made of them in the text), but international organizations fare much worse. What are stated to be the structures of the International Commission on Radiological Protection (ICRP) and International Commission on Radiological Units and Measurements (ICRU) "at the present time" are, in fact, many years out of date. There is no reference to any ICRP publication later than 1959, and although ICRU reports up to 1963 are cited their recommendations are not incorporated in the text, because the author still speaks of "exposure dose", gives a long outmoded definition of the roentgen, and nowhere even mentions "quality factor" or "dose equivalent". A book with these deficiencies which also calls liquid scintillation counting "a rather recent innovation" and has no reference to the rapidly developing subject of thermoluminescent dosimetry later than 1960 cannot be recommended for use in the 1970s.

J. R. GREENING

FUNDAMENTALS OF PHYSICS

Fundamental Principles of Modern Theoretical Physics

By R. H. Furth. (International Series of Monographs in Natural Philosophy, Vol. 24.) Pp. xv+351. (Pergamon: Oxford, London and New York, April 1970.) 90*s*; $12.

THE principal object of this book is to give an introductory development of quantum theory, relativity (special and general) and statistical mechanics. It is not a specialist textbook, in the strict sense of the word, but a presentation of the fundamentals with emphasis on ideas rather than rigour. As one who thinks that nowadays practical applications tend to overshadow the basic ideas of a subject, I applaud the author's approach. Of course applications are vitally important and are not entirely neglected in this book.

Dr Furth has properly concentrated on the more standard topics but, in an introductory text of this kind,

there should be more discussion of more recent or more advanced areas in order to whet the reader's appetite. One need only give a taste. Often a single word would do. The author has tried to do this as, for example, in the section on the Dirac equation (itself a formalistic titbit) where the exotic words "spinor calculus" appear. More of this is needed.

There would be no sense in discussing omissions in what is an admittedly selective text, but I think it regrettable that nowhere do the words group theory occur. The ideas can hardly be classed as recent or difficult and a few words only would have served to link vectors, tensors, spinors and angular momentum in a significant way and could have provided a tempting foretaste of symmetries and the classification of elementary particles.

Nevertheless within the limits set by the author this is a really excellent text and can be highly recommended either for teaching or self study. The style is lucid and the various sections are nicely arranged.

Finally, one error of detail caught my eye: the "four" in the four dimensional Dirac matrices is not the same "four" that occurs in the four dimensions of space-time, as the author seems to imply on p. 213.

J. S. DOWKER

SATELLITE PROBLEMS

Principles of Celestial Mechanics

By Philip M. Fitzpatrick. Pp. xvii+405. (Academic: New York and London, June 1970.) 119*s*.

THE title of this book does not adequately indicate its contents: although Professor Fitzpatrick certainly expounds the principles of celestial mechanics, his principal concern is with the problems of artificial satellites and the treatment is directed to that end. The first six chapters are chiefly occupied with the fundamentals, two-body orbits, expansions in elliptic motion and systems of coordinates. Chapters seven to eleven cover Lagrange's planetary equations, Hamiltonian functions and Hamilton-Jacobi theory. In chapter twelve gravitational potential is discussed, and in chapter thirteen the drag and gravitational perturbations of Earth-satellite orbits. The final chapter is really quite separate, being an account of the rotational motion of an artificial satellite.

The early sections of the book provide a sound introduction to the various concepts and problems of celestial mechanics, and the bias towards space will be welcomed by the majority of students of the subject who will make their careers in the space industry rather than in pure astronomy. Occasionally the mathematical treatment is a little laboured, or even clumsy, but again many students may like an intelligible step-by-step approach better than smart but mystifying tricks. The part of chapter twelve on the Earth and its gravity field is not entirely satisfactory: there are several misleading remarks, particularly on page 287, where it is implied that satellite orbital theory has not been developed for high-order harmonics. In fact there have been many generalized treatments covering all harmonics such as those of Groves (1960) and Kaula (1961), and also explicit formulae for the variations in orbital elements, such as those of Merson (1963). Chapter thirteen also has faults: the lengthy discussion of atmospheric drag effects, although correct as far as it goes, remains entangled in academic complications instead of exposing the practical problems. But the final chapter is particularly thorough and is a valuable addition to the material.

All in all, this is a competent and useful university textbook. Although it has defects, they are outweighed by the virtues of its clear and straightforward exposition.

D. G. KING-HELE

Short Notices

The Upper Thames.

By J. R. L. Anderson. (T Regions of Britain.) Pp. 301+40 plates. (Eyre a Spottiswoode: London, July 1970.) 75*s*.

FOR a river which has been associated with hum settlements for as long as a quarter of a million ye or more, it seems a little strange that the exact origin the River Thames is still disputed. The author of t readable and informative book seems, however, to fav a spot in a clump of trees at Trewsbury Mead, n Cirencester, and so does the Thames Conservancy, for has appropriately marked the place with a statue of Father Thames which once adorned the Crystal Pal in London. From Trewsbury Mead, the river wande with a few unexpected twists, to London and the s Mr Anderson, however, has restricted his book to Thames's upper tracts, well westward of London, ir region bounded by the hill country of its source to west, Banbury in the north, Thame in the east, a Newbury and Kennet in the south. The emphasis is the history of human settlements in this remarka unified region, but the author also describes the charac of today's towns and villages. The book is supplied w a splendid set of photographs, several maps, a gazett and an itinerary for a river cruise. It is a delight read.

Collins Guide to Tree Planting and Cultivation.

H. L. Edlin. Pp. 349+59 illustrations. (Collins: Lond September 1970.) 42*s*.

IF you have a drooping oak which looks near to colla it may be time to call in the tree surgeon to bolster it using his armoury of antiseptic creosote, cables, s and hatchets. If, however, there is a well establis stump to be removed he may have to bring his explosi and stump gobbler. Although H. L. Edlin advises a teurs against attempting any but the simplest surg he explains the techniques in this thoroughgoing gu But this is not just a book for amateurs; it is pac with every sort of information for tree planters, whet operating on a tiny scale in the back garden, or o large scale in the forest. Half the book is taken up w a catalogue telling which trees should be planted wh and when, and how they should be tended thereaf If this advice is heeded, many trees might be saved fr certain death as a result of being planted in unsuit conditions.

Geological Highlights of the West Country: A Nat

Conservancy Handbook. By W. A. Macfadyen, v contributions by A. W. G. Kingsbury. Pp. (Butterworth: London, July 1970.) 60*s*.

MOTORISTS with a bent towards geology who are head for the south-western counties of Britain may like tuck this new handbook into a handy pocket in t motor-cars. By planning their route to take in som more interesting geological features to be found in Son set, Gloucester, Dorset, Devon and Cornwall, they may find they have missed the traffic jams. This guide descr ninety interesting sites, with their special inter indicated, and several caves, some noted for their asso tion with Palaeolithic man. Exact locations are gi and Dr Macfadyen has also included maps of each cou with the described sites marked on them so as make the planning of a geological itinerary all the ea There are also plenty of helpful map sections stratigraphic successions, but the six photographs poor.

Correspondence

Chinese Restaurant Syndrome

SIR,—There is a major criticism that can be raised against the conclusion presented by Morselli and Garattini[1] that there is no connexion between the ingestion of monosodium glutamate and the Chinese restaurant syndrome. This is that they did not use susceptible subjects. From the very first reports of this condition it was apparent that only a relatively small proportion of normal individuals was susceptible[2], the proportion being higher among women than among men[3] (most of the subjects in the experiments reported by Morselli and Garattini were men). Obviously a study of non-susceptible subjects only will not reveal significant reactions.

My own interest in the Chinese restaurant syndrome arises from my suffering from this condition for at least twelve years before the original description of it by Ho Man-Kwok[4] in 1968. The onset of my recognition of the symptoms occurred when I moved from England to Boston and started to eat in the many excellent Chinese restaurants in that city. Like others[3] I was able to prepare a list of "safe" and "to be avoided" Chinese restaurants.

From my personal experiments (unfortunately with only one subject) I can assure the authors that a susceptible subject is capable of recognizing whether monosodium glutamate has been ingested or not: to some extent the amount ingested can also be judged from the severity of the symptoms.

Yours faithfully,

JEAN HIMMS-HAGEN

Department of Biochemistry,
University of Ottawa,
Ottawa 2, Canada.

[1] Morselli, P. L., and Garattini, S., *Nature*, **227**, 611 (1970).
[2] Schaumberg, H. H., and Byck, R., *New Engl. J. Med.*, **279**, 105 (1968).
[3] Amos, M., Leavitt, N. R., Marmorek, L., and Wolschina, S. B., *New Engl. J. Med.*, **279**, 105 (1968).
[4] Man-Kwok, Ho, *New Engl. J. Med.*, **278**, 796 (1968).

Milk Secretion

SIR,—Wooding, Peaker and Linzell[1] seem to portray the knowledge of milk secretion as involving little enlightenment and continuing controversy. In my opinion progress in this field has been impressive and substantial agreement regarding the process is emerging. The two basic milk secretion mechanisms discovered by Bargmann and Knoop[2], one involving expulsion of fat globules by envelopment in plasma membrane and the other emptying of Golgi vesicles containing milk proteins through the plasma membrane, are widely accepted as fact. Wooding *et al.*[1] (among others) have reported crescents of cytoplasmic material attached to some (1 to 5 per cent) secreted milk fat globules. They combine this observation with their detection of cytoplasmic fragments (concentration ?) in milk as evidence supporting and reviving the theory of apocrine secretion; that is, secretion by cell decapitation with loss of cytoplasm. The unpublished findings of at least three additional groups confirm their observations regarding crescents and all will agree that average milk contains minor amounts of cellular debris.

It seems important to ask how quantitatively significant are these apocrine manifestations. In the case of the cytoplasmic crescents, it can be assumed that average bovine or caprine milk contains about 4 per cent by volume of fat globules. Using the figure by Wooding *et al.* of 1 to 5 per cent of globules with crescents attached, and assigning an average volume of one-fourth globule volume to the crescents, this aspect of the apocrine mechanism concerns 0·01 to 0·05 per cent of the milk volume. There appears to be little loss of crucial cell machinery into milk such as nuclei, pointed out by Wooding *et al.*[1], or mitochondria[3]. In fact the very low levels of phospholipid in milk (0·02–0·05 per cent) indicate that relatively little cytoplasmic membrane material of any kind is secreted.

Yours faithfully,

STUART PATTON

Division of Food Science and Industry,
The Pennsylvania State University,
University Park, Pennsylvania 16802.

[1] Wooding, F. B. P., Peaker, M., and Linzell, J. L., *Nature*, **226**, 763 (1970).
[2] Bargmann, W., and Knoop, A., *Z. Zellforsch. Mikrosk. Anat.*, **49**, 344 (1959).
[3] Patton, S., Hood, L. F., and Patton, J. S., *J. Lipid Res.*, **10**, 260 (1969).

A Definition of Intelligence

SIR,—In the course of work on cybernetic machines we became increasingly concerned with the problem of intelligence in machines and intelligence in man. An adequate definition of intelligence could not be found in literature and a new one relevant to this problem seemed to be necessary. After due consideration the following definition was formulated:

> "Intelligence is that faculty, of mind, by which order is perceived in a situation previously considered disordered."

This definition resolves the potential conflict between so-called intelligent machinery and man since it ascribes, by definition, intelligence only to organisms which have a larger set than a machine can have. Thus machines may have intelligent-like performance but cannot be intelligent because the machine's instruction set is a proper subset of man's instruction set.

Yours faithfully,

H. A. FATMI
R. W. YOUNG

Department of Electronics,
Chelsea College,
University of London,
London SW3.

Phenolase Inhibitors

SIR,—J. R. L. Walker reported isolation of an unknown phenolase inhibitor from cultures of *Penicillium expansum* (*Nature*, **227**, 298; 1970). We have found that penicillamine (a degradation product of penicillin) is a potent inhibitor of both mammalian and plant phenolase[1]. It is likely that small amounts of penicillamine accumulating in the culture medium cause the inhibition of *o*-diphenol oxidase.

Yours faithfully,

K. PRABHAKARAN

Research Department,
US Public Health Service Hospital,
Carville, Louisiana 70721.

[1] Prabhakaran, K., Harris, E. B., and Kirchheimer, W. F., *J. Bact.*, **100**, 935 (1969).

Obituary

Academician M. M. Shemyakin

MIKHAIL MIKHAILOVICH SHEMYAKIN, who died on June 26, was one of the leading organic chemists and biochemists in the Soviet Union.

He was born in 1908 in Moscow, and was educated at Moscow State University, where he graduated in 1930. In the years that followed, he was attached to a number of research institutes and establishments: the Institute of Organic Semi-products and Dyes (1930–35), the Moscow Institute of Precision Chemical Technology (1935–45), the All-Union Institute of Experimental Medicine (1937–59), the Moscow Textile Institute (1945–47) and also the Institute of Biological and Medical Chemistry of the Academy of Medical Sciences of the USSR. Since 1964 he had held the post of Academic Secretary of the Department of Biochemistry, Biophysics and Chemistry of Physiologically Active Compounds of the Academy of Sciences of the USSR; since 1960 he had been Director of the Institute for the Chemistry of Natural Compounds of the Academy of Sciences of the USSR.

Shemyakin's research covered the chemistry of natural compounds and molecular biology. He made outstanding contributions to the study of the structure and function of the principal biopolymers and bioregulators, and carried out fundamental research into the problems of proteins and peptides, biological membranes, antibiotics and vitamins. He was author or co-author of a number of definitive works in these fields, including *The Chemistry of Antibiotics* (1953), *Oxidizing-Hydrolytic Transformations of Organic Compounds* (1957) and *Stereochemistry and the Mechanism of the Witting Reaction* (1963).

He played a considerable part in the development of biochemistry and organic chemistry throughout the Soviet Union, taking an active part in the organization of new research centres and institutes and in stimulating the work of biologists, chemists and physicists engaged on the solution of many currently pressing problems of Soviet science and the Soviet economy. For his services to the economic progress of his country, he had been awarded a number of Soviet orders and medals, and had been made a Hero of Socialist Labour. He also devoted much attention to the fostering of international links in science and was one of the Soviet representatives on the Scientific Advisory Panel of the Ciba Foundation. Shortly before his death, he had been Honorary President and Director of the International Symposium on the Chemistry of Natural Compounds in Riga.

Academician A. V. Shubnikov

ALEKSEI VASIL'EVICH SHUBNIKOV, the leading Russian crystallographer, died on April 27, in his eighty-fourth year.

Shubnikov was born in Moscow and began his research in 1910, while still a student at Moscow University. During the following sixty years, he carried out wide-ranging investigations of the theory and practice of crystallography. His research into symmetry led to the formulati of the concept of generalized symmetry (antisymmetr; He made a considerable contribution to the developme of the theory of piezoelectricity, and was responsible the discovery and application of a number of pie electric materials. His outstanding work on the growi and processing of crystals made him a pioneer of t synthetic crystals industry of the Soviet Union.

In addition to his research work, Shubnikov was sponsible for the initial organization of the Institu of Crystallography of the Academy of Sciences of t USSR, of which he was director for more than twen years. He founded the Department of Crystal Phys of Moscow University, and was founder and first editor-chief of the journal *Kristallografiya*. He was the auth of a number of standard works on crystallograp including original monographs and textbooks.

For his discoveries in crystallography and his servi to Soviet science and industry, Shubnikov was award a number of prizes and honours, including two St Prizes, the Order of Lenin (twice), the Order of the R Banner (twice) and the title of Hero of Socialist Labo

Dr M. M. Gerasimov

MIKHAIL M. GERASIMOV, one of the most outstandi figures in modern Soviet archaeology, died on July after a prolonged, unspecified illness.

Gerasimov, who was born in Leningrad in 1907, co bined his studies as an anthropologist and archaeolog with a considerable talent as an artist and sculpt In 1927, following his discovery of the upper Palaeolitl settlement of Mal'ta (80 km from Irkutsk), he beca interested in the possibility of the reconstruction of t soft tissues of the face, using the principles of anthropolo and the techniques of sculpture.

In 1927, he began work on a series of "portraits" various anthropological types and historical figures, t latter including Timur (Tamerlane), Andrei Bogolyubsl Yaroslav the Wise, Admiral Ushakov, Ivan the Terril and Friedrich Schiller. His Palaeolithic and Neolitl reconstructions covered the main archaeological sites the world; special emphasis was placed, however, on t Mal'ta settlement and also Trepole (Ukraine).

His work established plastic reconstruction as a rec nized part of Soviet archaeological practice, and a spec laboratory for this was founded under his directorsh a post which he held to the end of his life.

His work has also found an application in foren science.

His principal publications include *The Principles Facial Reconstruction on the Basis of Skull Formati* (1949) (for which he was awarded a Stalin Prize in 195 *Facial Reconstruction on the Basis of Skull Formati Modern and Fossil Man* (1955) and *The Mal'ta Pala lithic Settlement and its Place among the Palaeolithic Rel of Siberia* (1961).

Announcements

University News

George B. Field has been appointed chairman of the Astronomy Department at the **University of California** (Berkeley), in succession to **Ivan R. King. Dr Harold M. Agnew** has assumed direction of the **Los Alamos Scientific Laboratory**, New Mexico, operated by the University of California for the US Atomic Energy Commission. He succeeded **Norris E. Bradbury**, w has retired.

Michael B. McElroy, formerly at Kitt Peak Natio Observatory, has become Abbott Lawrence Ro professor of atmospheric science at **Harvard Universi Dr Neal Balanoff** has been appointed professor instructional development and director of a ne

established Office for Instructional Development at the **Harvard School of Public Health.**

Dr E. A. Faulkner has been appointed to the professorship of solid state electronics recently established in the Department of Physics, **University of Reading.**

Professor Walter E. Meyerhof has been appointed chairman of the Physics Department at **Stanford University,** in succession to Professor **Arthur Schawlow.**

Dr R. W. Coles, formerly at the Claremont Colleges, California, has been appointed director of the **Washington University Tyson Research Center,** near Eureka, Missouri.

Appointments

Dr DeWitt Stetten, jun., dean of the Rutgers University Medical School, has been appointed director of the **National Institute of General Medical Sciences,** US Public Health Service.

Sir Roger S. Falk has been reappointed chairman and **Mr J. G. Jenkins** deputy chairman of the **Central Council for Agricultural and Horticultural Co-operation. Alderman F. T. Hussey, Mr A. Norton, Mr H. R. Kemp** and the **Hon. J. Addington** have been appointed independent members of the council. The following members have been appointed from nominations by the farmers' unions and central cooperative bodies: *England,* **Mr R. Butler, Mr G. D. Robinson;** *Scotland,* **Mr W. Peat, Mr Goodfellow;** *Wales,* **Mr D. L. Carey Evans, Alderman T. Jones;** *Northern Ireland,* **Mr F. M. B. Loane, Mr W. J. Crawford.**

Professor Norton Ginsburg, professor of geography at the University of Chicago, has been elected president of the **Association of American Geographers.**

Miscellaneous

Dr W. M. Hutchison, University of Strathclyde, has been awarded the **Robert Koch prize and medal for 1970** for his work on toxoplasmosis infection.

Dr Marvin Legator, chief of the Cell Biology Branch, US Food and Drug Administration, has received the Key lecture award of the **American Genetics Association** for his contributions to the implementation of genetics for human welfare.

Professor J. J. Weiss has been awarded the Marie Curie medal of **l'Institut du Radium,** Paris, in recognition of his contributions to radiation chemistry and radiobiology, and to mark his retirement from the chair of radiation chemistry, in the University of Newcastle upon Tyne.

Dr Irvine H. Page, director of the Research Division of Cleveland Clinic Foundation, and **Sir George Pickering,** master of Pembroke College, Oxford, share the **1971 Stouffer prize** for their investigations and leadership in the field of heart research.

Sir Alexander Haddow, former director of the Chester Beatty Research Institute, has been awarded the **1970 Sidney Farber Medical Research award** by the Children's Cancer Research Foundation, Boston, for his contributions to the development and support of medical care and research.

The **International Union of Food Science and Technology** was formally inaugurated on August 14 in Washington DC. The Union is intended to foster international cooperation and to support progress in research and technology in food science. Membership is open to all nations on the basis of one representative body from each nation. Further information can be obtained from the Secretary General, Professor Erik von Sydow, Swedish Institute for Food Preservation Research, Kallebäck, Göteborg S, Sweden.

Applications are invited by the Banting and Best Department of Medical Research, University of Toronto, for the **C. H. Best Foundation postdoctoral fellowship** which is part of a new research programme placing special emphasis on hormones and other agents that regulate body chemistry. The fellowship is for two years and carries a basic stipend of $8,000 a year. Further information can be obtained from the Department of Information, University of Toronto, Toronto 5, Ontario, Canada.

Lalor Foundation postdoctoral research awards are available to investigators concerned with mammalian reproductive physiology—in particular with uterine phenomena as they may relate to early gestation, the dysgenic aspects of such phenomena, and better and safer means of prophylactic termination of pregnancy. The awards are open to workers of any nationality under the age of 41, and can be used for work at the candidate's own institution or elsewhere for any period up to one year. Further information can be obtained from the Lalor Foundation, 4400 Lancaster Pike, Wilmington, Delaware 19805, USA. The final date for receipt of applications for 1971 awards is January 15, 1971.

ERRATUM. In the article "Single Hit Aetiology of Human Minor Congenital Malformations unassociated with Major Malformations" by E. B. Hook and J. J. Perry (*Nature,* **227,** 847; 1970), the second and third sentences of the footnote to Table 3 should read: If unilateral manifestation is independent, then the expected bilateral incidence is the product of the probability of occurrence on either side. This cannot be calculated from the data in their present form, but can be shown, for these data, to be less than the square of the total observed unilateral incidence which is given in this column.

ERRATUM. In the article "The Classical Charged Photon", by P. Dolan (*Nature,* **227,** 825; 1970), the following changes should be made: the fourth sentence of paragraph 1 should read "In Bonnor's solution L^i, the propagation vector of the electromagnetic field, is always parallel to J^i . . ."; the second sentence of paragraph 2 should read "(I write $\tilde{\pi} = -N_{i;k}\bar{M}^i L^k$ to avoid confusion with the transcendental number π.)"; in equations 6 all π except those with factor 2 in front of them should read $\tilde{\pi}$; φ_t should be φ_1 and a should read α.

International Meetings

October 6–7, **Agricultural and Allied Industrial Tractors,** London (Institution of Mechanical Engineers, 1 Birdcage Walk, London SW1).

October 6–9, **Corrosion and its Prevention by Non-metallic Composite Materials,** Cardiff and Avonmouth (Peter Oldham, Plastics Design and Engineering, Culcheth, near Manchester).

October 13–14, **Environmental Improvement—Agriculture's Challenge in the Seventies,** Arlington, Virginia (Carol L. Rogers, Office of Information, US National Academy of Science, 2101 Constitution Avenue NW, Washington DC 20418, USA).

October 13–16, **Industrial Reciprocating and Rotary Compressors: Design and Operational Problems,** London (Institution of Mechanical Engineers, 1 Birdcage Walk, London SW1).

October 14–16, **Earth Station Technology,** London (Mrs Ann Cook, Manager, Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2R OBL).

October 20, **Acoustics as a Diagnostic Tool.** London (Institution of Mechanical Engineers, 1 Birdcage Walk, London SW1).

British Diary

Monday, October 5

Scientific Film Evening (6.30 p.m.) Society of Chemical Industry, at Shell Centre Theatre, Shell Centre, London SE1.

Tuesday, October 6

Agricultural and Allied Industrial Tractors (two-day symposium) Institution of Mechanical Engineers, Automobile Division, at 1 Birdcage Walk, London SW1.

How We Learn About the Ultimate Nature of Matter (5.30 p.m.) Professor C. C. Butler, FRS, Royal Institution at 21 Albemarle Street, London, W1. (Lecture for Sixth Form pupils from Schools in London and the Home Counties. To be repeated on October 7, 13 and 14.)

Just What are Plastics ? (7 p.m.) Mr A. G. Jones, Society for Analytical Chemistry, at the Eden Arms Hotel, Rushyford, Ferryhill, Co. Durham.

Manufacture of Wallpaper (7.30 p.m.) Mr A. O. Sutherland, Oil and Colour Chemists' Association, jointly with the Society of Dyers and Colourists, at the Griffin Hotel, Boar Lane, Leeds.

Preventing Wind-Induced Oscillations of Structures of Circular Section (5.30 p.m.) Mr D. E. Walshe and Mr L. R. Wootton, Institution of Civil Engineers, at Great George Street, London SW1.

Wednesday, October 7

Colour Education and Training (2 p.m. seminar) Colour Group (Great Britain), in the Mechanical Engineering Department, Imperial College, Exhibition Road, London SW7.

The Development of Flame Spectrophotometric Detectors for Gas-chromatography (7 p.m.) Professor R. Hermann, Society for Analytical Chemistry, in the Scientific Societies Lecture Theatre, 23 Savile Row, London W1.

Urban Motorway Planning and Elevated Structures (6.15 p.m.) Mr R. L. Wilson and Mr P. A. Brown, Institution of Civil Engineers, at Great George Street, London SW1.

Thursday, October 8

Optical Methods in Electrochemistry (6.30 p.m.) Mr R. Parsons, Mr J. Albery and Mr M. Stedman, Society for Analytical Chemistry, at the School of Chemistry, The University, Bristol.

Practical Applications of an HF Plasma Torch to Emission Spectrography (6.30 p.m.) Mr S. Greenfield and Mr P. B. Smith, Society for Analytical Chemistry, Midland Section, in the Large Lecture Theatre, Henry Wiggin and Co., Ltd, Holmer Road, Hereford.

The South East Regional Plan (4 p.m. informal discussion) Institution of Civil Engineers, Transportation Engineering Group; and the Regional Studies Association, at Great George Street, London SW1.

Friday, October 9

Industrial Espionage (6.30 p.m.) Major R. B. Matthews, Oil and Colour Chemists' Association, at the Manchester Literary and Philosophical Society, 36 George Street, Manchester 1.

Mammalian Responses to Cold (2 p.m. symposium) Society for Low Temperature Biology, at the British Institute of Radiology, Welbeck Street, London W1.

The Development of Flame Spectrophotometric Detectors for Gas-Chromatography (6.30 p.m.) Professor R. Hermannn, Society for Analytical Chemistry, in the Chemistry Tower, The University, Salford.

Monday, October 12

Jacking of Railway Bridge Abutments (5.30 p.m. informal discussion) Institution of Civil Engineers, at Great George Street, London SW1.

Reports and Publications

(not included in the monthly Books Supplement)

Ministry of Transport: Road Research Laboratory. RRL Report LR 320: Chest Injuries in Unrestrained Vehicle Occupants Who Survived a Road Accident. By E. Grattan, Nancy G. Clegg and J. A. Hobbs. Pp. 13. (Crowthorne, Berkshire: Road Research Laboratory, 1970.) [278

Modern China Studies—International Bulletin No. 1: Current Post-Graduate Research. Pp. viii+87. (London: *The China Quarterly*, Contemporary China Institute, School of Oriental and African Studies, London University, 1970.) 20*s*; $2.50. [288

Countryside Commission. Disused Railways in the Countryside of England and Wales—A Report to the Countryside Commission. By J. H. Appleton. With a Section on Disused Railways and Agriculture by Richard J. Appleton. Pp. 82+13 plates. (London: HMSO, 1970.) 20*s* net. [288

Careers Research and Advisory Centre. Degree Course Guide: Veterinary Science. (A Guide to First Degree Courses in UK Universities and Colleges, 1970/1971.) Pp. 18. (Cambridge: Careers Research and Advisory Centre, 1970.) 8*s* (40*p*). [288

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OCTOBER 10, 1970

Turn Up for the Book

NOTHING could more vividly demonstrate the futility of sciency policy in the United States than the way in which, at the end of the Congressional season, it should turn out that the Mansfield amendment to the Military Appropriations bill should have been thrown away. It now seems that the full rigours of the Mansfield amendment, with its insistence that research supported out of military funds should have a "direct and apparent relationship" to military operations, will not in future be applied, but enough of the financial year has gone for damage to have been done. After all, the watering down of the Mansfield language has not been accompanied by the appropriation of sums of money to allow abandoned projects to be restarted and, even if Congress had thought of setting aside the money, the chances are that the Administration would have declined to spend it. To be sure, next year there may be more freedom for the Pentagon to support basic research. Certainly, other agencies will feel less threatened than in the past year by the presence of the Mansfield amendment on the books. In retrospect, then, there is a possibility that the whole episode will be regarded as a temporary setback—a storm that came and went. That would be a great misfortune.

The lasting value of the fuss about the Mansfield amendment is that it has demonstrated as clearly as anybody could wish the anomalies in the present system for supporting research with public funds in the United States. Senator Mansfield was, of course, right to protest at the way in which defence money has in the past several years been spent on the support of basic research. Who at this stage will carry a torch for Project Themis, the Pentagon's ill-judged attempt to set up in competition with the National Science Foundation as a sponsor of excellence in American universities? And who is happy at the way in which a great deal of the encouragement of basic research in the fifties and sixties was dependent on the willingness of the military services to listen less critically to appeals for help from academics than the regular grant-giving agencies, particularly the National Science Foundation? The truth is that too many academic institutions in the United States have become too dependent on military support for basic research. A great many of the chickens which have come home to roost in the past few months, among which the trouble at the Instrumentation Laboratory at MIT stands out, show that it would be hard even if it were desirable to recreate the old pattern of military expenditure on research.

What is the alternative? The first thing to be said is that military support for academic research has been necessary in the past only because the agencies set up for just this purpose have been badly provided with money. It follows that last week's events in Congress do not make less urgent the need to strengthen the National Science Foundation. The hope that the foundation's budget will be much larger in the coming financial year (for which plans are already being made in Washington) is known to recur annually and the experience of the recent past suggests that it is only improbably fulfilled. Between 1970 and 1971 the National Science Foundation's budget will have been increased by some 7 per cent, enough to make up for inflation but not to make good the neglect of recent years. If there is not a budget of more than $700 million for the financial year which begins in July 1971, the whole scientific community in the United States will be right in thinking that it has once more been asked to accomplish the impossible with impossibly meagre resources. The danger now is that the softening of the effects of the Mansfield amendment will be represented by the Administration as a sign that the National Science Foundation can go short of funds for yet another year. That would be intolerable.

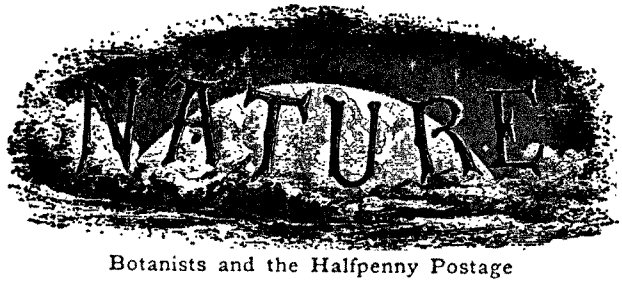
Two Ways to the Moon

THE success of last week's Russian rocket to the Moon—Lunar 16 as it was called—has presented the United States National Aeronautics and Space Administration with something of a problem, particularly now that the end of the present series of Moon landings is in sight (see page 110). Since the beginning of the Apollo programme, there have been fierce arguments that the project for landing men on the Moon has been too expensive and in any case unnecessary. Although at first, in the early sixties, the Russians were clearly at pains never to suggest that the first astronauts would not be Russian, more recently they have been extolling the virtues of automatic go and return vehicles to the Moon. They are probably right in claiming that a single visit to the Moon by Lunar 16 costs 5 per cent or less than the cost of a single Apollo journey, but the relative usefulness of the two methods for the exploration of the Moon is not, of course, as easily determined as a comparison of costs suggests. The utility of the Russian method will depend on the extent to which samples of the surface can be collected with discrimination and, ultimately, on the ease with which it will be possible to move instruments from one place to another on the surface of the Moon. Evidently the Russians have a long way to go before their technique is properly developed. The prospect that it could be used for collecting regular samples from the Moon and bringing them back to Earth must, however, be a source of serious disquiet in Washington. The folly

of the Apollo programme, with its excessive dependence on cumbersome techniques of launching people into space, is now plain for everybody to see. The practical question is how quickly NASA will be able to duplicate the work the Russians have been carrying out, for there seems no doubt that competitiveness will decree that there must be such an attempt. Most probably, it will be two or three years before the first automatic Moon landing from the United States is carried out. By then, the memory of Apollo 11 will have begun to fade, if it has not done so already.

The implications of this dilemma for the future of the development of space flight in the United States are several. First of all, there can now be very little doubt that the ambitious plans for launching space shuttles and permanently habitable space stations are at once too ambitious and too cumbersome. As with the rest of physical research, so with space research, the best instruments are the most delicate. What this implies in practice is that there should be a greater concern in the United States with the potential scientific benefits of the exploration in space. There may be something in the view that it would have been better if the comparatively modest amounts which the Russians have spent on Lunar 16 had been spent on more mundane research.

100 Years Ago



Botanists and the Halfpenny Postage

FOR several years I have been in the habit of sending herbarium specimens by "book post," by merely placing the plants between sheets of white cardboard, which were invariably fastened by string, without wrappers, and the address written on the cardboard itself; so that, in fact, the parcel was open at both ends and sides. On Tuesday last I did up a small packet of green or living plants, and sent them to the village post-office. As the parcel weighed under four ounces, I affixed two halfpenny stamps. On calling at the post-office the next day, imagine my disgust at seeing my parcel of plants stuck up in the window to be sun-dried! Upon inquiry, I found that the postman had very *wisely* declined taking it to the borough office, as "several similar parcels were lying about there now, and would not be forwarded until the senders had prepaid the postage at letter-rate."

I say the postman acted wisely in refusing to take my parcel, because, on taking it to the Newbury post-office myself on Thursday, I found that unless I paid eightpence, or rather put on seven more penny stamps, *double* that amount would have actually been charged to the recipient, at which I should have felt much grieved, as the specimens were really not worth half the amount.

Another gentleman of my acquaintance sent a parcel to the same office with a fourpenny stamp on; this the clerk kindly *defaced*, and returned the parcel as "not sufficiently paid"!

I have read the new rules, and can see no clause bearing on this subject—either for or against herbarium specimens, or other objects of natural history, being sent between cardboard with open ends at the old rate of four ounces and under for one penny, and should therefore be glad of any correct information from you or the readers of NATURE.

East Woodhay, Oct. 7 HENRY REEKS

From Nature, **2**, 474, *October* 13, 1870.

OLD WORLD

SEWAGE

Epidemic Fears Exaggerated

IN the face of gloomy predictions of disease and disaster to follow the strike last week by sewage workers and other local authority employees in Britain, independent assessments of the risk to health by microbiologists were surprisingly and reassuringly in agreement with the official view of the situation. It seems, in the short term at any rate, that little danger to health should ensue from the inadvertent or inevitable discharge into rivers of untreated or partially treated sewage, even if the effluent is voided into rivers from which drinking water is drawn.

Mr G. E. Eden, assistant director of the Water Pollution Research Laboratory, said last week that the chief danger was not so much the immediate risk of epidemic arising from polluted drinking water but the long term prospect of mechanical failure in either the sewage pumping stations or purifying plants, resulting from an inevitable decline in the standard of maintenance while the machinery was being manned by unskilled volunteers. But failure or closure of the sewage pumping stations in the London area would precipitate a very grave situation indeed in which the risk of the flooding of low lying land would be added to the danger of epidemic.

Professor F. Fulton of the London School of Hygiene and Tropical Medicine pointed out that drinking water in Britain is tested and monitored so rigorously for faecal and bacterial contaminants that the immediate risk of water-borne infection is small. It is, of course, true that viral contaminants are very much harder to detect than those of a bacterial nature, and that certain water-borne viruses such as those causing infectious hepatitis are comparatively resistant to simple hygienic precautions such as boiling. Nevertheless, Professor Fulton thought viral infection to be a relatively slight risk.

London, among the first of the areas affected by the strike, is particularly well protected against water-borne infection, for drinking water supplied to the capital is stored, after treatment, for five to six weeks in special reservoirs before being fed into the domestic water system. These conditions are particularly unsuitable for the survival of microorganisms, and so this procedure is remarkably effective in maintaining the purity of the water supply.

Earlier this week the situation seemed to be well under control in the affected areas. In Bristol, where untreated sewage was being discharged directly into the River Avon, a local authority spokesman claimed that reports that people living near the river were "terrified" were based on misinformation. A spokesman for the Greater London Council said that all sixteen pumping stations in the London area were functioning, and that preparations had been made to keep them operating for at least a further week. It is to be hoped that by the time this article is printed, these preparations will have proved unnecessary, for all shades of opinion are in agreement that if the present situation continues for more than a few weeks, the consequences will be grave.

TOXICOLOGY

Government Chemist allays Fears

THE accumulation of pesticide residues in British food seems to be under control. This can be concluded from the *Report of the Government Chemist for 1969*, published last week (HMSO, price 18*s*). A survey during the past few years has shown that the concentrations of organochlorides such as dieldrin and DDT in milk, butter, beef kidney fat and mutton kidney fat have remained very low, and that in many cases they are diminishing. For this reason the examination of milk, butter and home produced beef fat has been discontinued. The details of a recent survey of organophosphate residues in the diet may also give rise to optimism, but the report points out that wildlife is still in danger from pesticides.

In his survey of the organophosphate pesticides in the total British diet (samples of the total diet were devised on the basis of data supplied by the Ministry of Agriculture), the Government Chemist found that the most common was malthion. This is probably because malthion is used in the treatment of Australian and Argentinian grain before shipment. But malthion has a low mammalian toxicity compared with other organophosphate pesticides, and after degradation during transportation and processing, the amounts present in bread, cakes, flour and biscuits are much less than the maximum safe amount recommended by the Food and Agriculture Organization of the United Nations and the World Health Organization. These findings are similar to those of a survey carried out in the United States.

The report also dismisses fears that mercury, a metal highly toxic to living organisms, may be accumulating in foodstuffs because mercurial compounds are being used on crops as fungicides as well as being carelessly discarded by industry. In cereals, fats, fruits and milk, mercury was present below the detectable level of 0·01 part per million. Between 0·01 and 0·015 p.p.m. was found in samples of meat, root vegetables and green vegetables. The report says that these results should "allay some of the concern about mercury contamination in the national diet".

Pesticides are also accumulating in tobacco. This is not surprising because they are used extensively on the tobacco crop. Virginia tobacco, for example, contains up to 11 p.p.m. of DDT, but although these concentrations are considerably greater than those usually found in foodstuffs, only 8–12 per cent of the residue is inhaled, and of this, much remains in the smoke exhaled. Nevertheless, the report says, this could be an important source of DDT to the heavy smoker.

The Laboratory of the Government Chemist has also been examining wildlife tissue on behalf of the Nature Conservancy. The principal pesticide residues found in the eggs, organs and tissues of birds were DDT (in a metabolized form) and dieldrin. The greatest concentration of polychlorobiphenyls (PCB) found so far has also been recorded. As much as 900 p.p.m. were found in herons. These chemicals were first regarded merely as contaminants in the analysis of the pesticide residues. They were identified in 1967 and have since been recognized as another serious threat to wildlife because their effect on reproductive metabolism is similar to that of the pesticides themselves. Together with the organochloride pesticides, they may be responsible for the aberration in calcium metabolism which has been observed in many species of birds since the Second World War. Calcium metabolism in birds is intimately related to reproductive metabolism and is to a large extent regulated by the steroid sex hormones. The organochlorides are thought to induce hepatic enzymes that degrade the sex hormones.

ACADEMIC FREEDOM

Flying Start for New Council

THE view that career prospects are worsened for unorthodox or nonconformist academics wasput forward by Professor John Griffith of the London School of Economics at a conference at Imperial College, London, on October 3 to launch the Council for Academic Freedom and Democracy (see *Nature*, **227**, 1180; 1970). "It is very depressing that many colleagues have shown a great unwillingness to support those under attack," he said, saying that a member of the staff at LSE last year confided that although he deplored the way the authorities dismissed Mr Robin Blackburn from his lectureship last year, he could not say so publicly with a wife and two children to support.

The chief purpose of the new council will be to provide machinery to investigate cases such as those of Mr Blackburn and Mr Richard Atkinson, the man who was refused a lectureship at Birmingham University on what seem to be clearly non-academic grounds. In the minds of the 150 university teachers who attended the conference, the "Atkinson case" crystallized the dissatisfaction felt about the structure of universities and institutes of higher education in Britain. The plain fact seems to be that the Academic Appointments Board at Birmingham had refused Mr Atkinson a lectureship in sociology on the grounds that he had associated with students who were demanding the implementation of plans to democratize the structure of the university. The departmental selection committee had voted by five votes to four to offer him the post, and his former colleagues at the University of Manchester had warmly endorsed his ability as a teacher. His former students also found him an excellent teacher and found no political bias in his methods.

The debate about the posture which the new council should adopt turned out to be a lively encounter between those who saw it as a pressure group closely tethered to the Association of University Teachers, and those who wanted it to be a more independent body which would attract "middle of the road" lecturers. In the event, the conference settled on a compromise by which the CAFD, linked to the National Council for Civil Liberties, would draw on the NCCL for administrative and legal advice but would work closely with the AUT.

The chief danger of linking arms with the AUT and the Association of Teachers in Technical Institutions, Professor Griffith warned, is that members of these unions may themselves be involved on opposite sides of a dispute. Mr A. Booth of Harlow Technical College recalled that in many technical colleges the Principal, who wields more power than in most universities, was often also an official of the ATTI.

Mr W. Stephenson of Bedford College, London, pointed out that the AUT already has a commitment to democratization of university structures and that

it had shown its teeth by financing the defence of the three lecturers dismissed from the LSE, two of whom were later reinstated. This had cost the AUT £2,000. Another speaker warned that the public might see the CAFD as a weapon for fighting student militancy rather than government and university oppression. Encroachment of freedom from governing bodies and indeed industry was much more subtle and harder to combat.

Mr Richard Atkinson counselled against making the CAFD too specialized a body. "We must see ourselves as having interests intimately bound up with people in technical colleges and with students, people who have far more institutionalized unfreedom than we have," he said. He saw a danger of the same chasm developing between left wing students and liberal staff as had appeared in the United States.

RESEARCH PROGRAMMES

Space and Atomic Energy in India

An inadequate road and rail system seems to be one of the chief obstacles to the construction of nuclear power stations in India, according to a document now published by the Indian Atomic Energy Commission (*Energy and Space Research: A Profile for the Decade 1970–80*). Without a programme for the improvement of roads, the strengthening of bridges and so on, heavy items of equipment cannot be transported from the factories to potential sites for power stations. In the circumstances, it is anomalous that India is planning a space programme which is expected to cost £91·5 million during the next ten years. India's ambitions roughly match those that European organizations such as the European Launcher Development Organization and the European Space Research Organization have been mulling over during Professor Bondi's tenure as director-general. By the end of the 1970s India hopes to be able to launch 1,200 kg satellites into geostationary orbits.

Since 1961, space research in India has been under the wing of the Department of Atomic Energy. The programme is based on about 75 sounding rockets per year, chiefly for straightforward studies of the upper atmosphere and for X-ray astronomy. The reasons for turning this modest and reasonable programme into an attempt to join the countries with an indigenous launcher ability—at present the Soviet Union, United States, France, Japan and China—is vague. There are the usual unsupported platitudes about applications of satellites and, to be fair, India has a lot to gain from applications such as direct television, but there is also the argument that a space programme will drag Indian metallurgy, electronics and computing into the second half of the twentieth century.

Against this background, the plans for the development of atomic energy in India, the tidiest way of generating the electrical power that a developing country needs, have more sense in them. Even so, it is clear that the programme is not going well. If the present targets are attained, then by 1980 only six per cent of the electrical power in India will be generated by nuclear stations, compared with 22 per cent in Japan and nearly 30 per cent in Britain and the United States.

The Atomic Energy Commission is working on the principle that it is economic to use nuclear power in areas more than 600 miles from a coalfield. During the next ten years the plan calls for the building of reactors using natural uranium and moderated by heavy water, which will provide the plutonium for the fast breeder reactors to be developed for the 1980s. Between 1980 and 1985 the fast breeder reactors will be supplying ^{233}U for use in thorium cycle reactors. These reactors will be developed in the early 1980s for use after 1985. The reserves of thorium in India are thought to be roughly ten times the uranium reserves.

During the current five-year plan which runs from 1969 to 1974, about £141 million is earmarked for atomic energy, and Table 1 shows how the Atomic Energy Commission would like the spending to increase by 1980. By then the intention is to have 2,700 MW of installed capacity, which means that before 1974 building will have to start on five new stations totalling 1,700 MW.

ESTIMATED COST OF THE ATOMIC ENERGY PROGRAMME
(in millions of rupees, £1 sterling = 18·04 Rs)

	1970–75	1975–80
1,000 MW constructed or under construction	1,010	290
3 × 235 MW	440	1,860
2 × 500 MW	50	2,700
Design of 500 MW advanced thermal reactors	50	—
Fast breeder test reactor and research centre	320	230
Augmentation of heavy water production	750	200
500 MW fast breeder reactor	—	1,250
Gas centrifuge technology	100	1,000
Narwapahar uranium mines	40	140
Nuclear fuel complex	130	—
Reprocessing plants for plutonium	90	140
Bhabha Atomic Research Centre	650	1,000
Isotope applications	20	40
Total	3,650	8,850

The space research programme is more esoteric. At a cost of about £1·7 million for the development of the launcher, and slightly less for the development of a scientific satellite, India hopes to launch a 30 kg satellite into a 400 km orbit during 1974. The launcher for geostationary satellites is expected to cost about £8·6 million during the decade, and about £15 million is to be spent on a communications satellite. During the same period about £1·4 million will be spent on guidance systems and onboard computers. Much of the work will be done at the Space Science and Technology Centre which was established in 1965 close to the Thumba rocket range, on the geomagnetic equator in South India. Thumba is a sounding rocket range, however, near a densely populated area, and a range for satellite launches is being built on Sriharikota island, about thirty miles north of Madras on the coast of the Bay of Bengal. By December this year the range is expected to be in use for sounding rockets, and a year later for larger multi-stage rockets. Sriharikota will also contain the plant for the manufacture of the satellite launchers and testing laboratories. By 1980 the report estimates that 7,000 people in India could be involved in space research in India.

RESEARCH FUNDS

Selectivity to Stay

THE Science Research Council will continue to concentrate funds on a few centres of high activity in universities, applying the test of usefulness to industry. The council's chairman, Sir Brian Flowers, made this clear on October 2 when he opened the new computer laboratory at the Control Systems Centre of the University of Manchester Institute of Science and Technology. The laboratory has been equipped by means of a grant of £360,000 from the SRC, which has concentrated its support for control engineering on Manchester, Cambridge and Imperial College, London. Such selectivity, Sir Brian said, would continue, being all the more necessary in the present conditions of financial stringency.

He explained that the SRC recognized three phases of research. First there was the stage of testing new ideas—the most important and least predictable stage, and also the least expensive to support. Next came the recognition and promotion of useful ideas—the development stage. This was the stage when selectivity came in. The SRC's two major criteria in judging the worth of a project at this stage were the likelihood of it being a major breakthrough and its industrial significance. The third stage, Sir Brian said, was the application of a new development, when its scientific basis was well understood. By this time concentration of resources in a few centres was absolutely vital. Unless selectivity and the test of industrial usefulness were applied, the SRC would need three and a half times its present budget, according to Sir Brian.

SRC grants to the Control Systems Centre at UMIST have now totalled about £450,000. The centre was set up in 1966, to run an MSc course and provide services to the institute for control work. Since then the centre, under its director, Professor H. H. Rosenbrock, has acquired its present complement of four computers and attendant equipment. The new laboratory, which has been named after Sir Harold Hartley, a pioneer of control systems, should be the scene of increasing application of the centre's work to industry.

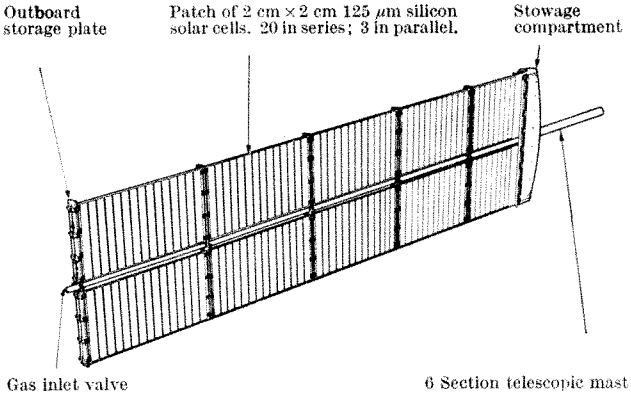
Work in progress includes the development of a mathematical model for the control of a new steel making plant for the British Steel Corporation. Other projects are concerned with problems in the control of processes connected with the production of glass, cement and plastic sheets. There is also a growing interest in the application of control to medicine, and work is well advanced on the control of a kidney machine, in collaboration with the Manchester Royal Infirmary. A small laboratory at the centre is to be devoted to medical engineering work.

SATELLITES

Lightweight Solar Sails

THE Royal Aircraft Establishment at Farnborough has now gone far enough with the development of a lightweight solar cell array to exhibit a prototype flight model. Such an array is meant to provide power for the X4 experimental meteorological satellite due to be launched by the 1972 Black Arrow firing. But it is possible that an additional technological satellite so powered for the Black Arrow programme will be inserted in the schedule between next year's X3 and the X4.

Compared with the original Farnborough concept of the lightweight array, generally called the "toilet roll", the new version is better described as a "sail". The cells, fairly conventional thin silicon wafers in themselves, are mounted on heat resistant plastic sheet only two-thousandths of an inch thick. This material, 'Kapton' (made by Dupont), besides being tough and light, has the advantage of being able to withstand temperatures from −200° C to 400° C. The array is folded like a concertina into a cushioned compartment for launching, and is then deployed pneumatically by a light telescopic mast in orbit (see diagram). The prototype array, similar to that to be flown on X4, will have an area of about one square metre.



The advanced solar cell array developed at the Royal Aircraft Establishment.

The advantage of the development (thought to be without rival) is that it has a power to weight ratio three times greater than normal solar cell arrays—53 kW per kg. Multikilowatt arrays could provide power for the next generation of communications satellites and for long-life unmanned probes to the planets. In conjunction with the ion engine, which yields low thrust over periods of months but with high demands on power, it has been suggested the new device could also help to wind a low orbit communications satellite out to geostationary distance if time were no object. It might take some months, taking into account periods of "coast only" when the satellite was eclipsed from sunlight by the Earth.

LABOUR PARTY

All Quiet on Science

THE Labour Party conference in Blackpool last week produced little to excite technologically minded members of the community, with the exception of a lively debate on pollution. It was disappointing to see matters apparently ignored which were considered of prime importance during the term of office of the last Government; there was singular lack of reaction, for example, to the now very compelling rumours about the fate of the Ministry of Technology and the possible amalgamation of some of its functions with those of the Board of Trade. And chemical and biological warfare, although on the conference agenda, was not

eventually discussed. Is the role of science and technology now so well accepted within the Labour Party that it can be overlooked with impunity?

Mr Anthony Wedgwood Benn, formerly Minister of Technology, gave stern warnings at the end of the nationalized industries debate against moves to sell into private hands profitable public sectors such as telecommunications with its well developed research and development facilities. He said that a future Labour Government would not compensate purchasers of denationalized industries.

A seven point plan for the prevention of pollution was put forward by Nottingham Central Labour Party and, during the debate, the present Conservative Government was criticized both for making only a junior minister responsible for a matter of such basic importance and for failing to see the problem as one to be handled by long term planning. Industry, as one of the chief villains, would be considerably restricted under the Nottingham plan; factories would have to treat their effluent on site and a severe limit would be imposed on the industrial emission of sulphur dioxide. At a more personal level, the further control of vehicle exhaust and measures to tighten up tests on chemicals and drugs before marketing figured prominently.

MEDICAL RESEARCH

MRC—University Symbiosis

THE comparative affluence of the Medical Research Council is leading to a shift in its role as a funding and organizing agency. Traditionally it has been a somewhat passive sponsor of research, taking the initiative whenever there was felt to be some gap in the national effort, but otherwise occupied with the care of its own units within the hospitals and universities and the provision of grants to those who came knocking on its door. The MRC is now to establish at its headquarters office a Universities, Grants and Training Awards Division with the specific objective of promoting liaison with the universities and strengthening the more informal means of communication which already exist.

So far the MRC has not suffered as much as most agencies—including the University Grants Committee—in the limitation of funds and this makes it one of the more attractive organizations with which to liaise. None the less, there is clearly an image of remoteness to be removed, and Dr Brandon Lush, who is to head the new division, will seek to bridge this gulf. The aim is not necessarily to make it easier to gain access to the coffers of the MRC, but to tell the universities more about the council's policies and to hear more directly about the needs of the universities.

The changes necessitate reorganization at head office. Last year saw innovation in the form of grants committees to help assess the merit of applications for support and there has always been a grants section at MRC headquarters. Even so, there have been signs of strain and the new division is at once a symptom of the increasing call on MRC funds and an acknowledgment of remaining inadequacy in the machinery for making awards. So long as rumours of the government's disenchantment with the research councils remain unfounded, the MRC can hope to improve both its efficiency and its public image and extend its influence as a power in the land.

COUNTRYSIDE

Save Britain's Coast

by our Planning Correspondent

THIRTY-FOUR stretches of coast, representing the finest coastal scenery in England and Wales, have been selected by the Countryside Commission from the 2,054 miles of largely underdeveloped coastline for designation nationally as "heritage coasts". The commission wants such areas, which total some 27 per cent (730 miles) of the English and Welsh coastline, and which are all more than a mile long, to be specially managed by local authorities so that they are protected from incongruous development and from pressures imposed by too many visitors. The commission's policies for the management of these coastal areas are set out in the two final reports (*The Planning of the Coastline*, 17*s* 6*d*; *The Coastal Heritage*, £4, HMSO) in its study of coastal preservation and development. These are the last of a series of reports which have been appearing since 1967.

The commission does not want to keep visitors away from the heritage coasts, which include the Seven Sisters and Beachy Head and the Isles of Scilly—indeed it recognizes that the demands for recreation in some of its proposed special areas will be particularly heavy because of their attractions. But to reconcile these demands with the safeguarding of the area's natural scenery, there must be positive policies for management. *The Coastal Heritage* suggests some fundamental principles to ensure effective management. One suggestion is that the scale of recreational activities and of the provision of facilities for visitors should be related directly to an optimum level of use. To achieve this, each stretch of coastline should be divided into three zones with an agreed level of use. There should also be regulations of access for people and vehicles at places where an excessive concentration is likely to cause damage. Rigorous control over development, emphasis on the opportunities for different forms of recreation, and information services are also urged. Each local authority should also appoint a conservation officer who could advise a special committee on the management of the heritage coasts.

In preparing *The Planning of the Coastline* the commission has looked more generally at the English and Welsh coastline in terms of the changing patterns of holidaymaking in Britain, and the effects of these changes on the coast. In addition to recommending compact new holiday centres, it wants the government to give it powers to designate regional parks in coastal areas which offer good opportunities for recreation, such as Chichester Harbour. The powers and organization of the authorities set up to administer these parks could, the commission suggests, be modelled on the present national parks. The commission is also critical of the eyesores which remain on the coast, in particular the colliery wastes on the foreshores of Northumberland and Durham, and defence structure in many places, and it says that target dates should be set for the removal of the messes by local authorities. The commission repeats its request, made in 1968, to look into the exploitation of reserves of gas and oil off the coast, saying that such a commission is an essential preliminary to the formulation of a national policy on the planning aspects of these industries.

NEW WORLD

Mansfield Amendment Cut Down

by our Washington Correspondent

THE law requiring that all the research sponsored by the Department of Defense should be of direct military relevance has become a dead letter, although it will probably continue to exert its influence from beyond the grave. The law, known as the Mansfield amendment to last year's military procurement authorization act, was re-included in the bill for the present financial year by the Senate but not by the House of Representatives. In negotiations between the two chambers reported last week, the delegates from the Senate were forced to yield on the Mansfield amendment in return for House concessions on other points in the bill, notably the Senate's stand on limiting the Safeguard anti-ballistic missile system to defending only missile sites. The importance of the amendment is that it knocked away the most conspicuous pillar of American science policy, the practice whereby the bulk of Federal support of science is channelled through the large mission oriented agencies such as the Department of Defense, instead of through the relatively puny National Science Foundation.

The price paid by the Senate for its position on the ABM was to allow the House to sabotage the Mansfield amendment beyond repair and to adulterate a corollary provision that the budget of the National Science Foundation should be increased. The crucial wording in the Mansfield amendment, that no research project should be supported by the Department of Defense "unless such project or study has a direct and apparent relationship to a specific military function or operation", has been replaced by the jejune stipulation "unless such project or study has, in the opinion of the Secretary of Defense, a potential relationship to a military function or operation".

This language was arrived at after rejection of a compromise Senate proposal that would have distinguished between applied and basic research, using a test of strict relevancy for the former but asking only for "clear potential relevancy" on the latter. But the House conferees were adamant that no restriction should be placed on the Pentagon's freedom to conduct whatever basic research it wanted, and insisted that the concept of military relevancy "should not be used as the determining factor in the support of basic research efforts of contractors, universities, or non-profit institutions".

The House conferees also took exception to the Senate's indication that the budget of the National Science Foundation for the financial year starting in July 1971 should be increased by a fifth. The reason given was that "this matter had not been a subject of House Committee hearings and therefore was not a matter on which the House members were prepared to act". The House Subcommittee on Science, Research and Development has held extensive hearings on the general issues involved, though not on the Senate proposal specifically, but these do not seem to count for much with the House Armed Services Committee, whose conferees were in charge of the negotiations. As a result the Senate's call for a stronger role to be played by the National Science Foundation has come out much watered down.

The Senate also had to give way on its proposal for an Interagency Council on Domestic Applications of Defense Research, which would have sought to convert the resources of defence industries, now underutilized because of budget cutbacks, to the solution of domestic problems. The House conferees argued that this type of coordination was the job of the Federal Council for Science and Technology but in any case they were unwilling to accept the Senate view of the matter since the House Armed Services Committee had not held hearings on it.

In the debate on the conferees' report Senator Mike Mansfield made no secret of his disappointment at the concessions that had been made. The alteration to his amendment, he said, is worse than eliminating it altogether since it "affirmatively states that the Department of Defense will solely determine what research is beneficial to it. This language, in my opinion, is a legislative act of abdication to the Secretary of Defense . . ." The Senator still hoped for "an alignment not only in what is necessary for military and scientific development . . . but also in that which can be allocated to the National Science Foundation and other civilian agencies engaged in basic research. . . ." Mr Mansfield gave notice that his fight to achieve this reallocation was far from ended, it had just begun again.

The virtual repeal of the Mansfield amendment will give the Pentagon less freedom to continue its support of the full spectrum of basic research—its "backdoor National Science Foundation", as Senator Mansfield has termed it—than in the days when Congress happily disbursed whatever the generals said was needed. But although in the short term the demise of the amendment may allow more funds for basic research, the long term prospects, even if the amendment is not resurrected next year, are less certain. Mr Mendel Rivers, the chairman of the House Armed Services Committee, has gelded the Mansfield amendment not because he thinks it may harm science but because it does no obvious good to the Pentagon. In Senator Mansfield's mind too, the thought of cutting the Pentagon down to size may loom larger than that of building up the National Science Foundation, but at least the Senator has laid out a logical and constructive design of how he envisages science should be supported. The whole question of science funding has now been thrown back into the political arena where it stands to be decided not on its own merits but as part of the tussle between the friends and foes of the Pentagon. To this extent the Senate's sacrifice of the amendment is a doubtful benefice.

NAS, AAAS to Report on Vietnam Defoliation

by our Washington Correspondent

NINE years after the start of the defoliation programme in South Vietnam, the Department of Defense is to commission a study of the programme's physiological and ecological effects. A clause in the military procurement authorization bill requires the Secretary of Defense to ask the National Academy of Sciences to conduct an appropriate survey. The clause came to light last week in the report of the agreement between House and Senate conferees on the rival versions of the bill passed by each chamber.

The language of the clause (Subsection 506c of the bill) calls for a study of the "danger inherent in the use of herbicides" and mentions the defoliation programme specifically. Apart from the general reference to herbicides it is not clear how far the proposed study will include the crop destruction programme, which may be being pursued no less actively than the defoliation of forests and mangrove swamps.

Preliminary discussions have taken place between the Office of Senator McIntyre, chairman of the Armed Service's Committee's Subcommittee on Research and Development, and the National Academy, but a formal proposal has yet to be made. Agreement will have to be found on such questions as whether the members of the study will include Vietnamese scientists and, in particular, on how far the findings should be made public. Although the academy rarely refuses to undertake research projects requested by the government, there has been vigorous discussion among members on the propriety of undertaking classified research.

Any survey the academy may agree to undertake is likely to run into problems of military secrecy since the Pentagon, at least on present showing, is intent on withholding certain information essential to a scientific study of the use of herbicides in Vietnam. A pilot study sponsored by the American Association for the Advancement of Science is being seriously impeded by the refusal of the Department of Defense to allow access to the record of the crop and forest spraying missions conducted in South Vietnam since 1965.

A study group appointed by the AAAS visited South Vietnam in August after receiving a formal invitation from the Vietnamese government. The members of the group were Matthew Meselson, professor of biology at Harvard University, Arthur Westing, a forester knowledgeable in the use of the herbicide 2,4,5-T; Robert E. Cook, a graduate student of ecology at Yale University, and John D. Constable, professor of surgery at the Harvard Medical School. In company with Vietnamese scientists, including a professor of botany, the group spent four and a half weeks surveying sprayed areas, interviewing farmers and taking samples of soil, fish, mother's milk, hair and various links in the food chain.

The group's instructions from the AAAS were to "study the long and short term effects of the military use of herbicides on the land and people of Vietnam". Findings will be reported at the annual meeting of the AAAS in December, but unless the Department of Defense releases its records of spraying missions the scientific value of the samples and observations collected by the group will be seriously limited.

The crucial records consist of a hand-kept log book, maintained in Saigon, in which are entered the date and map coordinates of all spraying missions flown since July 1965, together with the nature of the mission (whether crop spraying or defoliation), the number of hectares covered and the number of bullet holes counted in the aircraft on return to base.

Meselson said last week he is puzzled at the willingness with which the AAAS group was told the time and place of spraying missions carried out immediately before and during its time in Vietnam—information which could possibly be of military value to an enemy—compared with the refusal of defence officials to release the same data for all earlier missions, which it is hard to see could be of any military value. The refusal is the more surprising since Ambassador Ellsworth Bunker supported the group's request for access to this information.

Whatever the detailed conclusions of the AAAS group turn out to be, there is no doubt that any untoward ecological effects of the herbicide programme will be or are being felt extensively throughout South Vietnam. Since 1965, when the defoliation programme began, a third of the country's forests—amounting to 15 per cent of the total land area—and about half the mangrove swamps have been sprayed. The AAAS group has confirmed a survey conducted in 1968 by Fred Tschirley, a scientist with the US Department of Agriculture, that extensive areas of dead forest have been invaded by bamboo. Destruction of the forests is serious not only ecologically but also because in Vietnam's future economy forest products could play a leading role. In the mangrove swamps, by contrast, there is little or no evidence of regeneration in sprayed areas. Under the crop destruction programme half a million acres of crop land had been sprayed by 1968. Probably some 30,000 herbicide-spraying sorties have been flown in South Vietnam by fixed wing aircraft (there is no central record of helicopter missions) since 1964.

The principal herbicides used in the two programmes are picloram, cacodylic acid, 2,4-D and, until recently, 2,4,5-T. Besides any harm it may do on its own

account, 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) contains small quantities of an impurity created in the manufacture, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The dioxin compound appears to be the most poisonous chemical known, excluding certain protein toxins but not the nerve gases. In the guinea-pig dioxin is lethal in concentrations of less than 1 mg/kg, or one part per million, which is comparable with the toxicity of VX nerve gas. A perturbing feature of dioxin is that, unlike other toxins, it is most surprisingly quite inert chemically. Its planar structure suggests that it could be forming complexes with important molecules such as nucleic acids. Whatever its mode of action, dioxin may be stable enough to work its way up food chains and to accumulate in body tissues. Commercial preparations of 2,4,5-T contain amounts of dioxin that vary from one manufacturer to another (that from Dow Chemicals contained the least since the corporation had been aware of some of the untoward effects of the impurity since 1964), but it can be estimated that something of the order of 100 lb of dioxin has been sprayed over South Vietnam since the herbicide programmes began. It is not yet known what has happened to this material.

The biological samples brought back by the AAAS group may hold some of the answers, although Meselson emphasizes the necessary limitations of a study undertaken "in the middle of a hot war, by a group of people who are not experts in tropical ecology". There has been an unusually direct connexion between the scientific discovery of the chief herbicides and their use for military purposes. The herbicides 2,4-D and 2,4,5-T were developed during the Second World War by E. J. Kraus, chairman of the Department of Botany at Chicago University, with the deliberate intent that they should be used for crop destruction. This was opposed at the time on humanitarian grounds, chiefly by Admiral Leahy, and the same arguments prevailed during the Korean war. The question of using the herbicides for military purposes arose again in the early stages of American involvement in the war in Vietnam, and after an initial refusal President Kennedy gave permission for a programme of spraying herbicides alongside roads. Large scale spraying of crops was not begun until 1964, and the defoliation programme the following year. At the height of the defoliation programme some 4,000 square kilometres were being sprayed a year, but, following the replacement of General Westmoreland as American military commander by General Creighton Abrams, the strategic need for clearing large forest areas diminished and the defoliation programme was reduced by about a fifth. In particular a review of the programme initiated by Ambassador Bunker in 1968 resulted in a switch from the block spraying of forests to spraying in strips, a practice which it was thought would allow the sprayed areas to be reseeded from the unsprayed. But the crop destruction programme is still proceeding actively.

Whatever the ecological consequences of the herbicide programmes turn out to be, they are unlikely to be as irreversible as the effects on society. On one occasion the AAAS group witnessed the spraying of rice fields which, on the basis of aerial reconnaissance, had been judged to be cultivated by or for the Vietcong. Visiting the area by land a few days later, they learnt the fields belonged to Montagnard tribes people who were abandoning their land because of the evident pestilence under which it had fallen. Overdue though they may be, the studies by the AAAS and the National Academy of Sciences are at least a start towards understanding some of the possible long term effects of the pestilence.

WATER POLLUTION

One Detergent for Another

by our New York Correspondent

DETERGENTS have recently become a dirty word, politically at least, as more and more individuals and organizations have jumped onto the ecological bandwagon. Nelson Rockefeller, governor of New York, who is running for re-election in November, joined the band last week when he announced that he would propose in the 1971 legislature a ban on the sale of detergents containing phosphates in New York State by 1972.

Many environmentalists claim that phosphates are the main contributors to excessive eutrophication in lakes. Phosphates are considered to be the nutrient that controls the growth of algae. Excessive algae consume the available oxygen, and other life forms in the lakes eventually die. There is now some research to show that phosphates are not always the limiting nutrient and that often carbon performs the same function in some lakes, but in either case sewage wastes are still to blame. Disregarding the carbon theory, Governor Rockefeller and others seem to believe that if only phosphates can be removed from detergents, one of the major sources of phosphate wastes found in industrial and municipal sewage, the problem would be solved. Unfortunately things are never that easy.

The detergent manufacturers in turn claim that they are well aware of the problems and public concern over phosphates, and that they have been spending most of their research money searching for substitutes with little to show for it. They also claim that the proverbial American housewife, not to mention users such as hospitals and restaurants, would be unwilling to return to the "tell-tale grey" and germs of pre-detergent days.

Present detergents, the manufacturers point out, have been studied and tested for many years, and their ecological effects are well understood if not totally under control. Until possible alternatives have been subjected to equally rigorous testing, the companies argue that it would be foolhardy to invest large sums in new projects and equally impossible for legislatures to ban the present detergents before there are adequate substitutes.

In the meantime the manufacturers are being urged to promote detergents with lower amounts of phosphate and to experiment with nitrolotriacetic acid (NTA) as a partial substitute. NTA certainly seems to work well in conjunction with lower phosphate levels, but it, too, is running into problems. In the first place experiments on rats at Procter and Gamble over the past two years have shown that massive doses of NTA can cause nephrosis, a kidney disease. Procter and Gamble argue that this does not imply that the chemical is toxic for humans. Dr Samuel Epstein, a pathologist at the Harvard Medical School, has suggested that the nitrates produced by bacterial breakdown in sewage may combine with secondary amines to form nitro-

amines, some of which cause cancer. Detergent manufacturers seem confident that these fears will prove unfounded, but they agree that much more research needs to be done on the effects of NTA before it becomes widely used.

In any case, NTA is not being produced in sufficient quantities to substitute fully for the two billion pounds of phosphates at present used yearly in detergents. The two major producers of NTA in the country, Monsanto Company and Grace's Hampshire Chemical Unit, only manufacture about one hundred and fifty million pounds a year. By 1971 Monsanto expects to be producing only 350 million pounds a year. P and G, the company most heavily committed to NTA, expects to replace NTA for phosphates in nearly a third of its detergent production by 1972 while Lever Brothers, another major producer, said recently that "the question here is that we are still awaiting federal research on the safety of NTA if it is used in large quantities by industry as a replacement".

Whatever the final answer is on NTA it will be very difficult for any legislation banning phosphate detergents to be effective by 1972, no matter how politically expedient such a ban would be in an election year. In any case detergents are not the only villains in this piece. While it certainly simplifies things for the government when it can blame one industry for the problems caused by many, simply eliminating detergent phosphates still leaves many sources of phosphates to ruin the lakes. A far more effective though more expensive solution is adequate sewage treatment; by treating all wastes before they are discharged back into the water supply, all such problems would be solved at their source. Unfortunately, this would be another toll on the taxpayer, something that Governor Rockefeller is well aware will not win him votes on election day, even in a supposedly ecological era.

LUNAR SCIENCE

NASA Aims for Putrid Marsh

by our Astronomy Correspondent

LAST week the National Aeronautics and Space Administration announced that the destination of the Apollo 15 mission next July will be the Hadley rille (26° 52′ N, 3° 00′ E), which meanders through a flat region with the unfortunate name Palus Putredinis (Marsh of Putridity). This leaves only the two final sites to be decided before the American exploration of the Moon ends for the foreseeable future in June 1972. Of these two sites the first (Apollo 16) is expected to be Descartes (10° 10′ S, 16° 35′ E), a highland area marked by signs of volcanic activity, although this is dependent on good photographs of the area being taken during the Apollo 14 flight to Fra Mauro which is due to be launched on January 31. In spite of the tight schedule of modifications to Apollo 14 that are being made as a result of the Apollo 13 accident, Dr Rocco A. Petrone, the Apollo programme director, sees no problem in meeting the January 31 deadline.

Following the cancellation last month of a further two Apollo flights, which among other things is expected to free between $600 million and $900 million for the controversial space shuttle programme, the selection of landing sites has become even more important. The Hadley site should provide samples of the Mare Imbrium material and of the Apennine mountains, but it is disappointing that material which would give clues to the origin of the rille will not necessarily be forthcoming. The rille is 600 feet deep, and as far as the planning goes at present the astronauts are not expected to do more than scramble a few feet down the side. Even that depends on the slope—the steepest that can be tackled is probably 20°—and on the state of the ground.

NASA sees no problem in landing considerably further from the lunar equator than the previous sites or in the approach to the landing site which will be over the 8,000 feet high Apennine mountains. Apollo 15 is the first of the J missions which have an enhanced scientific potentiality chiefly because of the addition of a simple vehicle to the payload. Two of the three traverses planned for the mission will use the vehicle, taking the astronauts up to 8 km away from the lunar module along the edge of the rille and to the foothills of the Apennines. Apollo 15 will also carry an improved laser reflector with 300 instead of 100 corner reflectors so that it can be used by smaller observatories, and a seismometer which may throw more light on the positive gravity anomaly in Mare Imbrium. And this will be the first flight of a package of instruments which fit into bay 1 of the service module. The package includes a mass spectrometer, X-ray, gamma ray and alpha particle spectrometers, a laser altimeter, cameras for accurate mapping of the Moon, and a small satellite to be released into lunar oribit, chiefly to map the gravity field but also for particle and magnetic field measurements. The presence of the cameras in part of the spacecraft that is discarded before the return to Earth implies that there will have to be a spacewalk by one of the astronauts during the flight home to retrieve the film.

Awards for Heart Research

Sir George Pickering, Master of Pembroke College at the University of Oxford, and Dr Irvine H. Page, of the Cleveland Clinic Foundation, were named on September 23 as the joint winners of this year's $50,000 Stouffer Prize for Research in Heart Disease. It is to be awarded in recognition of their contribution to the understanding of arteriosclerosis (hardening of the arteries) and hypertension (high blood pressure). This is the fifth year that the award has been given.

Sir George was professor of medicine at the University of London and director of the Medical Clinic at St Mary's Hospital from 1939 to 1956. Until 1969, he held the chair of medicine at the University of Oxford, once held by Sir William Osler. Dr Page has studied the heart and circulation since 1937 when he became director of the Lilly Laboratory for Clinical Research at the Indianapolis City Hospital. He joined the Cleveland Clinical Foundation in 1945 and became president of the American Heart Association in 1946.

Their work has led to many of the therapies that have been so effective in recent years in the treatment of hypertension, the most prevalent of all cardiovascular diseases.

NEWS AND VIEWS

Hot and Cold from Nimbus

THE cause of improved temperature mapping of the atmosphere and, as a consequence, improved weather forecasting, was taken one step further by the launching in April of this year of Nimbus 4, one of six satellites in the NASA Nimbus programme. The cooperation between research groups at the Universities of Reading and Oxford has enabled a radiometer of British design, and embodying several novel principles, to be used as a self contained piece of equipment in an American satellite. Development work on this radiometer was started in 1961 and it is now possible to observe a large number of infrared lines in the CO_2 spectrum simultaneously. Data on atmospheric temperatures are extracted from the ratios of measured vertical intensities of radiation energy emitted at the infrared wavelengths together with the known variation of the absorption properties of the atmosphere with wavelengths. The results of its first few months of operation have come right up to expectations and some rather unexpected facts have been brought to light, which are described on page 139 of this issue of *Nature*.

The importance of atmospheric data (particularly temperatures) for weather forecasting is a consequence of the inherent difficulty of the study of medium scale air motions affecting areas about five or six hundred kilometres across. By contrast, air motions over areas of continental size are fairly well understood and predictions can be made by computer methods although there is a continual need for larger and faster computers.

The Reading/Oxford infrared radiometer has been used to plot the temperature distribution in several layers of the atmosphere (each typically 5–10 km deep) with an accuracy better than 2° C. The results so far indicate that at the 45 km level the temperature distribution is not as expected around the poles and that at the south pole, in particular, there are two distinct cold spots where intuitively only one would be expected. The shape of the temperature distribution in this layer seems to change dramatically with time, but these changes are not reflected to any great extent in lower atmospheric layers. Explanations of the cold spots are not yet forthcoming, but such an unexpected phenomenon would certainly have considerable bearing on the problems of weather forecasting. Recent data from Nimbus 4 have also revealed other examples of strange behaviour around the south pole. Quite large volumes of air in the upper stratosphere have been observed to warm up rapidly in only a few hours and have subsequently moved round the pole and mixed with regions of cold air. The temperature rises in these "sudden stratospheric warmings" have been as high as 20° C and their effect can be seen down to about 35 km. It is tempting and logical to speculate that the causes of all these disturbances can be traced back to the surface of the Earth. Mountain ranges such as the Andes are potential candidates, and future work will have to determine whether or not the fluctuations can be predicted easily and how much fine structure there is associated with them.

Plans for Nimbus 5 include large scale measurements of water and ice throughout the atmosphere as well as extensions and improvements of the present work. Future satellite equipment will eventually include microwave radiometers to replace the infrared types in use at present, but in the meantime detection of radiation in the far infrared will help to eliminate some of the problems of radiation absorption by clouds. The overall planning of the Nimbus programme continues to be a joint effort with members of the Goddard Space Flight Center, Maryland.

SELENOLOGY

What Happened to the Moon's H_2O?

by our Astronomy Correspondent

NOTHING much has been heard lately from the proponents of a one-time lunar hydrosphere. The debate had its heyday as a party game just before the first Moon landing, when the advocates of the water-on-the-Moon hypothesis were led with verve by J. J. Gilvarry. The hypothesis made some sense in accounting for the sinuous rilles, became esoteric when pressed into use to explain the mascons, and verged on the bizarre when the dark colour of the maria was attributed to decayed organic sediments.

With water now notable by its absence on the Moon, even as water of hydration, the game has turned full circle. In spite of the faint hope, kept alive chiefly in NASA offices, that ancient icebanks may be present in permanently shady corners of the Moon, and that there may even be a permafrost layer, the aim now is to account for the apparent lack of water.

E. Anders of the University of Chicago has said recently that the scarcity of lead, bismuth and thallium in the lunar samples indicates that not much water can be expected, anyway (*Science*, **169**, 1309; 1970). If conditions were such as to make these easily volatilized elements scarce, then water also is unlikely to have formed in any quantity. Anders's article is founded on the view that the terrestrial planets accreted in a cooling solar nebula, with the more volatile material condensing later, so that a planet's carbon, water, lead and bismuth, for example, would be acquired as a thin crust of material with a

composition like that of carbonaceous chondrites. On the basis of the abundances of lead, bismuth and thallium, Anders estimates that the amount of this chondritic material on the Moon is equivalent to a 9 metre thick layer which would contain 370 g cm^{-2} of water corresponding to a 3·7 metre deep covering if all of it were liquid. This relatively small amount of water is an overestimate, however, Anders says, because it fails to take into account the energy released by the impacting material in the final stages of accretion. This would be enough to vaporize virtually all of the water that it contained and would also account for the absence of the volatile element sulphur in the soil collected by Apollo 11.

Anders says therefore that the case for substantial amounts of water on the Moon seems to be weak, thus begging the question of how the Earth comes to be so wet. There are several possibilities—the Moon may have accreted closer to the Sun, for example—but Anders gives weight to the view that the dynamics of accretion processes were different.

With the Apollo 15 expedition therefore unlikely to pick up evidence for a lunar hydrosphere from Hadley rille next summer, the aqueous explanation of the sinuous rilles is becoming unfashionable. In the September issue of the *Geological Society of America Bulletin*, S. A. Schumm of Colorado State University extends the fluidization hypothesis of lunar features to the rilles, explaining how the outgassing from the lunar interior that has been put forward to account for flat-floored craters may also have created other shapes (**81**, 2539; 1970). Schumm has set up a fluidized bed in which a layer of sand a few centimetres thick represents the lunar regolith and compressed air is blown through a pattern of holes beneath the bed to produce fluidization. Using linear and sinuous arrays of holes, Schumm can create structures resembling crater chains and rilles in the artificial regolith.

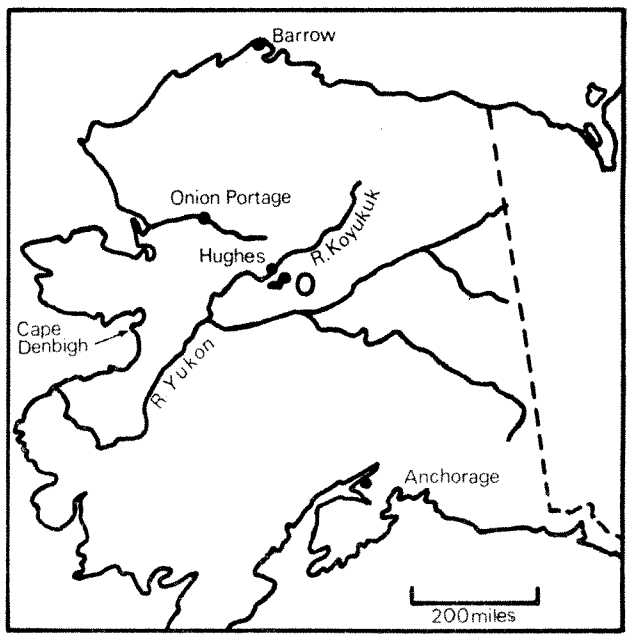
ARCHAEOLOGY

Sources of Obsidian

from our Geomagnetism Correspondent

THE philosophical and methodological parallels between geology and archaeology are well known; and recently both disciplines have benefited from the wider utilization of techniques once reserved for the physical sciences. The direct application of geological exploration to archaeological problems is still comparatively rare, however, probably because contact between students of the two disciplines is a matter of chance rather than a systematic attempt to discover and cooperate on common ground. How Patton and Miller (*Science*, **169**, 760; 1970) came to see their geological investigations as a solution to a problem in Alaskan archaeology will perhaps never be known, but archaeologists should be pleased that the connexion was made.

The archaeological problem is simply stated. In recent years artefacts and chippings of obsidian have been found in conjunction with chert and slate artefacts at several archaeological sites in Alaska, notably the excavations at Cape Denbigh and Onion Portage (see map). But whereas chert and slate are widely distributed in north-western Alaska, no one has ever discovered the source of the obsidian. As a result, archaeologists have had to rely on unproven hypotheses which envisage the transport of obsidian from as far away as the Aleutian Islands. Giddings (*Arctic Anthrop.*, **1**, 6; 1962), for example, even related the variations in obsidian abundance at various archaeological levels at Onion Portage to "the ebb and flow of coastal-oriented and inland-oriented cultures along the Kobuk valley". Clearly, the source of the obsidian is an important factor in the elucidation of cultural ties and trading routes in ancient Alaska.



Patton and Miller have put an end to speculation by their discovery of obsidian deposits near Hughes, Alaska, only a hundred miles or so from the Onion Portage site. The mere proximity of the source to the ancient settlements does not, of course, prove a physical connexion; but closer comparison of the excavated and indigenous obsidians goes a long way to doing so. On the descriptive level, most examples of the two obsidians compare well in colour, opacity and the absence of hydration and devitrification. And, quantitatively, use has been made of recent techniques for matching archaeological samples with bedrock deposits by chemical composition. Of particular interest in this connexion is the ratio of sodium to manganese, both elements which vary widely between obsidian flows. The ratios of sodium to manganese of the Onion Portage and Hughes obsidians are similar. The scientific and circumstantial evidence for the connexion is thus strong.

Unfortunately, Patton and Miller were unable to carry out a systematic search for evidence of ancient "mining" of the bedrock deposits. Even so, several artificially chipped obsidian flakes were found among the natural residual fragments. Furthermore, chipped fragments found on nearby hillsides and ridge tops could only have been carried there by human agency. The only question now is whether or not the Hughes site was the only source of obsidian for the ancient Alaskan communities. No further sources have been discovered, but geological associations are favourable along much of the Koyukuk and lower Yukon Rivers.

MATERIALS

Between Crystals

from a Correspondent

Two aspects of grain boundary structure came under scrutiny in Amsterdam recently. Although most useful materials occur in the form of polycrystalline aggregates, relatively little is known of the atomic structure in the vicinity of the boundaries between the various crystals. The available information was reviewed on September 22 during the meeting of the Institute of Metals.

There seem to be two distinct aspects to grain boundary structure. There is a general structure which is a direct consequence of the super-position of the periodicities of the adjacent crystals, and superimposed on this there may be imperfections, or irregularities, which can critically affect certain materials parameters. Indeed, considering the importance of dislocations within the crystal, it is tempting to draw an analogy to postulate the existence of dislocations in, or intersecting, the grain boundary.

Two approaches were adopted in the theoretical description of the general structure. Dr W. Bollman (Battelle, Geneva), from purely geometrical arguments, and Dr G. Hasson (École des Mines, St Étienne), by computing the minimum energy configuration for an assumed interatomic pairwise potential, concluded that a grain boundary of arbitrary orientation would be expected, on the atomic scale, to consist of straight sections, where the matching between the crystal lattices is good, connected by sections of poor matching.

Dr H. Mykura (Warwick University) pointed out that adjacent crystals normally bear no special crystallographic relationship to each other. Nevertheless, the observations of macroscopic facets (of good intercrystalline fit) by optical, electron and field-ion microscopy indicate that boundaries tend to be arranged in a direction such that they contain a high proportion of atoms common to both lattices (coincidence boundaries), which is clearly energetically reasonable. Precipitation of second phase particles occurs preferentially at grain boundaries containing ledges where the atomic fit is bad and at dislocations in the grain boundary.

Several contributors, using transmission electron microscopy, had observed linear defects at the grain boundary. Dr D. Dingley (University of Bristol) compared some electron micrographs with a computer simulation of the image expected from the combined strain fields of a dislocation and grain boundary, and confirmed the defects to have simple lattice burgers vectors. On the other hand Drs D. Smith (University of Oxford) and B. Loberg (Chalmers University) by studying specimens by both electron and field-ion microscopy have observed, at a grain boundary, dislocations with burgers vectors which are not simple lattice vectors of either crystal, which suggests that segments of the dislocation lie in the plane of the boundary.

Certain phenomena seem to depend sensitively on the structure of the grain boundary. For example, Dr M. Biscondi (École des Mines, St Étienne) showed that, for a given shear stress, the rate of grain boundary sliding is slower for boundaries of high coincidence than for the more disordered boundaries. Dr C. A. P. Horton (Central Electricity Research Laboratories, Leatherhead) suggested that the occurrence of grain boundary sliding and crystal deformation were closely related. Presumably, dislocations generated within the crystal can migrate to the grain boundary, and grain boundary sliding is achieved by displacement of segments of the dislocation in the plane of the grain boundary.

Grain boundaries are known to act as efficient sources and sinks of vacancies during the high temperature creep of polycrystalline materials. This behaviour cannot be explained in terms of the general equilibrium structure, and implies the existence of a self-perpetuating defect. Dr E. D. Hondros (National Physical Laboratory) showed that such vacancy sources can be rendered inactive by the adsorption of certain impurities to the grain boundary, while in other circumstances even low angle subgrain boundaries seem to act as efficient source/sinks.

There was therefore substantial evidence, supported by many other contributors, that both aspects of grain boundary structure are necessary, although the precise nature of the defects is still a subject for debate.

SOLID STATE

More and Better Devices

from a Correspondent

THE latest progress in solid state devices was the theme of a conference held at Exeter by the Institute of Physics and the Physical Society from September 15 to 18. Most contributions were concerned with semiconductors, and covered topics from materials to computer aided design.

Three contributions from the group under Dr C. Hilsum (Royal Radar Establishment, Malvern) described pioneering work on indium phosphide. Miss A. Royle described the growth of large single crystals with electron densities from 10^{14} to 10^{18} electrons/cm^3 and mobilities up to 3,000 cm^2 V^{-1} s^{-1}. Semi-insulating InP had now been made. The preparation of vapour phase grown InP layers was described by Dr B. D. Joyce, who said that they had achieved low electron densities and a mobility of 4,740 cm^2 V^{-1} s^{-1} (60,000 cm^2 V^{-1} s^{-1} at 77 K), which is the highest reported for this compound. Dr J. R. Morgan described a three level transferred electron microwave oscillator using the ultrapure InP layers. Oscillations up to 28 GHz had been observed while at 8·5 GHz a 7 per cent efficiency had been obtained. No continuous wave output had yet been realized. A broad tuning range and reasonable efficiency indicate that this three level oscillator has good prospects.

Dr G. H. Schwuttke (IBM) showed, with the aid of X-ray topograms, the propagation of dislocations in a defect free silicon wafer, as it progressed through the various stages of oxidation, epitaxy and diffusion involved in the making of an integrated circuit. He was able to correlate the electrically defective devices with the highly dislocated regions in the wafer. When the wafer is laid horizontally in the furnace instead of vertically, as is the normal practice, and very rapid changes of temperature are avoided, the number of dislocations can be reduced considerably and a device yield as high as 90 per cent is achieved. He said that 1970 was the year of large scale integration (LSI) for

such applications as solid state memories. As the emphasis in the industry was now on cost and thus on yield, and not the speed of operation, interdisciplinary activity was essential to remove the "art" from the processing and establish a sound process control. He expected to see a revolution from manufacturing research.

Dr G. E. Moore (Intel Corp.), surveying semiconductor memory, said that considerable progress had been made, and integrated memory devices were now cost competitive with conventional stores (for example, the magnetic core store) and also offered increased performance. As the yield increases with decreasing area, considerable effort is being made to increase the packing density by research into the metal semiconductor transistor technology. He also mentioned dielectric storage devices, ovonic switches and magnetic bubbles as other promising approaches for memory systems. Memory development as a whole is in a state of flux, and manufacturers are in a dilemma as to which line of research to pursue. Several contributions on dielectric storage devices indicated that these are still far from being a practical memory.

Magnetic bubbles, small stable regions of reverse magnetization in a highly anisotropic ferrimagnetic sheet, were described by Dr F. C. Rossol (Bell Telephone Laboratories). A telephone dialler has been made using the bubbles and in the future a bubble store could replace the present disk files in a computer. A store containing 10^7 bits per cubic inch at a cost of "milli cents" per bit was projected.

Mr D. Baker (the Post Office), reviewing microwave transistor technology, emphasized the need for narrow emitter stripes and base widths and reduced base resistances. He anticipated that electron beam techniques for resist exposure will be essential for fine geometrical structures. More than ten contributions were concerned with transistor design and performance indicating a continuing and lively interest in bipolar structures.

Dr P. R. Selway (Standard Telephone Laboratories) gave the details of an aluminium gallium arsenide double-heterostructure injection laser. This is efficient enough for continuous operation at room temperature, and this opens up the feasibility of an optical transmission system.

Dr C. Sandbank (Standard Telephone Laboratories) explained the addressing on a moving high field domain in CdS. It is possible to initiate electroluminescence from a domain, and, by scanning, Sandbank ultimately hopes to obtain a display with the resolution of a television picture.

METALLURGY

Recovery and Recrystallization

from a Correspondent

STEEL was a dominant theme at the discussion of recovery and recrystallization held in London on October 2 under the auspices of the Institution of Metallurgists. The plastic behaviour of rimming steels, vacuum-melted iron of high purity, as well as of aluminium has been examined by Dr G. M. Sellars (University of Sheffield) by means of "hot" torsion tests. He showed that in the usual industrial hot-working conditions the critical strains at which recrystallization set in were generally exceeded, particularly in rolling of steels. It occurred discontinuously, in "spurts" separated by periods of intense hardening of the structure, during deformation. Recovery and recrystallization obliterated the structural changes so effectively that it was common to find equiaxed rather than elongated grains in steels strained in hot-rolling passes by 300 per cent. The size of subgrains seemed to be a measure of the strength of the material at all times; the kinetics of their migration, generation and annihilation were, however, still obscure in detail.

The less "drastic", secondary type of recrystallization, essentially grain growth controlled by the grain interface energy, had been studied by Dr T. Healey (Nuclear Laboratories, Berkeley) in niobium-stabilized 25/25 Ni/Cr steels containing 0·017–0·043 per cent (by weight) of carbon. A theoretical interpretation of the observations based on the Wagner–Lifshits concepts of particle coarsening, and embodying Zener's model of grain-growth inhibition by precipitate particles, developed by him, accounted reasonably well for the observations, particularly for the occurrence of an incubation time $t = A \exp(E/kT)$, in which A increased with carbon content in the range of temperatures used, that is, 920°–990° C. E was independent of the carbon concentration; its value, 5·5 eV, was unexpectedly high, lying well above the activation energy of vanadium diffusion (3·2 eV) in the material. Its precise significance was still a matter for speculation.

Dr I. D. McIvor (British Steel Corporation) illustrated with two examples what he considered to be a general principle, namely, that fine precipitates with dimensions less than about 200 Å, coherent with the matrix, tended to inhibit recrystallization and subboundary displacements. Coarser ones, such as vanadium carbides or nitrides which had "grown", were, however, found to be effective recrystallization nuclei. Again, he considered the resistance of the material to plastic deformation to be largely a function of subgrain size; a Hall–Petch relation seemed to hold.

A different aspect of hot deformation was discussed by Dr W. B. Hutchinson (University of Birmingham). He pointed to the shortcomings of the pole-figure representation of rolling textures. The former were two-dimensional, while crystals had three rotational degrees of freedom. A more informative description of textures consisted of a series of "intensity" maps, drawn on standard stereographic triangles, each referring to a section through the sheet, each inclined by a different angle in the range 0°–90° to the rolling plane. Such maps could be made and used with ease, once the computer program for drawing them was set up.

Dr E. D. Hondros (National Physical Laboratory) showed that in metal sheet which was thin compared with the diameter of the grains—that is, it contains "pancake" grains—the difference between the surface energies of adjacent grains was the chief driving force for boundary migration, provided that "Zener drag" due to impurity absorption into the boundary was absent. Chemisorption provided means for modifying surface energy anisotropy. He demonstrated this with measurements on (3 per cent) silicon iron sheet, in which grain growth had taken place in the absence of chemisorbed oxygen (partial pressure $2{\cdot}5 \times 10^{-27}$ atm.), and in the presence of chemisorbed oxygen (partial pressure 10^{-16} atm.) respectively. In the latter case the desirable (100) texture prevailed over the customary (110) orientation. Sulphur, nitrogen and

other gases were also effective in modifying surface energy anisotropy.

Professor P. Feltham (Brunel University) showed that stress relaxation provided a method for studying recovery at low temperatures, for example, at 20 K in nickel. In principle a single relaxation curve sometimes sufficed for determination of the magnitude of the energy barrier to dislocation movement. He illustrated this for the case of the Peierls barrier in α-iron. The method was potentially most useful in the design of alloys, but these potentialities had not as yet been generally recognized, he said.

BIRDS

Magnetism of Navigation

from our Animal Behaviour Correspondent

THE way in which migrating birds find their way continues to be a puzzle to zoologists. Until quite recently, however, it was thought possible to discount at least one theory—that birds made use of the Earth's magnetic field for navigational purposes. Experiments in which magnets were attached to the wings of homing pigeons and found not to interfere with their homing ability at all seemed to show that they could not possibly be using magnetism. Then, in a series of articles, W. Wiltschko (*Z. Tierpsychol.*, **25**, 537; 1968) claimed that European robins could be made to reorient their migratory activity with respect to a static artificial magnetic field, putting the magnetism theory once more firmly in the running.

S. T. Emlen (*Anim. Behav.*, **18**, 215; 1970), at Cornell University, on the other hand, has recently reported his failure to confirm these results with indigo buntings (*Passerina cyanea*). In the absence of visual cues, these birds seemed totally disoriented, and they also failed to learn that they could avoid a brief electric shock by responding to a change in the direction of the magnetic field in a classical conditioning experiment.

These negative results do not, of course, rule out the possibility that indigo buntings may be able to use magnetic cues, and should be viewed in the light of Wiltschko's claim that his birds only responded to changes in a magnetic field when the strength of the artificial field closely corresponded to that of the Earth's field. The robins did not orient to stronger fields. This intriguing problem is still open.

CELLULOLYTIC ACTIVITY

Municipal Refuse Composting

from our Microbiology Correspondent

ONE of the major limitations to the effectiveness of composting solid waste is the incomplete degradation of cellulosic materials, or the long period required for its removal. The results of a research programme carried out at the US Department of Health, Education and Welfare and East Tennessee State University on cellulolytic activity in municipal refuse composts are therefore interesting and timely. Stutzenberger and his colleagues (*Canad. J. Microbiol.*, **16**, 553; 1970) have studied the factors effecting cellulose decomposition in refuse dumps and defined precisely the kinetics of the process.

Municipal solid waste, after being ground, was mixed with concentrated sewage sludge and piled into low ridges or windrows. The windrows, about 9 feet wide, 3 feet high and 150 to 250 feet long, were sampled during a seven week composting period for cellulolytic activity. Measurements of temperature indicated the course of the composting process. Thus the windrow heated up at a linear rate and usually exceeded 60° C by the end of the first week. A temperature gradient of 10° to 15° was quickly established in the windrow and maximum heat was developed in the middle of the fermenting refuse. The peak temperatures held for about two weeks and thereafter the windrows cooled gradually but even after the end of the experimental period the temperature was about 50° C.

Cellulase activity was measured using soluble carboxymethyl cellulose as the substrate and, although the resulting data clearly indicated the progress of cellulolysis, it seems a pity that a cellulosic substrate closer in physico-chemical properties to that encountered in solid waste was not used. Nevertheless, a consistent temperature optimum of 65° to 70° C for the cellulase activity of compost extracts was observed and this optimum was independent of the age of the windrow. Temperatures considerably greater than 60° C often develop in composts, and Stutzenberger accordingly tested the activity of compost extracts at 75° C. Although activity declined 70 per cent within three hours, the residual cellulase remained very stable for up to twenty-four hours at the hotter temperature. Experiments designed to follow the selection of thermostable cellulase producers were not carried out, but it is likely that such development would occur within a natural microflora. Specific cellulase activity, however, increased exponentially during the seven week composting period, while the total cellulase content decreased by half. The final product was described as having the appearance, texture and odour of soil.

Of the microorganisms responsible for cellulose decomposition in the windrow composts, *Aspergillus fumigatus* and a *Bacillus* species grew at 45°–50° C, and a third, not surprisingly an actinomycete, was more thermophilous and grew at 55°–60° C. The crucial question now is: what limits the extent of cellulose decomposition? Heat denaturation of the enzymes and product inhibition do not seem to be implicated to any large extent. The cause of compost termination is much more likely to be substrate heterogeneity, a condition which is also responsible for the decline of glucose production by enzymatic treatment of wood cellulose (*Nature*, **218**, 320; 1968), and the complexing of cellulose with other materials, such as lignin, which blocks its hydrolysis. The 40 per cent of undegraded cellulose remaining after composting could be reduced by more extensive physical and/or chemical pretreatment of the raw waste or by seeding the partially degraded material with lignicolous microbes. Alternatively, it might be used directly as a substrate in commercial mushroom farming as Block suggested (*Appl. Microbiol.*, **13**, 5; 1965).

SENSORY PATHWAYS

Active and Passive

from our Experimental Psychology Correspondent

FOR several generations the large tracts of fibres which ascend to the brain in the dorsal columns of the spinal

cord have been thought to convey information necessary for proprioception, and for fine tactile discriminations of stimulus location, separation, size and texture. But "One must now question the validity of the generally accepted view of dorsal column function". This is the opening of an article by P. D. Wall (*Brain*, **93**, 505; 1970) which critically attacks the view that has been held with great conviction since the first fumblings towards neurological localization of function in the nineteenth century.

For some time Wall has been ransacking the scattered literature on this problem. Curiously, all the available evidence seems to contradict the universal view, but the idea has been so thoroughly accepted that mere evidence has not been sufficient to displace it.

Relevant data are mostly of two kinds; from clinical observations, and from experiments on dorsal column section, mostly in monkeys, which have been followed by behavioural testing. In ten types of experiment reviewed by Wall, section of the columns was followed by no deterioration of any of the faculties that should have been affected according to the accepted view. Operated animals were still able to perform discriminations of weight, vibration, roughness and stimulus location just as well as normal animals. Rather than challenge the classical notion, however, investigators confronted by such embarrassing findings preferred to suppose that although the discriminations in question were normally accomplished on the basis of information ascending through the dorsal columns, in the operated animals the information acquired by other routes was sufficient for the tasks. This leads to the puzzling conclusion that the dorsal columns, which in man comprise about 27 per cent, and in the rhesus monkey about 23 per cent, of the entire cross-sectional area of the cord (compared with 1·5 per cent in teleosts), have no function other than to transmit information that can perfectly well be handled by other pathways.

Wall has supplemented his review with two experiments of his own. In one, rats had all the fibre tracts in the cord except the dorsal columns severed in the lower thoracic region. These animals never responded with the front half of their body to any stimulus applied to the hind legs, although single limb reflexes were intact, and the dorsal column fibres were found by electrophysiological measurements to be conducting. In the second experiment rats without dorsal columns, but with the rest of the cord intact, responded quite normally by arousal, startle and several other measures to stimuli applied to the back legs. But the frequency with which the operated rats oriented towards the location of the stimulus in order to explore the area was decreased in comparison with normals.

The retention of discriminative capacities in clinical cases and experimental animals, coupled with a rather more subtle deficit (noted in some reports) of a lack of ability to perform skilled exploratory movements, has stimulated Wall to propose a new idea of dorsal column function. He speculates that these tracts, enormously developed in higher mammals, act to initiate movements to explore the external environment, and transmit sensory information resulting from such exploration. This function is quite different from discrimination of stimuli that simply impinge on the surface of the body. Such passive sensory experience is in any case rather rare except in neurological examinations and psychological experiments.

TRANSFER RNA

Are Differences Significant?

from our Cell Biology Correspondent

In 1964 Sueoka and Kano-Sueko reported that during the infection of *E. coli* by phage T2 a leucyl-tRNA of the host cell is modified. Since then the idea that the amount and activity of tRNAs or their aminoacyl synthetases may be a limiting factor in the translation of messenger RNAs and affect the regulation of protein synthesis has been widely discussed. Direct evidence for such regulation is hard to come by, but it has been variously claimed that changes in the patterns of aminoacyl-tRNAs accompany at least some cases of cellular differentiation, bacterial sporulation, phage infection and virus infection. Furthermore, differences in the amounts or activities of tRNAs and their charging enzymes have also been reported between identical cells growing in different media or environmental conditions. This last observation means, of course, that comparisons of the aminoacyl-tRNAs in cells which have been cultivated for long periods need to be interpreted with great caution.

Gallo and Pestka (*J. Mol. Biol.*, **52**, 195; 1970), who have just reported an analysis of the aminoacyl-tRNAs in normal and leukaemic human lymphoblasts, are clearly well aware of the pitfalls of such analyses. To minimize spurious differences they studied newly established lines of lymphoblasts derived from a normal person and a person with acute lymphoblastic leukaemia. Furthermore, they grew the cells for the same generation times in identical media and collected them at the peak of the log phase of growth. In both the normal and the leukaemic lymphoblasts there are at least fifty-six tRNA species, and not surprisingly Gallo and Pestka detected very few differences between most of these molecules in the two types of cell. Reverse-phase partition column chromatography revealed only small but apparently reproducible differences in the leucyl, seryl, threonyl and prolyl-tRNAs, and more pronounced differences between the tyrosyl and glutaminyl-tRNAs.

The leukaemic cells apparently lack one of the three species of tyrosyl-tRNAs present in the normal cells, and the relative amounts of the two species common to both types of cell differ. The differences in the glutaminyl-tRNAs seem to reflect differences in the charging enzymes rather than differences in the tRNAs themselves. After charging the glutaminyl-tRNAs of both normal and leukaemic cells with the aminoacyl synthetases from normal cells, three species can be detected. On the other hand, after charging with the enzymes from leukaemic cells, only two species of glutaminyl-tRNAs can be resolved. Human leukaemic lymphoblasts may therefore lack a glutaminyl-tRNA synthetase which is present in normal cells. Until other lines of normal and leukaemic cells have been compared, however, the significance of these results is obscure; they may only reflect a normal variation among lymphoblasts rather than differences resulting from leukaemia.

In the same issue of the *Journal of Molecular Biology* (*ibid.*, 247), Morris, Wagner and Roizman report their failure to confirm earlier claims that herpes simplex virus specifies arginyl and seryl-tRNA during productive infections of HEp-2 cells. Morris *et al.* find no

differences between the tRNAs for these two amino-acids in infected and uninfected cells and purified arginyl and seryl-tRNAs from infected cells fail to hybridize with herpes simplex DNA. There is a remote possibility that the virus specifies tRNAs specific for derivatives of serine and arginine, but it seems more likely that the virus uses only cellular tRNAs during its replication.

ENZYMES

Synthetases Analysed

from our Molecular Biology Correspondent

"NUCLEIC acid-protein interactions" is an incantation which is just now widely held to possess talismanic powers. A particularly desirable case is that of the transfer enzymes, not least because so much is now known about the structure and reactions of their substrates. There is hope that the crystallographic structures of one or two tRNA species will in time be solved, and the possibility of co-crystallizing the transfer enzymes with their tRNA cannot be far from the crystallographers' minds.

A number of transfer enzymes from *E. coli* have been prepared in a highly pure state, and it comes as something of a surprise that within this group of molecules, which differ so subtly in specificity, there should be considerable structural differences. The latest study, for example, on valyl and leucyl-tRNA synthetases (Berthelot and Yaniv, *Europ. J. Biochem.*, **16**, 123; 1970) bears out early reports that these enzymes consist of single polypeptide chains with the unusually high molecular weight of 110,000. Binding plots show that each chain possesses only one amino-acid binding site, and measurement of the stoichiometry of the complex with the amino-acid and AMP gives the same answer. Other synthetases reported to contain only one chain are those for lysyl-tRNA, with 85,000 molecular weight, and leucyl-tRNA, with 100,000. Several others, however, are dimers or tetramers, and these all appear to have subunit molecular weights close to 50,000 (or at least 40,000, it not being at this juncture clear whether —no offence intended—such a difference is significant).

One of this group is methionyl-tRNA synthetase, which seems to exist in the cell as a tetramer, but Cassio and Waller reported some time ago that it is dissociated irreversibly in the presence of a cytoplasmic factor into a fully active dimer. Methionyl-tRNA synthetase has now been taken in hand by Bruton and Hartley (*J. Mol. Biol.*, **52**, 165; 1970), who have applied to its study every artifice of the protein chemist's craft. Working with the dimeric form, they have used equilibrium dialysis to construct binding plots for the amino-acid and for ATP, and find two binding sites per molecule, or one per chain. Binding of methionyl-tRNA, and also of the species that will accept formylmethionine, were measured by the accompanying changes in protein fluorescence which again led to two sites with identical intrinsic binding constants, that is to say with no mutual interaction.

By radioactive labelling of the α-amino group and proteolysis, an N-terminal peptide could be isolated and sequenced. The presence of a unique tetrapeptide sequence from both the dimeric and tetrameric enzyme, taken together with identical binding constants of the two sites in respect of the amino-acid and two tRNA species, makes it overwhelmingly likely that the enzyme in fact consists of identical chains.

Bruton and Hartley have also begun an exploration of the enzymatically essential residues. In the first place the free dimer has ten accessible cysteines, eight of which react rapidly with a thiol reagent (and with iodoacetamide). The reaction of the other two is accompanied by dissociation into monomers and inactivation, both effects being reversed when the blocking group is stripped off. (Similar essential thiols have also been reported for other synthetases.) Finally, Bruton and Hartley have brought off an active site labelling reaction, in which a reactive function, containing a nitrophenyl group, is attached to the α-amino end of methionyl-tRNA. This finds a nearby lysine, with which to react, and after enzymatic digestion, fragments containing the methionyl-tRNA were isolated. The sequence of an octapeptide containing the central lysine is given.

This is an admirable beginning to a complete characterization of the system. The relation between the dimer and the tetramer and the significance of the latter remains obscure. It is interesting to note that Rouget and Chapeville (*Europ. J. Biochem.*, **14**, 498; 1970) also found a cytoplasmic factor which modified a synthetase, in this case, however, the leucyl-tRNA enzyme, which is a 100,000 single chain. The factor was found to promote the interconversion between an active form, and one which, though it binds leucine tRNA is unable to transfer the amino-acid to it; its other characteristic activity, of exchanging pyrophosphate against bound ATP, remains intact, but with impaired specificity. There is perhaps more to the regulation of transfer enzyme function than is yet apparent.

CYCLIC AMP

Involved with Hormones

from a Correspondent

WIDESPREAD interest in cyclic adenosine 3′,5′-monophosphate, and in the enzyme systems which produce and destroy this compound, was clearly indicated by the size of the audience which assembled, during a meeting of the Biochemical Society in the University of Dundee, for a colloquium on adenyl cyclase and cyclic AMP on September 24 and 25. The first contribution was from Professor J. Kilpatrick and Dr P. W. Major (University of Sheffield), who outlined the development of interest in cyclic AMP as the mediator of many hormonally controlled processes. The list is now so extensive that one should ask which processes are not controlled or influenced by cyclic AMP rather than which are so controlled. Their report was illustrated with references to their own work on the interaction of luteinizing hormone with rabbit ovary adenyl cyclase. Dr D. G. Grahame-Smith (St Mary's Hospital, London) discussed the evidence which implicates cyclic AMP as the mediator of the effect of adrenocorticotropic hormone on the adrenal cortex, in respect of its steroidogenic and tissue maintenance properties. He emphasized the lack of understanding of the way in which cyclic AMP acts in the adrenal cortex, but mentioned studies with protein synthesis inhibitors which suggest that *de novo* synthesis of protein may be involved.

Dr J. I. Davies (University of Wales, Bangor) was concerned with the factors which control the intracellular concentration of cyclic AMP. Assuming these to be the enzymes adenyl cyclase (with or without hormonal stimulation) and cyclic AMP phosphodiesterase, he presented a theoretical kinetic relationship in the form of an equation which could be used to predict tissue levels of cyclic AMP. He showed most clearly that experimental results from many laboratories were compatible with his calculations.

Dr D. A. Stansfield and Mr D. J. Franks (University of Dundee) have approached the study of the properties of adenyl cyclase by attempting to separate luteal adenyl cyclase from the complex ATP hydrolysing system which is also present in plasma membrane fractions, and which competes very successfully with adenyl cyclase for the available substrate (ATP) in *in vitro* incubations. They emphasized the lack of understanding of the way in which fluoride ions activate adenyl cyclase and inhibit the "ATPase" and suggested that fluoride polyanions are responsible for some of the effects which vary with fluoride concentration. Studies with an ATP analogue, β,γ-methylene ATP, were also described.

The lack of convenient and accurate assay methods for cyclic AMP was underlined during the discussion, and by the presentation of two methods for determination of cyclic AMP in later sessions of the meeting. A method which seems to have very great potential was described by B. L. Brown, R. P. Ekins and W. Tampion (London); in this saturation analysis study a specific cyclic AMP binding protein from bovine adrenal cortex was used in combination with charcoal adsorption of unbound nucleotides. Several other communications dealt with kinetic studies of cyclic AMP phosphodiesterase. A. R. Sommerville, M-L. Rabouhans, A. A. Smith, I. F. Skidmore and S. T. Hindley (Macclesfield) have explored the kinetics of this enzyme at substrate levels usually used in *in vitro* assays (about 0·5 mM) and at very low substrate levels. The Lineweaver-Burk plots they obtained suggested the presence of two enzymes, with very different K_M values.

Discussion also brought out the unreliability of implicating cyclic AMP in a system solely on the grounds that methyl xanthines had an effect on the system. Caffeine and theophylline can, apparently, have effects other than the inhibition of cyclic AMP phosphodiesterase.

SPOROPOLLENIN

Interest in Exine

from a Correspondent

THE unifying influence of sporopollenin was clear at Imperial College, London, on September 23 to 25. Geologists, organic chemists and many others gathered for a symposium on the most chemically inert substance produced by plants. Sporopollenin forms the outer coat of pollen grains and spores of higher plants, and there is interest in its composition, its formation in living plants and its survival in ancient sediments.

Professor J. Heslop-Harrison (London) reported that centrifugation and treatment with colchicine at different stages of pollen formation interrupt the normal pattern of sporopollenin deposition, giving new insight into the controlling mechanism. In his experiments with *Lilium* fragments of microspore cytoplasm, lying within the pollen sac, but lacking a nucleus as a result of post-meiotic centrifugation, nevertheless had a normal sculptured exine deposited on their surface; while similar fragments derived from (diploid) tapetal cells received a layer of sporopollenin, but without the characteristic sculpturing pattern. He suggested that although most sporopollenin precursors must be supplied from the tapetum, these results are consistent with the view that the pattern is dictated by the cytoplasm of the haploid cell; and further, that this cytoplasm is already "programmed" (probably from the time of the first prophase of the meiotic division) for a pattern which is independent of the presence of a nucleus. The sporopollenin precursors, regarded by Dr G. Shaw (University of Bradford) and his students as carotenoids, seem to be polymerized *in situ* on the developing exine; the role of enzymes in this polymerization remains uncertain and Heslop-Harrison suggested that at least the final stages in the assembly of sporopollenin may be non-enzymatic.

The biological degradation of sporopollenin offers as many problems as its synthesis. Spores and pollen exposed to microbial activity in controlled natural soil environments by Dr A. J. Havinga (Wageningen, Holland), and fossil spore exines occurring in sedimentary rocks examined by Dr W. C. Elsik (Houston), all show evidence of degradation in which both fungi and bacteria seem to be involved. The role of soil fauna may be considerable, but this is less well documented. Sporopollenin is much more sensitive to raised temperatures and pressure for prolonged periods (diagenesis and, eventually, metamorphism) than mineral constituents of sedimentary rocks. Computer analysis of an extensive study of fossil sporopollenin in a wide range of lithologies subjected to different patterns of diagenesis, conducted by Drs M. J. Correia and G. Peniguel (Pay, France), offers a basis for evaluating the role of the many factors involved in the diagenetic process.

Drs J. Brooks (Sunbury) and Shaw emphasized the significance of the similarity of physico-chemical characteristics (infrared absorption spectrum, pyrolysis gas chromatography) of sporopollenin from living plants to those of the insoluble organic residues of ancient ($3{\cdot}7 \times 10^9$ year old) sediments and even of carbonaceous meteorites. Shaw advocated extending the term sporopollenin without qualification to all these substances, regarding their presence as evidence of life-activity, of both terrestrial and extraterrestrial source. Several botanists urged a narrower concept of sporopollenin, and would restrict its use to material demonstrably derived from the spores of higher plants.

In spite of the emphasis placed by chemists on the unity of sporopollenins from various sources, minor differences in composition may be clues of value in interpreting the history of fossil material. The use of ultraviolet fluorescence microscopy, described by Dr Van Gijzel (Nijmegen), of the new technique of cathodoluminescence on the scanning electron microscope, described by Drs M. Muir and P. Grant (London), and of centrifugation of fossil assemblages on a density gradient, described by Drs W. Chaloner and G. Orbell (University College, London), were all advocated as means of detecting small compositional differences, reflecting the origin and post-depositional history of fossil sporopollenin.

Glutamate and γ-Aminobutyric Acid in Brain

by
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Evidence is accumulating that, in the brain, the amino-acids L-glutamate and γ-aminobutyric acid (GABA) are essential agents of communication and not just elements of an alternate metabolic pathway, as was generally believed for some years.

It has long been known that slices of brain can metabolize L-glutamate[1] transforming it, in a process which involves transamination and oxidation, to α-oxoglutarate (Fig. 1)[1,2]. The addition of glutamate thus increases the oxygen consumption of the brain. Slices of cerebral cortex can absorb large amounts of glutamate from incubating fluid, and a great deal of K^+ is taken up at the same time[3–6]. Although glutamate stimulates cerebral metabolism, glucose is the principal source of energy utilized by the brain: glutamate therefore seemed to serve some unknown function in the transport of K^+ (ref. 4).

Like other nerve cells, cerebral neurones are normally rich in K^+ and poor in Na^+. But the inward flux of Na^+ and outward flux of K^+ associated with depolarization and spike activity tend to reduce the difference between the internal and external concentrations of these ions. The normal steep gradients of concentration across the membrane are restored by an Na-K pump, which, through the expenditure of energy, can extrude Na^+ from the cell in exchange for external K^+ (refs. 7, 8). The high content of K^+ in the brain is thus a function of the state of polarization of the cells and can only be maintained by metabolism. It is therefore significant that glutamate has a powerful depolarizing action on cerebral neurones.

Excitation by Glutamate

The excitatory action of glutamate was first apparent in Hayashi's experiments[9] on the cerebral cortex. The effect is so strong and so rapid that it may not be recognized as excitation. Gross applications of glutamate to the brain surface give rise to "spreading depression"—a slowly moving wave of intense neuronal depolarization and inactivation—and so abolish all spontaneous and evoked activity[10].

Excitation is clearest when glutamate is released within the brain in minute amounts (10^{-13} equiv/s), by iontophoresis from a micropipette (Fig. 2*A* and *B*)[11,12]. The evoked discharge, which is typically rapid in onset and stops sharply when the release ends, is associated with depolarization of the neuronal membrane[11,13,14]. Most central neurones have this excitatory effect, but other cells (neuroglia) are not depolarized by glutamate[14]. The fact that neurones in spinal ganglia are also not depolarized by glutamate[15] suggests that the sensitivity to glutamate depends on the presence of synaptic contacts.

To excite neurones, glutamate must somehow trigger the entry of Na^+. Like acetylcholine at the muscle endplate, it may do this by increasing the general permeability to cations. Alternatively, glutamate may activate a specific Na^+-carrier, similar to that which generates the nerve action potential[16]. There is some evidence against this, however. Tetrodotoxin, a specific inactivator of the Na^+-carrier[17], does not block very effectively the influx of Na^+ caused by glutamate[18].

Both glutamate and Na^+ are perhaps transported by the same carrier (Fig. 3), as in the mechanism suggested for amino-acid uptake in the gut[19]. In such a scheme, glutamate could be removed from its site of action without the need for a special glutamate pump, the energy being supplied by the electrochemical inward gradient of Na^+, maintained by the Na-K pump. It is assumed that the common carrier has separate sites for combination with

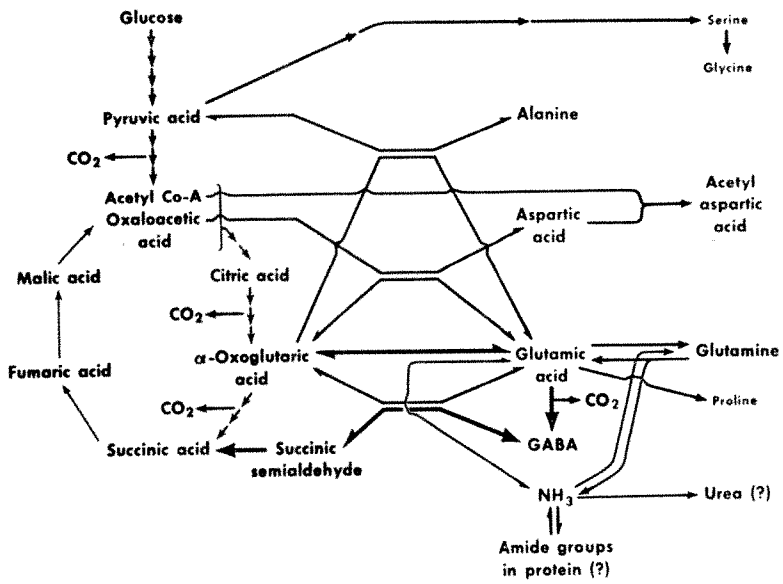


Fig. 1. Glutamate, GABA, and glucose metabolism in the brain (from ref. 90).

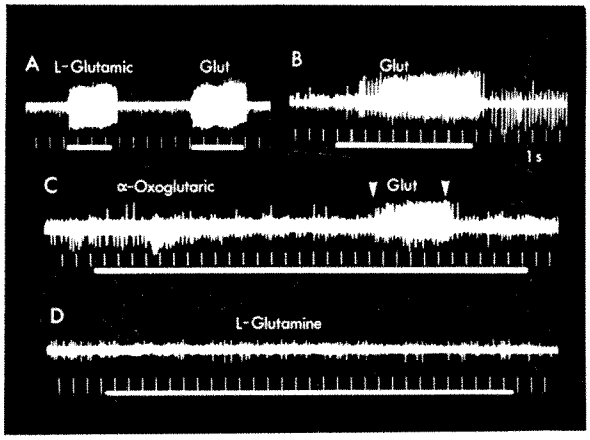


Fig. 2. Excitatory action of L-glutamate applied to cortical neurones by microiontophoresis (60–70 nA), at times indicated by white lines below oscilloscope traces. (*C*) α-Oxoglutarate (170 nA) had no effect and did not prevent action of glutamate applied between arrows. (*D*) Application of L-glutamine (100 nA) for a long time was also ineffective.

Na^+ and glutamate; that through a conformational change in the carrier, the affinity for Na^+ is much increased by the attachment of glutamate; and that the affinity for glutamate is itself a function of the Na^+ concentration. Thus external glutamate would promote the entry of Na^+, while being itself transported into the Na^+-poor cytoplasm, and the membrane would depolarize if this influx of Na cations exceeds that of glutamate anions. This hypothesis is given some support by the marked dependence of glutamate uptake on the presence of external Na^+ (ref. 5).

Glutamate excites so much more powerfully than much stronger chelators that its action cannot be ascribed principally to the binding of Ca^{2+} (refs. 12, 20).

Glutamate and Accumulation of Potassium Ions

Whatever its precise mechanism, the depolarizing effect of glutamate is probably caused by an influx of Na^+, and it should be followed by an efflux of K^+. What, then, is the reason for the observed increase in the K^+ content of brain slices?

An important additional observation is that glutamate causes a comparable increase in water content[4,21]. Because the cells swell, the internal concentration of K^+ remains approximately constant[21]. The complete sequence of events has been described by Harvey and McIlwain[8]. Within a minute of adding glutamate to brain slices, there is an increase in the Na^+ and a decrease in the K^+ contents of the "non-inulin" compartment (a useful index of the volume of intracellular water because bulky inulin remains outside the cells). At this stage there is no detectable movement of water. Thus the initial fluxes of Na^+ (in) and K^+ (out) can be explained as a direct consequence of the depolarizing action of glutamate.

In the next phase, the influx of Na^+ continues, but is accompanied by an influx of K^+, and there is an increase in intracellular water. These phenomena presumably follow the activation of the Na-K pump by the rise in internal Na^+. This active uptake of K^+ leads to a depletion of the high-energy phosphate compounds that provide the immediate energy requirements of the Na-K pump[22]. In spite of the continued rapid influx of Na^+ and K^+, a significant increase in internal osmolarity is prevented by the inflow of water. Glutamate causes similar changes in the Na^+, K^+ and water content of the isolated retina[4,23].

Glutamate as a Transmitter

The brain contains plenty of glutamate (about 10 μmole/g), more than its content of any other amino-acid[24,25]. This fact has perhaps militated against the idea that glutamate might be a transmitter—that is, a substance released by nerve endings at synaptic contacts to excite or inhibit other neurones. Neurotransmitters are often believed to be very scarce substances, difficult to extract or identify, and probably highly labile. Glutamate is the reverse of all these. Not only is it very abundant, but it is highly active in brain metabolism: if radioactive pyruvate, acetate or glucose is injected into an animal, labelled carbon is very rapidly incorporated into cerebral glutamate (Fig. 4)[26–31].

The general abundance of glutamate is not sufficient evidence of a transmitter function. The presence of adequate amounts of glutamate in excitatory nerve endings would be more significant. In an attempt to throw some light on this point, Whittaker and I[32] examined the excitatory properties of the contents of cortical nerve endings. Gray and Whittaker[33] had shown that nerve endings separate from nerve fibres when the cortex is homogenized and then centrifuged in a suitable density gradient. Suspensions of such isolated nerve endings were inserted into micropipettes and their contents released close to nerve cells in the cortex. Definite excitatory effects were observed, which could be ascribed to the glutamate content of the endings measured in separate experiments by Mangan and Whittaker[34]. Enough glutamate must therefore be present, at least in some nerve endings, for a significant synaptic action. Cerebral glutamate in general seems to be widely distributed, presumably in neuronal cytoplasm[35–37], but intracellular glutamate probably does not directly influence neuronal activity: unlike extracellular applications, intracellular injections of glutamate do not cause excitation[38].

The glutamate sequestered in isolated nerve endings forms *in situ* an active and readily accessible "pool". For example, it is rapidly labelled by systemic injections of

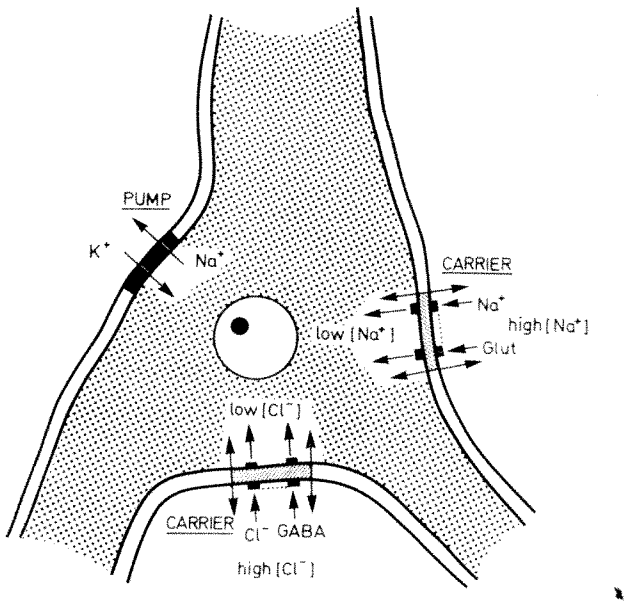


Fig. 3. Hypothetical model of glutamate and GABA carriers. Combination of glutamate with carrier on surface of cell promotes entry of Na^+, while combination of GABA with its carrier accelerates movement of Cl^- (see text).

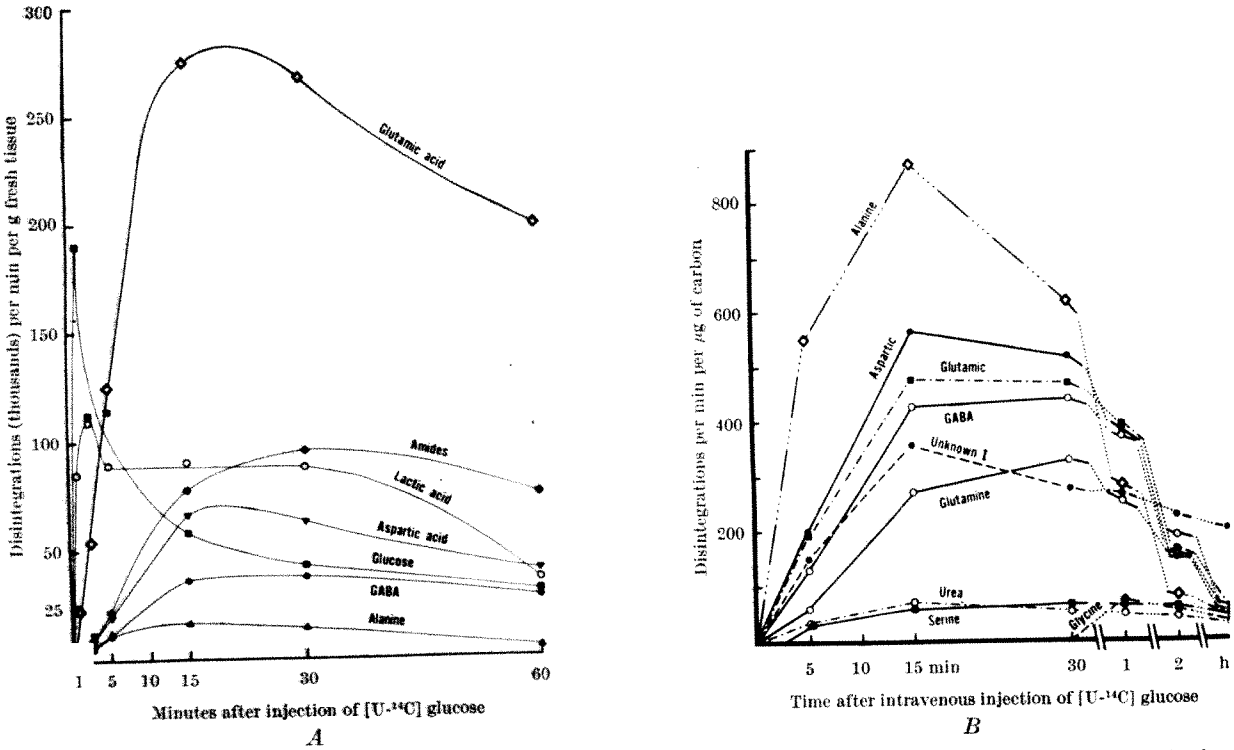


Fig. 4. Radioactive labelling of glutamate, GABA and other amino-acids in rat brain after intravenous injection of U-^{14}C-glucose. (*A*) Total radioactivity, (*B*) specific activity of ninhydrin-positive material (from ref. 31).

radioactive glucose[39]. And much of the uptake of glutamate into cortical slices finds its way into nerve endings[37]. By efficient synthesis and recapture, nerve endings evidently could supply the variable quantities of glutamate needed for synaptic release, without maintaining a large store.

Further evidence is required to confirm a possible transmitter function of glutamate: for example, that glutamate is released by excitatory nerve fibres. All that is known so far is that glutamate is continually being released at the surface of the brain[40]. The rate of release varies with cortical electrical activity, and it can be increased by stimulating the brain stem reticular formation. According to recent experiments[41], suspensions of isolated nerve endings selectively release glutamate and aspartate when they are stimulated electrically or with solutions rich in K^+. A serious problem is that re-uptake must prevent the escape of all but a small fraction of any glutamate released. If the process of uptake is driven by the Na^+ gradient, it will be difficult to block without abolishing all neuronal activity—except possibly by competitive inhibition.

Better evidence is needed to prove the identity between the action of glutamate and that of the natural excitatory transmitter. Some formidable technical problems are also likely to impede rapid progress in this direction. The reversal level of excitatory synaptic potentials is far removed from the resting potential, and therefore not easily attained with the small currents that can be carried by fine microelectrodes. Furthermore, many excitatory synapses may be situated remotely on the dendrites of pyramidal cells[42]; the corresponding synaptic events would give only small conductance changes and poorly defined reversal levels at the probable site of recording in the cell body. Finally, there are still no specific pharmacological antagonists of either excitation or of glutamate action.

Derivatives of Glutamate

L-Aspartic acid, the other dicarboxylic amino-acid found in the brain, has a strong excitatory action, comparable with that of L-glutamate[12,43]. But the longer-chain compounds, amino-pimelic and amino-adipic acids, are quite ineffective. The excitatory action depends rather critically on the presence of one amino and two acid groups: thus α-oxoglutaric acid (Fig. 2*C*), glutamine (Fig. 2*B*) and acetyl-aspartic acid are also practically ineffective; and the α-decarboxylation products of glutamate or aspartate (γ-aminobutyric acid or β-alanine) are powerful inhibitors of neuronal discharge discussed later. But a carboxyl group can be substituted with a sulphonic group; the resulting compounds are even more potent excitants than glutamate[12,43].

γ-Aminobutyric Acid and Inhibition

In contrast to glutamate, GABA was identified as an important brain constituent only relatively recently[44,45], probably because it is not found in any significant amount in other tissues, and its presence was therefore unexpected. Its functional role as a possible inhibitory transmitter became apparent when Elliott and his collaborators[46,47] identified GABA as the active agent in brain extracts which had a unique inhibitory action on crayfish stretch-receptors[48,49].

The powerful inhibitory effect of GABA on crustacean stretch-receptors and muscle was later shown to be indistinguishable from synaptic inhibition[50,51]. With the further demonstration that GABA is selectively released by stimulating inhibitory nerve fibres[52], it became probable that GABA is an inhibitory transmitter in crustaceans.

Hayashi[9] first noted that GABA and some other omega monocarboxylic amino-acids could inhibit mammalian cortical neurones. A depressant action was also observed by Purpura *et al.*[53] and Curtis and his collaborators[43,54], but neither group believed that GABA could be responsible

for the natural inhibition of central neurones. But further tests on the cerebral and cerebellar cortex[12] revealed such a powerful inhibitory effect (Fig. 5*A*) that GABA could not be ignored as a possible inhibitory transmitter. Later, intracellular recording and tests of membrane resistance[55,56] demonstrated a remarkable similarity between the effects produced by synaptic inhibition and by GABA. In both cases, there was a hyperpolarization—with a similar reversal level—and a great decrease in membrane resistance, chiefly attributable to an increase in Cl^- permeability (Fig. 6*A* and *B*)[38]. Comparable observations on neurones in Deiters's nucleus make it probable that GABA is the inhibitory transmitter released in the medulla by the endings of the cerebellar Purkinje cells[57].

Derivatives of GABA

Other short-chain omega-amino-acids and some related compounds such as guanidine derivatives are also potent inhibitors of central neurones (Fig. 5*B* and *D*)[9,12,43]. Physiologically, the most significant is glycine, which is probably an important inhibitory transmitter in the spinal cord[58]: many spinal neurones are more strongly inhibited by glycine than by GABA; and, like the best-known variety of spinal inhibition, the action of glycine is blocked by strychnine[59]. Glycine may be an inhibitory transmitter also in some parts of the brain stem[60,61]. But it is unlikely to have an important inhibitory function in the cerebral[62] or cerebellar[63] cortex, where it has only a very weak action (Figs. 5*C* and 6*C*).

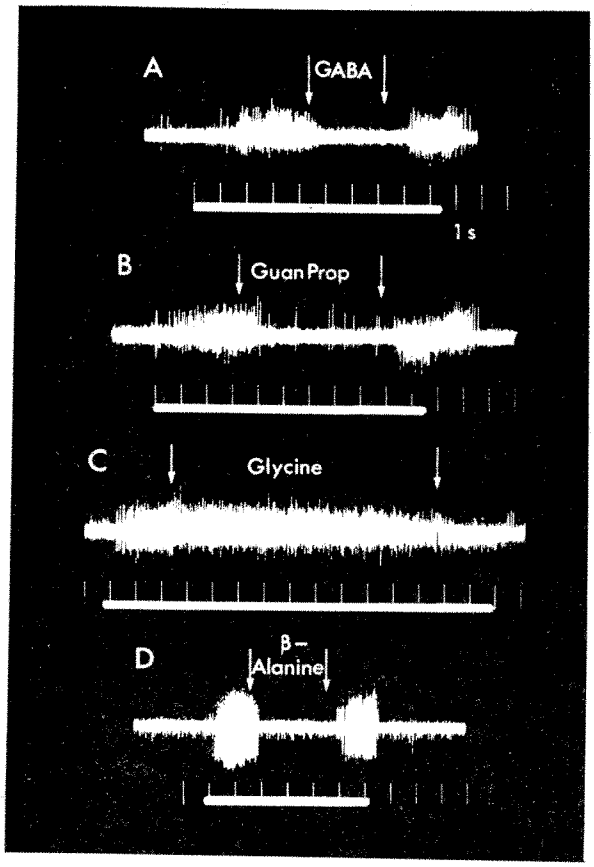


Fig. 5. Inhibitory action of GABA and some related amino-acids on cortical cells. In each case, neuronal discharge was initiated by release of glutamate (white line). (*A*) GABA (20 nA); (*B*) guanidino-propionic acid (20 nA); (*C*) glycine (100 nA), all tested on same group of cells; (*D*) effect of β-alanine (20 nA) in another experiment.

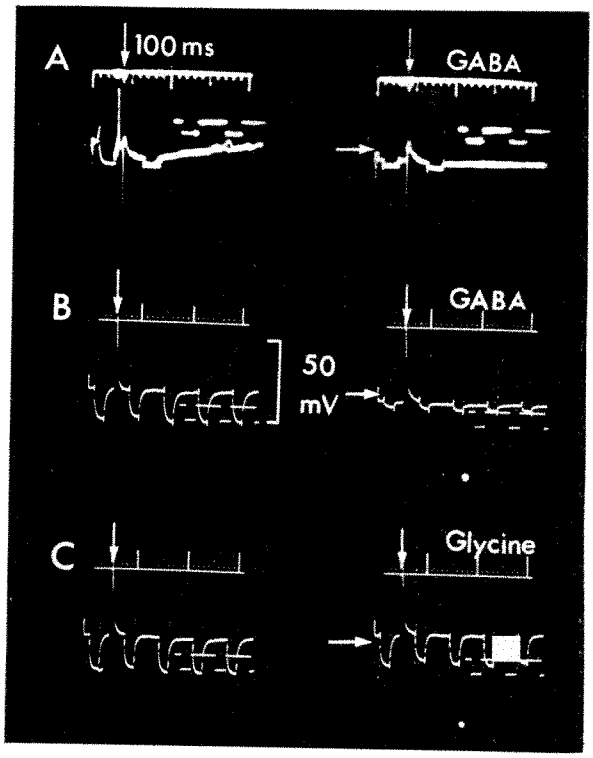


Fig. 6. Intracellular recording from cortical neurones showing inhibitory synaptic potentials (IPSPs) elicited by surface stimuli (at vertical arrows). Control traces are on the left. (*A*) IPSP shown after shock artefact as a downward (negative) deflexion, associated with a marked decrease in membrane resistance (note smaller voltage pulse developed by constant current pulses; the latter are monitored on another trace); when GABA was applied (140 nA), membrane potential became more negative (trace moved down) and resting resistance fell. (*B*) and (*C*) Traces obtained from another neurone: five resistance-testing pulses are visible in each. Note much smaller IPSP. GABA (28 nA) had a correspondingly smaller hyperpolarizing effect, but the membrane resistance was again much reduced. In contrast, a larger dose of glycine (56 nA) produced only a small change in resistance and some depolarization. Horizontal arrows indicate initial level of resting potential.

Metabolism and Localization in Brain

GABA is widely distributed in the brain[25,44,45]. Its production from glutamic acid (Fig. 1) is mediated by a highly specific enzyme, glutamic decarboxylase (GAD), which requires pyridoxal phosphate as coenzyme, and is inhibited by semicarbazide and hydroxylamine[64]. Other properties of this enzyme have been studied extensively[65]. A feature of some interest is that GAD is inhibited by Cl^-, so that an increase in the content of Cl^- in inhibitory cells, resulting from intense activity, could reduce substantially the rate of production of GABA[66]. GAD and GABA have an approximately similar distribution in the central nervous tissue[67,68]. The enzyme is not found in peripheral nerves or spinal roots and its concentration is greatest in areas of the brain stem that are exceptionally rich in GABA (such as the substantia nigra).

GABA-amino-transferase (GABA-T) is another relevant enzyme. By reversible transamination with α-oxoglutarate, it converts GABA to succinic semialdehyde, which, in the presence of an appropriate dehydrogenase[69], is oxidized to succinic acid (Fig. 1). Because succinic acid is a component of the tricarboxylic acid cycle, GABA is conveniently removed by this route. GABA-T is also present in the brain[70] but its distribution differs somewhat from that of GABA[67,71]. It seems that the content of GABA in a given region is determined by local GAD rather than GABA-T activity[67]. In the cerebellum and the hippo-

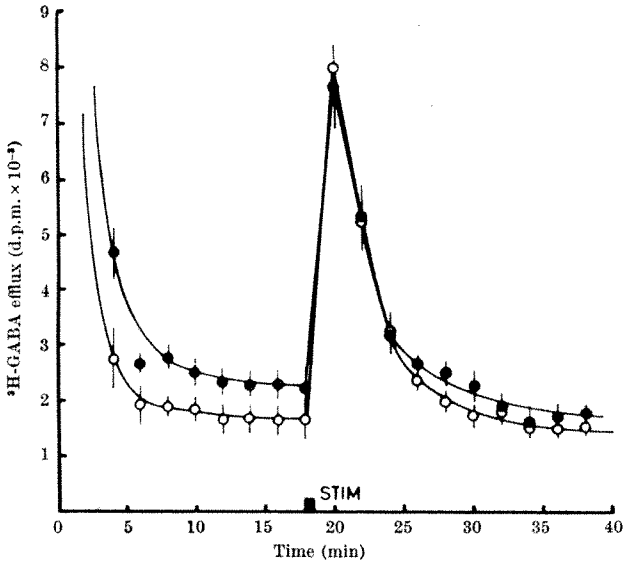


Fig. 7. Release of (^{3}H)-GABA from rat cortical slices by electrical stimulation (20 mA, 5 ms pulses at 100 Hz, for 30 s). ●—●, Normal; ○—○, Ca^{2+}-free. Mean values from seven experiments, with standard errors. (From Srinivasan *et al.*[84])

campus, the known situation of inhibitory neurones agrees with the distribution of GABA and the relevant enzymes[72,73].

GABA is too powerful an inhibitor to be freely distributed in the extracellular space of the brain. But GABA injected inside neurones does not alter their excitability[55]. GABA could therefore be stored inside cells. In fact, most of the GABA in the brain is probably actively kept in an intracellular compartment. Fluid left in contact with the surface of the brain contains at most only traces of GABA[40,74]. Slices of cerebral cortex absorb GABA, even against a very high concentration gradient[6,75]. This active process requires the expenditure of energy, and is therefore blocked by metabolic inhibitors.

Glutamate is apparently contained in more than one "pool" in the brain. One of these pools is in equilibrium with cerebral GABA but not glutamine; it is labelled very effectively through systemic glucose[30,31,39]. A second, smaller, pool seems to be accessible to systemic acetate, through which one obtains a high degree of labelling of glutamine but relatively little of GABA[27]; labelled glutamate injected directly into the brain[76] or systemically[77] also gives a high yield of labelled glutamine, probably by entering the same pool. These two pools may include, on the one hand, inhibitory nerve terminals which release or take up GABA and, on the other, excitatory terminals which release or take up glutamate. In the first pool, glutamate and GABA rapidly reach equilibrium through GAD and GABA-T activities. In the second, any excess glutamate may readily be stored in the inactive form of glutamine through glutamine synthetase.

Mechanism of Action and Uptake

A large increase in permeability to Cl^- fully accounts for the inhibitory action of GABA, because the gradient of Cl^- across the membrane is close to that for electrochemical equilibrium when the cell is in its resting condition; inward (repolarizing) movements of Cl^- therefore act as a powerful brake against any depolarizing (exciting) tendencies.

The precise mechanism by which GABA facilitates movements of Cl^- is not known. By analogy with glutamate, GABA might combine with a membrane carrier for Cl^-, increasing its affinity for the anion, and therefore accelerating Cl^- fluxes across the membrane (Fig. 3). If the binding of GABA to the carrier is a function of the ambient concentration of Cl^-, the usual low internal content of Cl^- would promote a net uptake of GABA. There is no direct evidence for this scheme, but some support is given by the observation that cortical slices absorb much less GABA in the absence of Cl^- or other halide anions (personal communication from Z. Gottesfeld and K. A. C. Elliott).

Although the active uptake of GABA by brain slices has been studied for some years, the precise localization of the absorbed GABA has been demonstrated only recently. According to Neal and Iversen[78], like endogenous GABA, nearly half of the labelled GABA taken up by cortical slices is found in nerve endings, which also contain most of the GAD activity in the tissue. These results thus confirm the presence of both GABA[32,34,39,79] and GAD activity[36,79,80] in cerebral nerve endings. Presumably at least part of the GABA absorbed by the slices is taken up directly by nerve endings, for even isolated nerve-ending particles can bind and take up external GABA[81,82]. In any case, when extracts of cortical nerve endings are tested directly on cortical cells[32], endings can be shown to contain enough GABA for a significant inhibitory action.

Release of GABA during Inhibition

If GABA is indeed an inhibitory transmitter, one should be able to demonstrate its release from inhibitory nerve endings, although only very small amounts are likely to escape from the tissue, even during the strongest inhibition, until a means is found of preventing the rapid uptake of extracellular GABA.

Some release of GABA from brain tissue has been demonstrated in experiments on brain slices, on isolated nerve endings or on the brain *in situ*. For example, if brain slices are incubated in a medium containing radioactive GABA, the subsequent efflux of labelled GABA into non-radioactive medium is very much accelerated by stimulating the slices electrically or with 40 mM K^+ [83,84]. Stimulating in a similar way a suspension of nerve endings—isolated by the technique of Gray and Whittaker[33]—causes a markedly greater proportional loss of GABA than of other amino-acids (except glutamate and aspartate)[41]. Jasper and his collaborators[40,74] have shown that appreciable amounts of GABA can sometimes be collected from the surface of the cortex; this efflux apparently depends on the functional state of the animal, being relatively large during sleep and practically undetectable after arousal. A more direct correlation with inhibitory function is evident when the efflux of cortical GABA is specifically accelerated by the activation of known inhibitory pathways; for example by stimulating the cortical surface, the thalamus[85], the caudate nucleus or the pyramids (personal communication from H. Jasper and I. Koyama).

Finally, Obata and Takeda[86] have shown that cerebellar stimulation increases the leakage of GABA into the fourth ventricle. This is particularly significant because the cerebellar cortex has a solely inhibitory action on nuclei adjacent to the ventricle[87]. Both in cerebellar and cerebral experiments, the yields of GABA are substantially greater when animals are pre-treated with inhibitors of GABA-transaminase.

In crustaceans the inhibitory action of GABA is blocked very effectively, and specifically, by the convulsant agent

picrotoxin[47,91,92]. Unfortunately, such a clear antagonism of GABA has not so far been observed regularly in the vertebrates, with picrotoxin or any other compound. As already mentioned, the inhibitory action of glycine is prevented by strychnine; but strychnine has little effect on inhibition by GABA[59,93,94]. Picrotoxin may antagonize the action of GABA on certain neurones in the brain stem and spinal cord[93,95], but really convincing antagonism has not yet been demonstrated in the cerebral or cerebellar cortex. Curtis *et al.*[96] have stated that the alkaloid bicuculline blocks selectively the inhibitory action of GABA in the brain and spinal cord, and that this drug should be useful for detecting GABA-mediated inhibition. But repeated tests by prolonged direct applications on cerebral cortical neurones have failed to confirm these observations[97]: it seems unlikely that bicuculline can be of great value as a specific antagonist of GABA.

In summary, there is now much evidence to suggest that the most important functions of L-glutamate and GABA are to act respectively as excitatory and inhibitory neurotransmitters. That the glutamate-GABA metabolic pathway (Fig. 1) can use up a substantial fraction of the total oxygen consumed by the brain[88] may be an indication not of its importance for general metabolism, but rather of the large amount of energy needed to maintain an adequate supply of these essential transmitters. Thus although cerebral glutamate first became well known because of its effects on the oxygen consumption and the sodium and potassium contents of the brain, these are probably secondary manifestations of transmitter functions. Of course, glutamate is also involved in the synthesis and breakdown of cerebral proteins[29,89]. But even these slower processes may be considered aspects of neurotransmission, for they probably determine the long-term synaptic facilitations or inactivations required for learning and memory.

I thank the Canadian Medical Research Council for its financial support.

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Synthesis of 5S Ribosomal RNA in *Escherichia coli* after Rifampicin Treatment

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Experiments involving rifampicin show that 5S RNA is generated from a transcription unit carrying between thirteen and thirty times the amount of genetic information necessary for the production of one molecule.

ACCUMULATION of higher molecular weight precursors of mature 16S and 23S ribosomal RNA (m16 and m23) has been demonstrated in bacterial cultures in which the maturation of these precursors is prevented by inhibition of protein synthesis[1]. More recently, polyacrylamide gel electrophoretic techniques have facilitated the demonstration of the transient existence of such macromolecular precursors (termed p16 and p23) in normally growing cultures of *Bacillus subtilis*[2] and *Escherichia coli*[3,4]. Woese and his collaborators[5,6] have shown that, in *B. subtilis*, the other two "stable" RNA species, 5S ribosomal RNA and 4S (transfer) RNA, are also secondarily derived from substantially larger macromolecules. In the case of *E. coli* 5S RNA, however, results from this laboratory[4] and a recent report by Adesnik and Levinthal[3] are in contradiction. The kinetic experiments of Pace *et al.*[4] suggest that *E. coli* 5S RNA is the product of post-transcriptional modification, while those of Adesnik and Levinthal indicate that it is not. We describe here an alternative approach to this problem using the antibiotic rifampicin, which inhibits the initiation of transcription by *E. coli* RNA polymerase, but allows completion of transcriptional events already initiated[7,8].

This approach is perhaps best understood in terms of two possible models for the molecular origin of 5S RNA (Fig. 1). Here we define a "transcriptional unit" as a segment of DNA bounded by (and including) a single transcription initiation site and a single transcription termination site, which is transcribed without interruption by any RNA polymerase molecule which effectively begins RNA synthesis at the initiation site. A transcriptional unit may comprise one or many genes. (If an operator region is included, then the transcriptional unit is properly called an "operon".) In model A (Fig. 1), 5S RNA is the product of a small transcriptional unit (5S DNA) which carries little or no additional genetic information. In model B, 5S DNA is included within, and near the termination site of, a considerably larger transcriptional unit. The primary transcription product of this unit is a large RNA molecule which is secondarily cleaved into 5S RNA and one or more other RNA species ("X-RNA"). It should be noted, however, that such cleavage might occur before transcription of the 5S DNA segment is completed. If new initiations are prevented by the addition of rifampicin, and isotopic label is added immediately, incubation being continued until all RNA synthesis has stopped, the amount of label incorporated into 5S RNA will depend on the size of the transcriptional unit from which it is derived or, more precisely, on the number of molecules of RNA polymerase bound to that transcriptional unit at the time of antibiotic addition. There are indications that the 5S DNA segment can probably accommodate no more than two simultaneously transcribing RNA polymerase molecules[9], and in our conditions (0·55 generations/h) it presumably carries only one such molecule at any instant. This single polymerase molecule will, on average, have traversed about one half (fifty-five nucleotide pairs) of the 5S DNA segment. Thus, if rifampicin and then label are added, this polymerase will incorporate approximately fifty-five nucleotides from the labelled pool into 5S RNA (see Fig. 3 for experimental details). Let this amount of label correspond to one half "unit" of radioactivity, where one unit is that amount of radioactivity expected in one fully labelled 5S RNA molecule.

If model A is correct, only this one half unit of radioactivity incorporated by the single polymerase bound to 5S DNA will be found in 5S RNA, because no further transcription of 5S DNA can be initiated in the presence of rifampicin. With model B, however, each of the n polymerase molecules bound to X-DNA at the time of

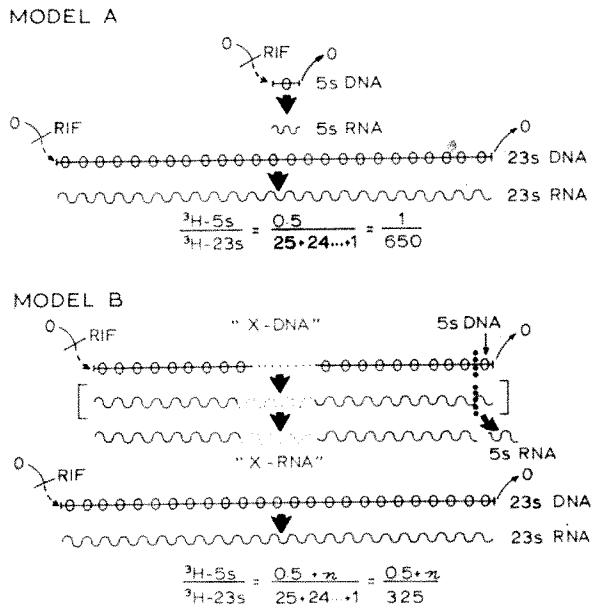


Fig. 1. Two models for the transcriptional unit containing 5S RNA. In model A, 5S RNA is the product of a small gene carrying no additional information. On average, one RNA polymerase molecule (oval) is bound to this gene at any given time, while 25 polymerase molecules are bound to the 23S transcriptional unit. In model B, 5S RNA is near the termination site of a larger transcriptional unit. Transcription is begun only at the initiation site of this DNA unit, producing a large RNA species which is cleaved to "X-RNA" and 5S RNA, possibly even before transcription termination and its release from the DNA. The expected ratios of labelled 5S RNA to labelled 23S RNA synthesized after inhibition of transcription initiation by rifampicin (RIF) are shown for both models. (See text for further explanation.)

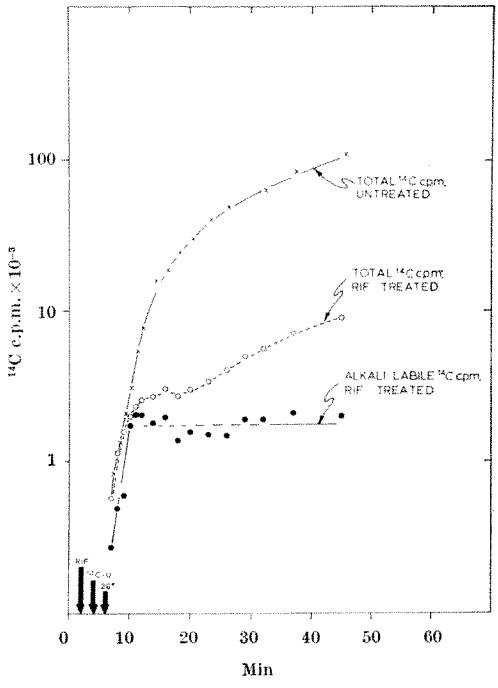


Fig. 2. Incorporation of ^{14}C-uracil into total TCA precipitable and alkali-labile TCA precipitable material. A culture of AB 257 at 26° C in methionine supplemented minimal medium[10] containing 20 μg/ml. uracil was chilled to 0° C and divided into two 20 ml. subcultures at zero min. After two min, rifampicin (Pittman-Moore lot D-1238) was added to one culture to a concentration of 300 μg/ml. and an equivalent volume of acetone (in which stock solutions of rifampicin were made) to the control culture. ^{14}C-Uracil (50 μCi, International Chemical and Nuclear Corp.) was added to each culture at 4 min and at 6 min; the cultures were rapidly warmed to 26° C. Samples (0·5 ml.) were taken during shaking at 26° C and either (*a*) directly precipitated with cold 5 per cent trichloroacetic acid (TCA) and collected on glass fibre filters for the determination of total number of c.p.m. incorporated, or (*b*) mixed with an equal volume of 0·5 M NaOH and incubated at 37° C for 24 h before neutralization and TCA precipitation as above. The lower curve was obtained by subtracting alkali-stable from total TCA precipitable counts.

antibiotic addition will transcribe the 5S DNA segment, producing n additional units of radioactivity in 5S RNA, and if polymerase molecules are distributed evenly across X-DNA and 5S DNA, as expected with only one initiation site, then n equals the ratio of the lengths of X-DNA to that of 5S DNA.

Although the absolute amount of 5S RNA labelled after rifampicin addition could be calculated if the specific activities of this species and of the precursor pool were known, it is simpler and perhaps more precise to consider the ratio of label incorporated into 5S RNA to label incorporated into a second, preferably larger, species of RNA. The second species we have chosen is 23S RNA, which, although not a primary transcription product, is of nearly the same size as the macromolecular precursor(s) from which it is derived[3,4] and which may be the "X-RNA" postulated in model B.

Because 23S RNA is approximately twenty-five times larger than 5S RNA and is normally produced in the same molar quantities in the cell, we expect twenty-five molecules of RNA polymerase to be transcribing 23S DNA at the time of antibiotic addition. This will result in the incorporation of $25+24+23+\ \ldots\ .+1=325$ "units" of label (as defined above) into 23S RNA. For model A, therefore, the ratio of label incorporated into 5S RNA to label incorporated into 23S RNA after rifampicin addition will be 1 : 650, and for model B will be $(n+0{\cdot}5) : 325$. The ratio of labelled 5S to labelled 23S RNA found in steady state or long pulse labelled RNA preparations is 1 : 25. Thus if model A is correct, one would expect a drastic reduction in this ratio in antibiotic treated cultures with essentially no incorporation of label into 5S RNA. In model B, however, the ratio depends on n, and can even exceed the steady state ratio if n is greater than 13. These predictions depend qualitatively neither on the exact spacing of polymerase molecules nor on gene dosage. That is, with model A, 5S : 23S ratios will always be considerably reduced by rifampicin treatment, and with model B they will be increased if n is sufficiently large.

Shut-off of Initiation by Rifampicin

It is first necessary to show that rifampicin, at an appropriate concentration, inhibits initiation of transcription in *E. coli in vivo*, as it is known to do *in vitro*. The strain chosen was AB 257 (*HfrC*, *metB*$^-$, *rel*$^-$), a K12 strain derived from 58–161, and similar if not identical to the strain EA2 used by Adesnik and Levinthal[3].

The kinetics of incorporation of ^{14}C-uracil into acid insoluble material after addition of rifampicin were determined in an experiment illustrated in Fig. 2. Several conclusions can be drawn from this figure: (*a*) rifampicin inhibits ^{14}C-uracil incorporation only after a lag of several minutes, as expected from the *in vitro* demonstration that chain elongation is not affected by this drug, (*b*) the incorporation of ^{14}C-uracil into TCA precipitable material is not completely inhibited by 300 μg/ml. rifampicin, but (*c*) this residual incorporation does not reflect RNA synthesis, for the labelled material is not rendered TCA soluble by alkaline hydrolysis. The lower curve in Fig. 2 represents the difference between alkali stable and total TCA precipitable counts incorporated by the rifampicin treated culture, and indicates that RNA synthesis is completely inhibited. Similar results were obtained using ^{3}H-uridine in place of ^{14}C-uracil.

Residual 23S and 5S RNA Synthesis

The relative accumulation of 5S and 23S RNA during transcription in the presence of rifampicin was determined in the following manner. A culture was prelabelled with ^{32}P-orthophosphate, chased by further growth in non-radioactive medium, chilled to 0° C and divided into two subcultures. Rifampicin was added to one culture, and then ^{3}H-uridine was added to both cultures, which were maintained at 0° C throughout this period to minimize RNA chain elongation during equilibration with the antibiotic and the isotopic label[11]. Incorporation of ^{3}H-uridine into RNA begins immediately on warming to 26° C (see Fig. 2) and incubation was continued at 26° C for 14 min, that is, for some 10 min after residual incorporation of radioactivity into RNA ceases. RNA was purified from both cultures by phenol extraction and individual RNA species were resolved by polyacrylamide gel electrophoresis. The resulting gel profiles are illustrated in Fig. 3, in which the radioactivity in each gel slice is plotted as a percentage of the total radioactivity retained in the gel. It is immediately apparent that the relative amount of label (^{3}H) in 5S RNA, as well as the ratio of labelled 5S RNA to 23S RNA, is greater after rifampicin treatment than with either the chased ^{32}P-prelabel or the 14 min continuous ^{3}H-uridine labelling in the control culture. This eliminates model A of Fig. 1, where ^{3}H radioactivity in 5S RNA should be considerably reduced by antibiotic treatment.

The percentages of total radioactive label in 23S RNA and 5S RNA for both experimental and control cultures are shown in Table 1. (Correction has been made for the loss of that material from 2·8 per cent gels which is retained on 8 per cent gels.) These results are consistent with model B, where the gene for 5S RNA is transcribed as part of a larger unit, and preceded in that unit by a segment of DNA of considerably greater size.

5S RNA is not Synthesized Preferentially

This conclusion rests, of course, on the assumption that the initiation of the synthesis of both RNA species is equally sensitive to rifampicin inhibition, and that 5S

RNA does not continue to accumulate during the labelling period. This assumption is verified by the experiment illustrated in Fig. 4. A culture was chilled and treated with rifampicin as described for the previous experiment, and then divided into subcultures to which 1 mCi of ^{3}H-uridine was added at various times after initiating transcription by warming to 26° C. The absolute amounts of both labelled 23S and labelled 5S RNA decreased very rapidly after the culture was warmed. It is clear that essentially no RNA of either type is synthesized in the period between 11 and 24 min after warming, and that if preferential residual synthesis of 5S RNA occurs, it is not sufficient to account for the increase in 5S : 23S ratios shown in Table 1.

Implications

The data presented can be interpreted in two ways, both of which require that 5S DNA be part of a larger tran-

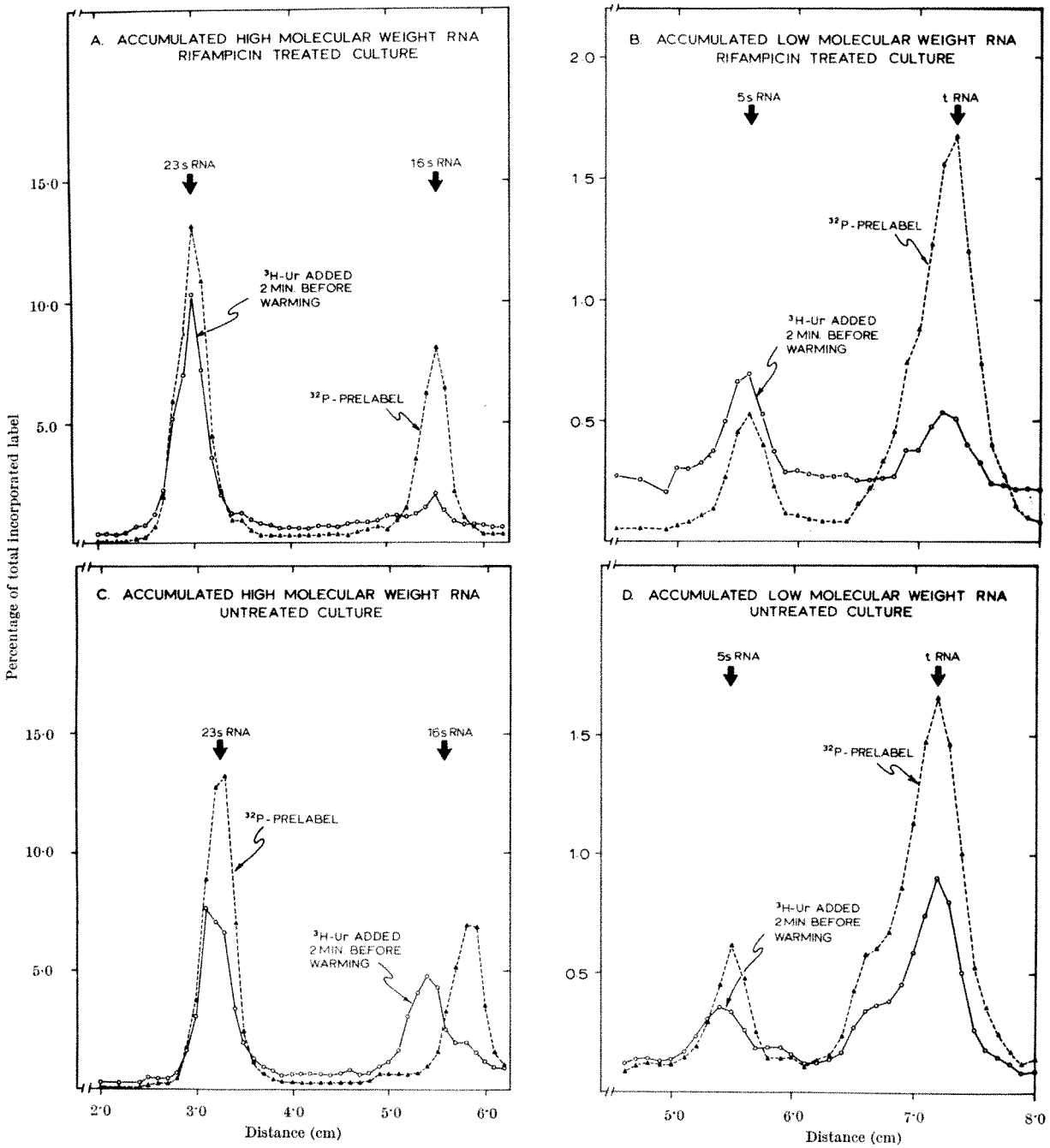


Fig. 3. Residual 23S and 5S RNA synthesis after rifampicin addition. A culture of AB 257 growing at 26° C in methionine supplemented minimal medium containing 0·025 mCi/ml. ^{32}P-orthophosphate (International Chemical and Nuclear Corp.) was washed and resuspended in non-radioactive medium containing 20 μg/ml. uridine. After a 30 min "chase" of the ^{32}P-prelabel the culture was chilled to 0° C and divided into two 4 ml. subcultures. 2 min after chilling, rifampicin was added to one subculture to a final concentration of 300 μg/ml., and an equivalent volume of acetone was added to the control subculture. 4 min after chilling, 1 mCi of ^{3}H-uridine (Schwarz Bioresearch, Inc.) was added to the rifampicin containing subculture, and 0·05 mCi of ^{3}H-uridine was added to the control. At 6 min, both cultures were rapidly warmed to 26° C and shaken vigorously at 26° C for 14 min. Both cultures were then rapidly chilled to 0° C in the presences of 10^{-3} M KCN, washed once in cold 10^{-3} M Tris (*p*H 7·4) and 10^{-3} M $MgCl_2$, and quickly frozen. RNA was purified by phenol extraction and resolved on 2·8 per cent and 8·0 per cent polyacrylamide gels as described previously[4,12]. (Note that during the relatively short, unchased pulse with ^{3}H-uridine, most of the labelled 16S RNA remains in precursor form in the control culture.)

scriptional unit. The first is that 5S RNA is generated from a transcriptional unit carrying considerably more genetic information than is necessary to specify 5S RNA alone and that the 5S DNA region is at some distance from the transcription initiation site of this unit. This does not necessarily imply that a large RNA molecule corresponding to the entire transcriptional unit exists independently in the cell for any appreciable length of time; indeed, 5S RNA could be cleaved from the remainder of this macromolecule ("X-RNA") before transcription of the 5S DNA segment is even completed. Data of the type presented in Fig. 3 and Table 1 make it possible to determine n, the ratio of the length of the postulated DNA segment preceding 5S DNA in the transcriptional unit to the length of 5S DNA. From Table 1, this value would appear to be about thirty. The high backgrounds of label observed across our 8·0 per cent gels, however, make precise measurement of the amount of 5S RNA difficult, and in two other experiments similar to that illustrated in Fig. 3, n is approximately twenty. In no case, however, is the ratio of labelled 5S RNA to labelled 23S RNA in rifampicin treated cultures as low as that observed in steady state conditions. According to this interpretation, 5S DNA is preceded by a segment of DNA at least thirteen times, and possibly thirty times, its own length in the transcriptional unit of which it is part. The other RNA product(s) of this unit cannot be identified by this approach.

The alternative interpretation of our results is that 5S RNA is derived from a transcriptional unit consisting of fifteen to twenty repeating genes for 5S RNA, there being only one such transcriptional unit per genome. Cleavage into individual 5S RNA molecules could occur either during or immediately after completion of transcription of this large unit.

This approach using rifampicin provides a method for measuring the size of transcriptional units in a manner complementary to the approach using actinomycin D developed by Bleyman *et al.*[6]. We are currently using rifampicin to examine the relationship, if any, between the genes for 16S and 23S RNA. It is apparent in Fig. 3*A*, and has been found in other experiments, that the incorporation of label into 16S RNA is much more inhibited by rifampicin than is incorporation of label into 23S RNA, but the high background radioactivity observed across these gels does not yet permit precise measurement. Further, it is possible to determine the size of the transcriptional units from which the transfer RNA species are derived. If each tRNA gene were itself a single transcriptional unit, then more label than expected is incorporated into tRNA after antibiotic treatment (Fig. 3*B*).

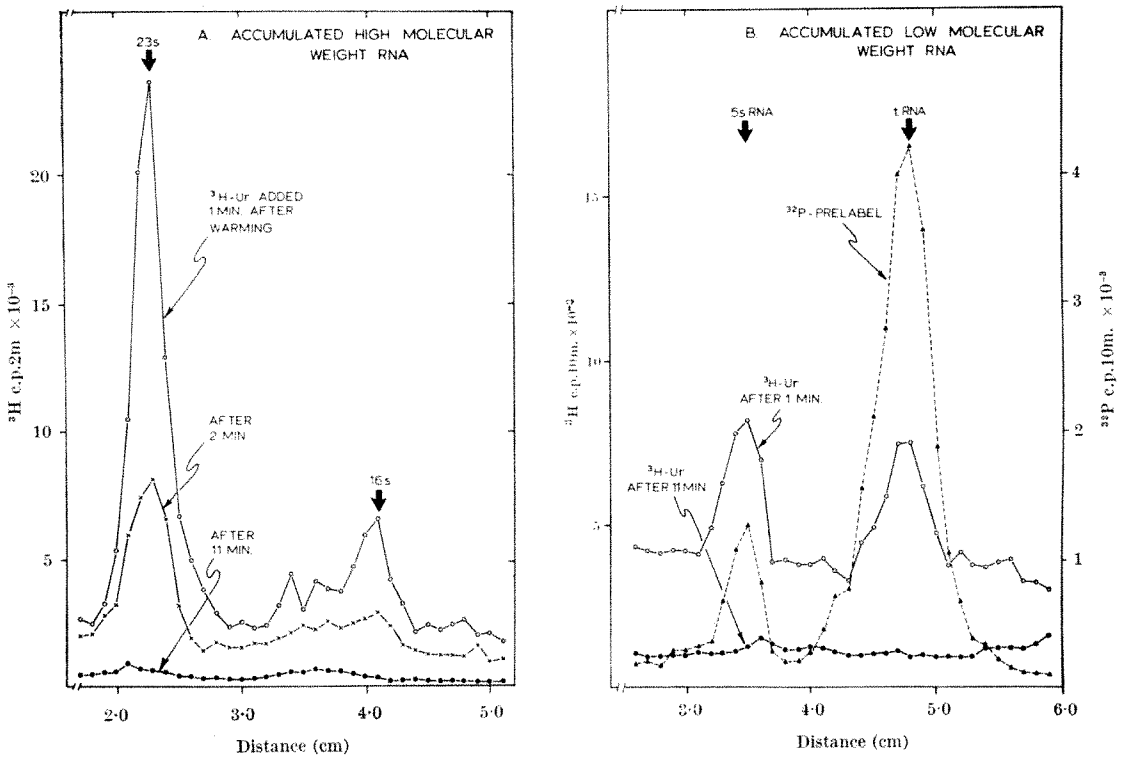


Fig. 4. RNA synthesized at various times after addition of rifampicin. A culture of AB 257 (at 26° C) was prelabelled with ^{32}P-orthophosphate, chased, and chilled as described for Fig. 3. 2 min after chilling, rifampicin was added to a final concentration of 200 μg/ml. and the culture was divided into eight subcultures. At 6 min, the subcultures were warmed to 26° C and vigorous shaking was begun. 1 mCi of ^{3}H-uridine was added to each subculture at times before and after warming. 24 min after warming, RNA was extracted from all cultures and fractionated on 2·8 per cent and 8·0 per cent gels. *A*, Representative 2·8 per cent gel profiles for RNA extracted from subcultures to which ^{3}H uridine was added at 1, 2 and 11 min after warming. *B*, Representative 8·0 gel profiles for RNA from subcultures labelled at 1 and 11 min. The profile of ^{32}P-prelabelled material is shown to indicate the positions of 4S and 5S RNA within the gels.

Table 1. RELATIVE ACCUMULATION OF 5S AND 23S RNA IN RIFAMPICIN TREATED AND CONTROL CULTURES

	^{3}H-labelled RNA (percentage of total)			^{32}P-prelabelled RNA (percentage of total)		
	5S	23S	5S/23S	5S	23S	5S/23S
Rifampicin-treated	2·27	22·4	0·101	1·52	37·8	0·040
Control culture	0·99	22·1	0·045	1·71	38·0	0·045

Fractions of total ^{32}P and ^{3}H radioactivity in the 5S and 23S RNA regions of the gel profiles (Fig. 3) were aggregated. In the case of ^{3}H radioactivity, a background, corresponding to radioactivity in gel slices adjacent to the 5S or 23S region was subtracted from each of the summed points.

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Effects of Gibberellin and Cytokinins on the Activity of Photosynthetic Enzymes and Plastid Ribosomal RNA Synthesis in *Phaseolus vulgaris* L.

by
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Applications of hormones to fully expanded leaves of dwarf bean stimulate the activity of carboxydismutase. The increase in enzyme activity is not paralleled by synthesis of plastid ribosomal RNA nor is there a demonstrable enhancement of synthesis of fraction I protein. These results are interesting in relation to the activities of other photosynthetic enzymes.

We have reported[1,2] immediate increases in photosynthetic rates of maize, bean and clover leaves of dwarf mutants after applications of gibberellin A_3 (GA_3) and kinetin. Moreover, the increases in light-saturated photosynthesis, measured by infrared gas analysis or $^{14}CO_2$ methods, were closely paralleled by enhanced specific activity of the photosynthetic enzymes ribulose-1,5-diphosphate carboxylase (RuDPC) and phosphoenolpyruvate carboxylase in clover and maize, respectively. Feierabend[3] has reported stimulated rates of synthesis of photosynthetic enzymes after application of kinetin during the greening of etiolated leaves exposed to light. The question thus arises as to whether hormones affect the synthesis or activity of photosynthetic enzymes.

The bulk of fraction 1 protein synthesis and RuDPC synthesis occurs during the expansion phase of the leaf and thereafter only a low turnover can be detected[4]. Moreover, synthesis of plastid ribosomal RNA soon declines after the early stages of leaf development, although synthesis of cytoplasmic RNA continues[5]. We therefore decided to carry out parallel investigations of plastid rRNA synthesis, fraction I protein synthesis and RuDPC activity with leaves of varying age, with effects of GA_3 and kinetin superimposed on this study.

The simultaneous study of plastid and cytoplasmic rRNA in the same extract is made possible by the high resolution obtained with the polyacrylamide gel technique of Loening[6]. Incorporation of the precursors ^{3}H-cytidine and $H_3{}^{32}PO_4$ was achieved by feeding through either the excised stems of seedlings or the cut petioles of leaves, for periods of 4–6 h. Immersed portions of plant material were discarded in order to minimize effects of bacterial contamination. Total nucleic acid was extracted using the phenol method[7] and fractionated on 2·4 per cent acrylamide gels at 5 mA/tube for 2–3 h in Tris-acetate buffer, *p*H 7·2. The gels were scanned at 265 nm and frozen and sliced into 1 mm sections which were hydrolysed with 1 ml. of 10 per cent piperidine at 80° C. Radioactivity of the gel sections was determined by liquid scintillation counting in toluene/'Triton X-100' (2/1 v/v) containing 0·27 per cent PPO.

The separations of the RNA components from leaves at three stages of development are shown in Fig. 1. In the very young leaves there was marked incorporation of precursors into both Pr-RNA and cytoplasmic rRNA; at this early stage the amount of plastid rRNA was small

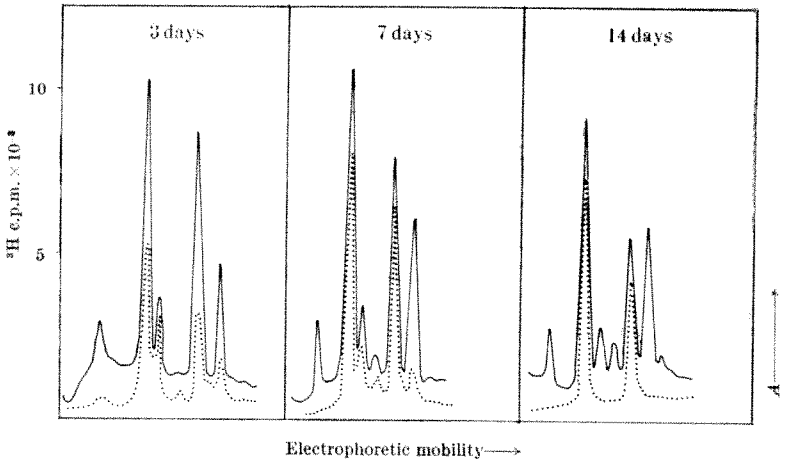


Fig. 1. Gel fractionation of nucleic acid prepared from leaves of *Phaseolus* at three stages of development. Electrophoresis was carried out for 2·5–3 h in Tris-acetate buffer, *p*H 7·2. ———, Absorbance; · · · · , ^{3}H-cytidine incorporated.

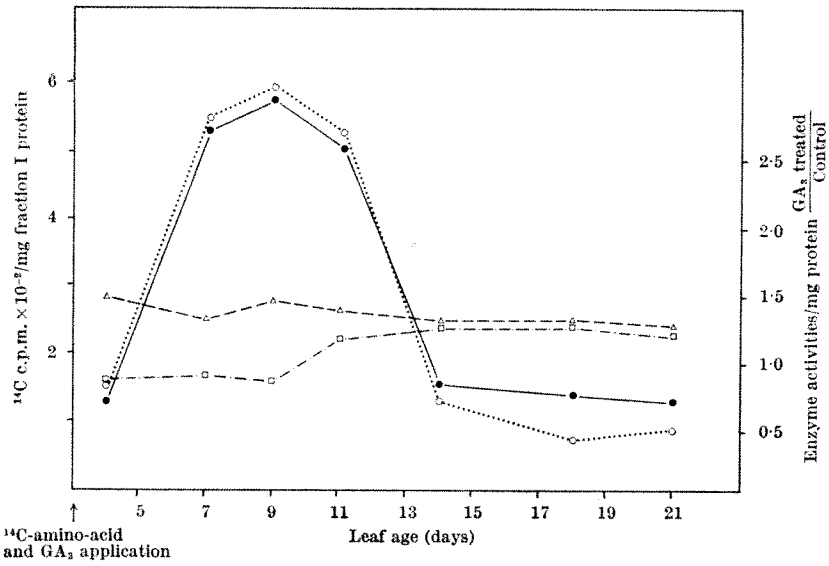


Fig. 2. Effects of GA_3 on incorporation of ^{14}C-amino-acid into fraction I protein (●—●), and activities of RuDP carboxylase (□ — · —□) and fructose-1,6-diphosphatase (FDP) (△ - - - △) in *Phaseolus vulgaris*. ○ - - - ○, Control.

in relation to the cytoplasmic fraction. The ratios of radioactivity incorporated and of plastid rRNA to cytoplasmic RNA content for the different stage of growth are given in Table 2. The plastid rRNA content was greatly increased in the 7–8 day old leaves relative to the cytoplasmic RNA component, but the absolute rate of incorporation of precursors into the plastid rRNA fraction was significantly lower at this stage. Both precursors gave identical results in these experiments and the data clearly indicate that the plastid rRNA is synthesized at early stages of leaf development, demonstrable with these labelled precursors. When 14 day old leaves were exposed to either ^{32}P or labelled base, however, there was no detectable incorporation into plastid rRNA, whereas massive amounts of label were located in the cytoplasmic rRNA fractions. This suggested that there is very little or no synthesis of plastid rRNA in fully expanded leaves of *Phaseolus*. Nevertheless, it was possible that failure to demonstrate incorporation of these precursors into plastid rRNA in expanded leaves could be due either to cytoplasmic dilution or to a change in the permeability characteristics of the chloroplast membrane, resulting in failure of the precursors to reach the site of synthesis. Consequently, both young and mature leaves were exposed to $^{14}CO_2$ and the labelling pattern of the ribosomal RNA bands examined. A marked incorporation into plastid rRNA was obtained with young developing leaves, but the label in this rRNA was negligible with expanded leaves, cytoplasmic rRNA being labelled at both stages of leaf growth. We conclude, therefore, that the bulk of plastid rRNA is synthesized in bean chiefly during the early development of the leaf. This is supported by observation on radish leaves and agrees with recent findings[4,5]. No significant effects of either GA_3 or kinetin on the specific radioactivity of the plastid rRNA components could be detected at any growth stage examined (Table 1). RuDPC activity, however, was enhanced by the addition of both GA_3 and kinetin to fully expanded 20 day old leaves, which therefore suggests that the mechanism of enzyme stimulation by the application of hormones does not act through the synthesis of plastid rRNA and presumably does not involve *de novo* plastid protein synthesis (unless it is occurring on existing ribosomes).

Table 1. EFFECTS OF HORMONE APPLICATIONS ON PHOTOSYNTHETIC ACTIVITY AND *in vitro* PROTEIN SYNTHESIS IN FULLY EXPANDED LEAVES OF *Phaseolus vulgaris* L.

Treatment of leaves (p.p.m. hormone)	Leaf PR (c.p.m./ cm^2/min × 10^{-3})	RuDPC activity /mg fresh weight ×10^{-4}	RuDPC activity /mg protein ×10^{-6}	^{14}C-leucine incorporated /mg fresh weight	^{14}C-leucine incorporated /mg protein
Control	4·17	1·37	1·71	1,875	546
GA_3 (100)	5·25	1·66	2·17	1,730	434
K (1)	5·13	1·60	2·13	1,810	458
BAD (30)	5·50	1·85	2·32	1,551	423

PR, photosynthetic rate.

This proposal was tested in experiments where incorporation of labelled amino-acids into fraction 1 protein was determined in the presence and absence of hormone supplements. In the initial investigation, cotyledonary leaves of bean were given a single treatment with a synthetic ^{14}C-amino-acid mixture (Amersham CFB-104) in a dose of $2{\cdot}2 \times 10^5$ c.p.m. 3 days after the appearance of leaves. The plants were maintained at 15° C in natural daylight with a 12 h photoperiod, one batch acting as unsupplemented controls and the other treated with a solution of 100 p.p.m. ($2{\cdot}9 \times 10^{-4}$ M) of GA_3. Matched pairs of treated and control plants were sampled at intervals for 18 days and extracted in Tris (0·04 M), *p*H 7·8, containing 0·01 M $MgCl_2$, 0·25 mM EDTA, and 4 mM GSH. After centrifugation of extracts at 20,000*g*, determinations of enzyme activities, soluble protein content (Lowry method) and radioactivity were made on the supernates. Samples of 100 μl. were subjected to electrophoresis on polyacrylamide gel slabs (6·5 per cent) in Tris-citrate buffer, *p*H 9·0, containing 5 mM GSH. The gels were run at 2° C in 0·05 M borate buffer, *p*H 9·0, for 3 h with a current of 15 mA per gel. A reference gel was stained with amido black and the corresponding fraction 1 zones were excised from the simultaneously run replicates. These gel sections were used (*a*) for determination of RuDPC activity, (*b*) for protein determination and (*c*) to estimate the amount of ^{14}C incorporated into fraction 1 protein from the precursor amino-acid supplied. Changes in the specific radioactivity of the fraction 1 protein for hormone treated and control plants are presented in Fig. 2. No significant effect of GA_3 on the specific radioactivity was observed during the first 10 days after treatment. Thereafter, GA_3 retarded the loss of radioactivity from the fraction 1 zone, which is consistent with the findings of Goldthwaite and Bogorad[8] who demonstrated that GA_3 retarded the loss of radioactivity from RuDPC in leaf disks of *Rumex crispus*. But this is not applicable to the young leaf, and it is notable that the short time courses for stimulatory effects of both GA_3 and cytokinins, in intact leaves, are not consistent with the much longer periods required for the manifestation of effects attributable to retarded senescence. Moreover, the pattern of active protein synthesis observed parallels the pattern of synthesis of plastid rRNA obtained in the previous experiments. The effects of application of GA_3 on RuDPC and fructose-diphosphatase (FDP) activities are also shown in Fig. 1, the data being presented as ratios of control to treated values. As was expected from our previous studies on hormone concentration effects, addition of GA_3 to the very young leaves, where endogenous concentrations of hormone are presumably high, resulted in depressive effects of RuDPC activity per unit protein. After the tenth day after treatment, however, all assays revealed stimulations in the order of 20–30 per cent after application of GA_3. It is interesting that the activities of FDP (Fig. 1) and phosphoribulokinase (data not shown) per unit protein were higher in the GA_3 treatment at all growth stages.

A further experiment was conducted to determine

Table 2. EFFECTS OF GA_3 AND KINETIN ON INCORPORATION OF ^{3}H-CYTIDINE INTO PLASTID rRNA AT THREE STAGES OF LEAF DEVELOPMENT IN *Phaseolus*

Leaf age (days after appearance)	Plastid rRNA / Cytoplasmic rRNA	c.p.m. incorporated into plastid rRNA / c.p.m. incorporated into cytopl. rRNA		
		Control	GA_3	Kinetin
3	0·25	0·73	0·70	0·75
7	0·41	0·15	0·16	0·14
14	0·58	Negligible rRNA	incorporation	into plastid

Table 3. EFFECTS OF GA_3 AND KINETIN ON FRACTION I PROTEIN SYNTHESIS AND RuDPC ACTIVITY AT FOUR GROWTH STAGES OF COTYLEDONARY LEAVES OF *Phaseolus vulgaris*

Leaf age days	Treatment of leaves	Homogenate ^{14}C (d.p.m./mg protein $\times 10^{-3}$)	Fr. I ^{14}C (d.p.m./mg protein $\times 10^{-3}$)	Per cent incorp. fr. I protein	RuDPC activity (c.p.m./mg protein $\times 10^{-3}$)	RuDPC activity (c.p.m./mg fr. I protein $\times 10^{-3}$)	Endogenous GA_3 (μg/g fresh weight)
3	C	218	20·5	63	4·9	7·3	0·28
	GA	155	12·2	56	2·8	4·0	
	K	310	24·3	64	4·9	6·6	
7	C	394	6·0	10	4·4	6·6	0·08
	GA	304	5·5	9	3·6	7·4	
	K	403	5·0	7	7·9	14·4	
12	C	28	0·15	3·3	4·0	5·9	0·05
	GA	37	0·18	3·7	4·5	6·0	
	K	39	0·21	5·6	6·3	6·3	
18	C	846	0·16	0·003	3·5	5·2	0·01
	GA	435	0·14	0·005	3·8	5·5	
	K	160	0·10	0·005	4·7	5·8	
Trifoliate 16–18 day old leaves	C	227	18·5	1·3	6·0	9·0	—
	GA	216	16·0	1·2	6·9	10·8	
	K	256	15·4	1·0	7·6	11·2	

rates of synthesis of fraction 1 protein in leaves at four stages of development, coupled with effects of GA_3 and kinetin. The hormones were applied to attached leaves followed by labelled amino-acids. Leaf extracts, enzyme activities and fraction 1 protein separations were carried out as in the previous experiment. Table 2 shows that there was active synthesis in the young developing leaves, some 60–65 per cent of the labelled amino-acid in the leaf homogenate being located in fraction 1 protein. The rate of synthesis became progressively lower with increasing leaf age, some 9–10 per cent of label being located in the fraction 1 component in the 7–8 day old leaves, while only 2 per cent of incorporated label was found in protein of 12 day old leaves. The gibberellin treatment depressed the incorporation of amino-acid into fraction 1 protein in the young leaves, confirming our previous result, but had no significant effect in the later stages. A similar pattern was observed with the activity of RuDPC, which was depressed by GA_3 in the young leaves, but enhanced in the two later growth stages. The endogenous levels of free gibberellin in the leaves at the four stages studied are shown in Table 3. The specific radioactivity of the fraction 1 protein of leaves treated with kinetin was about 20 per cent higher than the control values in the phase of very active synthesis, but when the figures were corrected for differences of uptake of amino-acid into leaves, no significant difference between control and hormone treatment was evident. Although there was no effect of application of kinetin on RuDPC activity in the very young leaves, there was a marked stimulation in activity at the later stages, with no corresponding detectable increase in incorporation of labelled amino-acid into fraction 1 protein. In view of the very low incorporation of radioactivity in the 18 day old leaves, the experiment was repeated using 18 day old trifoliate leaves with a larger dose of amino-acid. Hormone treatments enhanced the activity of RuDP carboxylase but again no detectable increase in rate of fraction 1 protein synthesis was observed.

These results are somewhat difficult to interpret. On the one hand, applications of GA_3 and cytokinin to mature bean leaves result in significantly higher RuDPC activity within 24 h. In view of the well known effects of both hormones on the synthesis of proteins, it might have been expected that their effects on the level of RuDPC activity could be ascribed to stimulation of enzyme synthesis. There seems, however, to be little or no synthesis of plastid rRNA or of fraction 1 protein in leaves of bean once they have fully expanded. Thus it would seem that the stimulated activity of RuDPC after the application of the hormones can be ascribed to activation of the enzyme rather than to synthesis of new enzyme, although how such activation could occur remains obscure. Alternatively, it might be that the increase in RuDPC activity is due to its release from an inactive form as in the well known conversion of trypsinogen to trypsin.

It should, however, be pointed out that the measurements of incorporation of labelled precursors into plastid rRNA and fraction 1 protein can be obscured by pool dilution or permeability effects. It is also interesting that the observation of little or no synthesis of plastid rRNA in mature leaves does not preclude the possibility that there may be synthesis of plastid messenger or transfer RNA, for acrylamide gel fractionation of leaf RNA does not separate the plastid and cytoplasmic components of these species. Furthermore, it is clear from our other work that the fraction 1 protein, which contains the RuDPC activity and is separated as one major band by gel electrophoresis, has various other enzyme activities, for example, malate dehydrogenase and peroxidase, associated with it. It may well be that *de novo* synthesis of RuDPC in response to hormone treatments is so small in relation to the total amount of fraction 1 protein that further synthesis of the enzyme might not be detected. By the same token, however, by expressing the activities of RuDPC in terms of unit fraction 1 protein we may well be underestimating the extent of stimulation produced by the hormone applications. Finally, it is possible that the active form of RuDPC is synthesized in the cytoplasm or in a soluble plastid fraction and then becomes associated with fraction 1 protein of the plastid. The possibility of hormone stimulated enzyme synthesis in the cytoplasm is certainly not excluded by the available experimental data, and support for the contention of synthesis of a minor soluble component has been provided by Wildner and Criddle[9]. The observed increases in specific activities of fructose diphosphatase and phosphoribulokinase, which are not associated with fraction 1 protein in our experiments, make it necessary to defer conclusions on the implication of protein synthesis in the effect of GA_3 until labelling of RuDPC can be separated from that of the other components of fraction 1 protein.

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Selective Synthesis of a Nuclear Acidic Protein in Liver Cells stimulated by Cortisol

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Differential labelling experiments establish that synthesis of a non-histone protein of molecular weight 41,000 is specifically enhanced in response to the injection of cortisol into rats.

THIS report is concerned with the metabolism of a particular class of acidic proteins associated with DNA in the cell nucleus. It is now well established that chromosomal proteins influence the synthesis of RNA. Histones, for example, have been shown to inhibit DNA-directed RNA synthesis[1–5], although their uniformity in composition[6] and their regular distribution in diverse cell types suggest that they are unlikely to be primarily responsible for all the features of the control of transcription in the chromosomes of differentiated tissues. Instead, much of the specificity seems to reside in more acidic proteins of the nucleus, which vary in amount and composition in different cell types and which stimulate[7–10] and modify[11] the synthesis of RNA in isolated chromatin fractions.

If selectivity in transcription depends on non-histone proteins associated with DNA, one would expect changes in the distribution or metabolism of such proteins to occur at times of gene activation and repression. High rates of synthesis and "turnover" are characteristic of many of the non-histone proteins of chromatin[11–13]. The content of acidic chromosomal proteins is high in metabolically active tissues[14,15], and it correlates with the RNA synthetic capacities of chromatin fractions from different cell types[15] and from different regions of the nucleus[16]. Nuclear protein phosphorylation is stimulated by mitogenic agents[17], and synthesis is influenced by hormone administration[18].

We now present evidence for the specificity of the response of DNA-associated proteins during gene activation induced by steroid hormones. The enhancement of RNA synthesis in the liver which follows the administration of cortisol to adrenalectomized rats[19–21] is reflected in a specific stimulation of synthesis of a particular acidic protein of the hepatocyte nucleus. Because no corresponding response has been observed in the histones of the nucleus or in the ribosomal proteins of cortisol-treated animals[22], these findings support the view that the acidic protein fraction of the chromatin includes components which may be specifically concerned in the regulation of chromosomal activity.

The preparation of non-histone proteins is commonly based on differences in extractability, solubility and electrical charge between the acidic proteins, the histones and other components of the nucleus. For example, in early procedures, the acidic proteins were solubilized in dilute alkali after previous extraction of the histones in dilute mineral acids (see, for example, ref. 23). Alternatively, strong salt solutions have been used to dissociate nucleoprotein complexes as a first step in their fractionation[27]. The resulting mixture of histones, non-histone proteins, DNA and RNA can be resolved by a variety of techniques, including density-gradient centrifugation, selective precipitation and chromatographic or electrophoretic separations[8,24–29]. We have used a different method of extraction based on the solubilization of certain acidic chromosomal proteins in phenol (Fig. 1). In this procedure, the acidic proteins are extracted from the residue which remains after removal of the histones (and some other protein fractions) from isolated rat liver chromatin. The method is a modification of the procedure developed by Vinuela, Algranati and Ochoa[30] for the solubilization and electrophoretic analysis of proteins associated with the nucleic acids of bacterial viruses.

The acidic proteins were prepared from rat liver nuclei isolated in 0·025 M citric acid (a method which has also been applied to nuclei isolated in 2·2 M sucrose solutions). The nuclei were washed with buffered saline (0·15 M NaCl–0·01 M Tris-HCl; *p*H 7·5) to remove soluble proteins, and dispersed by homogenization in distilled water. Adjustment of the salt concentration to 0·15 M NaCl precipitated the chromatin, which was again dispersed in water and precipitated as before. The chromatin pellet was extracted with 0·20 M HCl at 0° C for 1 h to remove the histones, and the residue was resuspended at *p*H 8·4 and treated with phenol as shown in Fig. 1. Subsequent treatments bring the phenol-soluble proteins back into aqueous solution to permit their electrophoretic analysis.

Acid-washed chromatin

↓ disperse in 0·10 M Tris-HCl (*p*H 8·4—0·01 M EDTA–0·14 M 2-mercaptoethanol (TEM)

↓ extract 3× with phenol buffered with TEM

↓ dialyse combined phenol fractions against 0·10 M acetic acid—0·14 M 2-mercaptoethanol

↓ dialyse against 0·05 M acetic acid—9·0 M urea—0·14 M 2-mercaptoethanol

↓ dialyse against 0·10 M Tris-HCl (*p*H 8·4)—8·6 M urea—0·01 M EDTA—0·14 M 2-mercaptoethanol under N_2

↓ dialyse against 0·01 M sodium phosphate (*p*H 7·2)—0·1 per cent SDS—0·14 M 2-mercaptoethanol

↓ dialyse against 0·01 M sodium phosphate (*p*H 7·2)—1·0 per cent SDS—0·14 M 2-mercaptoethanol

Soluble acidic chromosomal SDS-protein

Fig. 1. Procedure for the preparation of nuclear acidic proteins from isolated rat liver chromatin.

Components of the acidic protein fraction differ in their electrophoretic mobility and can be separated and characterized as individual bands on polyacrylamide gels. We have separated complexes of the proteins with sodium dodecyl sulphate (SDS)[31]. In these conditions, the dis-

tance of migration of individual proteins can be correlated with their molecular weights[32,33], as indicated in the upper curve of Fig. 2.

The phenol-soluble proteins of rat liver chromatin separate into eleven to sixteen principal bands on electrophoresis in 10 per cent acrylamide gels at *p*H 7·2 (Fig. 2). (By modified techniques, resolution has recently been improved to yield about thirty distinct bands arranged in a pattern which is highly reproducible and characteristic of the tissue of origin: unpublished experiments of C. S. Teng and V. G. A.) Estimates of the molecular weights of the proteins in different regions of the gel were based on comparison with the migration distances of standards of known molecular weight. Light (23,500) and heavy (51,000) chains of human γ-globulin (donated by Dr Myron Waxdal) were labelled with iodine-125 (ref. 34) and reduced and alkylated as described by Shapiro and Maizel[35]. The iodinated proteins were added to the nuclear acidic protein fraction and co-electrophoresed as described in Fig. 2. The position of ^{125}I in the gel marked the location of the standards, and permitted the calculation of the relationship between mobility and distance shown in the upper curve of Fig. 2.

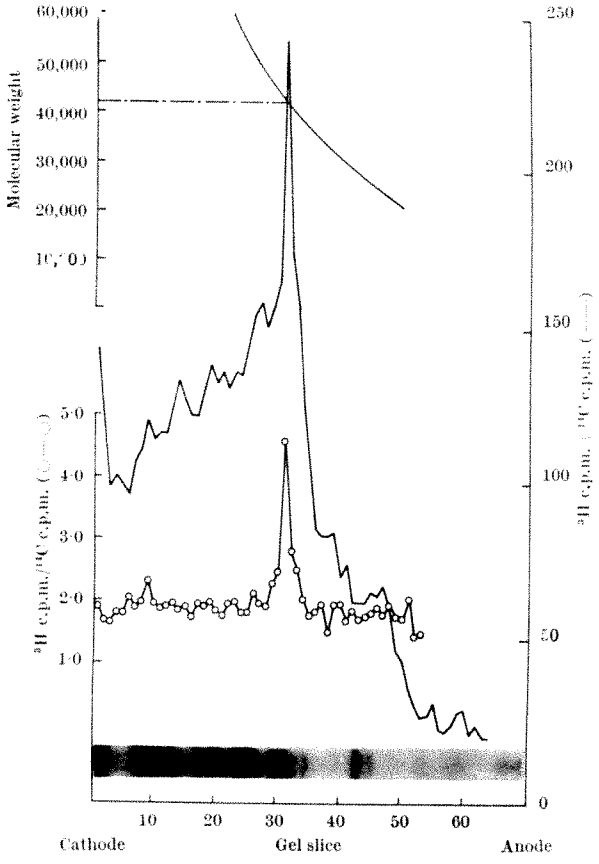


Fig. 2. Electrophoretic separation and analysis of labelling patterns for nuclear acidic proteins from livers of cortisol-treated and control rats. Matched pairs of adrenalectomized rats (180–220 g) were fasted for 20 h before administration of hormone. Hydrocortisone sodium succinate (solution Cortef, Upjohn; 20 mg) was injected intraperitoneally into two rats; a control pair received isotonic saline at the same time. At selected times, the hormone-treated animals were pulse-labelled for 1 h periods by the injection of 600 μCi of DL-4,5-^{3}H-leucine (specific activity 5 Ci/mmole). The control animals received 83 μCi of uniformly labelled L-^{14}C-leucine (specific activity: 0·28 mCi/mmole) in the same conditions. At the end of the 1 h labelling periods, the livers were removed from all animals for isolation of the chromatin and preparation of the phenol-soluble proteins. The livers from both hormone-treated and control animals were pooled, and all were homogenized together in 0·025 M citric acid. The nuclei were isolated and chromatin was prepared as described. This procedure ensured that the ^{3}H-labelled acidic proteins from the cortisol-treated animals and the ^{14}C-labelled proteins from the control animals were exposed to the same conditions throughout the course of their isolation as in Fig. 1. The mixed proteins (250 μg) from control and hormone-stimulated animals (7–8 h after cortisol injection) were co-electrophoresed on 10 per cent polyacrylamide gels with a running buffer of 0·1 M sodium phosphate (*p*H 7·2) containing 0·1 per cent SDS. The protein bands were fixed in 20 per cent sulphosalicylic acid and located by staining with Coumassie brilliant blue. Radioactivity in individual bands was determined by transverse slicing of the gel and scintillation spectrometry. Lower curve: ratio of $^3H/^{14}C$ incorporation into the acidic proteins at different positions in the gel. Middle curve: total radioactivity at different regions of the gel. Upper curve: relationship between mobility and molecular weight.

Table 1. AMINO-ACID COMPOSITION OF ACIDIC PROTEINS FROM RAT LIVER CHROMATIN

Amino-acid	Moles per 100 moles total amino-acid
Lysine	6·62
Histidine	2·25
Arginine	5·60
Aspartic acid	10·22
Threonine	4·79
Serine	5·77
Glutamic acid	13·92
Proline	5·59
Glycine	8·60
Alanine	8·08
Valine	6·35
Methionine	1·52
Isoleucine	3·98
Leucine	9·47
Tyrosine	3·08
Phenylalanine	4·10
Acidic/basic residues	1·67

Results are average values for two preparations of the phenol-soluble proteins of rat liver chromatin, not corrected for hydrolytic losses.

Amino-acid analyses of the phenol-soluble proteins of rat liver chromatin clearly indicate their acidic nature: the content of aspartic and glutamic acids greatly exceeds that of the basic amino-acids (Table 1). Chromosomal proteins prepared by the phenol procedure are also characterized by high rates of synthesis. For example, 1 h after the administration of ^{14}C-leucine, the specific activity of the nuclear phenol-soluble proteins was 9,550 c.p.m./mg, as compared with 5,450 c.p.m./mg for the cytoplasmic protein fraction and 2,640 c.p.m./mg for the acid-soluble proteins of the nucleus. Similar observations on the rapidity of ^{14}C-lysine incorporation into a phenol-soluble nuclear protein fraction were made by Steele and Busch[36].

The effects of cortisol were studied by observations on the incorporation of ^{14}C-leucine and ^{3}H-leucine into individual acidic proteins of rat liver chromatin as described in the legend to Fig. 2. The mixed acidic proteins were separated as SDS–protein complexes by polyacrylamide gel electrophoresis. The resulting pattern (Fig. 2) is a series of protein bands, each of which contains a mixture of the ^{3}H-protein from hormone-stimulated nuclei and the corresponding ^{14}C-protein from control nuclei. The total $^3H + ^{14}C$ activity of the bands is plotted as a function of the distance of migration in Fig. 2 (middle curve). The specific activities of individual proteins have not been determined in this experiment, but clearly not all bands are labelled to the same extent. A more significant analysis of the data is obtained by plotting the ratio of ^{3}H-activity to ^{14}C-activity at different positions in the gel. This ratio serves as an indication of the relative rates of synthesis of individual nuclear proteins derived from the chromatin of hormone-treated and control animals. It is plotted as a function of protein mobility in the lower curve of Fig. 2 and is remarkably uniform for all fractions in the gel except for the protein in a particular band located at gel slice 31. The rate of synthesis of this particular type of protein is specifically stimulated by cortisol administration. The extent of stimulation varies with time after administration of the hormone. It becomes evident within 2–3 h, increases at 4–5 h, and exceeds 200 per cent at 7–8 h after administration of cortisol, which is the time shown in Fig. 2.

When the administration of the isotopes was reversed—the cortisol-treated animals received ^{14}C-leucine and the control rats ^{3}H-leucine—there was a corresponding decrease in the $^3H/^{14}C$ ratio of the protein at that region of the gel which shows the specific response. The effect is not, therefore, an artefact arising from uneven quenching

or other counting anomalies which might occur at specific regions of the gel. Other control experiments have established that the proteins are nuclear in origin and are not a result of cytoplasmic contamination. For example, nuclei were isolated from non-radioactive livers in the presence of the radioactive cytoplasm from control and hormone-treated animals. The phenol-soluble proteins prepared from these nuclei had only slight ^{3}H or ^{14}C activity, and that was randomly distributed throughout the gel.

The mobility of a band in electrophoresis is a function of molecular size. According to the upper curve in Fig. 2, the acidic protein which specifically responds to cortisol has a molecular weight of 41,000. This is the first instance of such specificity in the response of nuclear acidic proteins to gene activation by steroid hormones. The function of this protein in the liver nucleus is not known.

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Preliminary Experiments on the Chemistry and Pharmacology of Cannabis

by

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There are at least six pharmacologically effective components of cannabis. Their effects on mice include a lowering of body temperature, catalepsy, analgesia and an extension of barbiturate sleeping time, with doses from 25 to 200 mg/kg.

In this investigation we have used tincture of cannabis BPC. This is a commercial product prepared by extracting the leaves and flowers of *Cannabis sativa* with cold alcohol, evaporating the solvent and dissolving the resinous residue in fresh ethanol. The plant material is grown in Pakistan and imported into Britain under licence. The crude extract, a complex mixture[1], was fractionated according to the procedures developed by Korte and Sieper[2]. Solvent was removed *in vacuo* and the resin was extracted with petroleum spirit. This fraction, soluble in petrol, was freed from chlorophylls and carotenes by chromatography on alumina and the fraction containing phenol was purified further by counter-current distribution. Three principal fractions were obtained: (1) Δ_1-tetrahydrocannabinol (Δ_1-THC) (Fig. 1*a*; R = n-C_5H_{11}), frequently identified as the major physiologically active component, constituting 2·4 per cent of the tincture base. (2) A second single component, very similar to Δ_1-THC, which was subsequently identified as the hitherto undescribed n-propyl analogue of Δ_1-THC (Fig. 1*a*, R = n-C_3H_7). The structure of this compound was deduced from its infrared, ultraviolet, NMR and mass spectrum and has been confirmed by synthesis. The compound represented 2·0 per cent of the crude tincture base and is therefore a quantitatively significant component of the crude extract. (3) The third fraction, least mobile in the counter-current system used, was a complex mixture, characterized by strong absorption at 1,600–1,800 cm^{-1} (suggesting that it

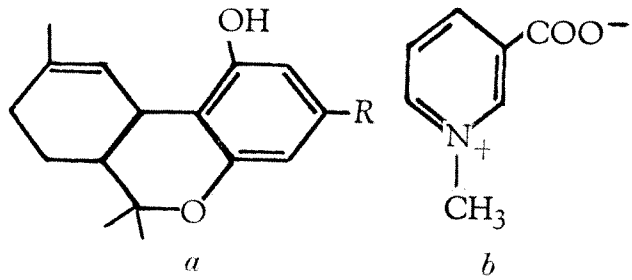


Fig. 1. Structures of (*a*) Δ_1-tetrahydrocannabinol ($R = n$-C_5H_{11}) or its n-propyl analogue ($R = n$-C_3H_7), and (*b*) trigonelline.

contained partially oxidized components), and has not been further fractionated.

The residue that was insoluble in petrol was extracted by shaking with water, and the aqueous extract was analysed by high-voltage paper electrophoresis. A strip of the paper was subjected to colour reactions, and the remainder divided into six parts and eluted.

An Atropinic Substance

We readily detected a spot with the mobility and colour reactions of trigonelline (Fig. 1*b*), a previously identified constituent of cannabis. Trigonelline was found to have little pharmacological activity, being about 10^5 times less active as an agonist than acetylcholine, and with no detectable antagonist activity. But in the eluate from this region of the strip, an acetylcholine-antagonizing material was found, with a slow course of action comparable with that of atropine, ineffective against histamine and such that the material from 1 ml. of tincture produced a fourteen-fold dose-ratio in a 5 ml. organ bath. This is equivalent to about 1 μg/g of resinous residue; but the atropine-like component is labile to acids and alkalis, and the losses, although known to occur, have not been estimated.

A second, more rapidly moving spot was also found, with an electrophoretic mobility comparable with that of the methyl or ethyl esters of trigonelline (but not that of choline). These esters are both gut stimulants; the methyl ester is like acetylcholine, but 1/200 times as potent; the ethyl ester is roughly 1/2,000 times as potent as acetylcholine, but has a flatter log-dose-response curve. Both are sensitive to lachesine but not (on the intestine) to hexamethonium. The eluate from the front of the fast spot resembled the methyl ester pharmacologically; that from the rear part had a log-dose-response curve flattened in the same way as that of the ethyl ester.

There is therefore evidence of the presence of an atropinic and two muscarinic substances in the watery extract as well as of the relatively inert trigonelline. None of these, however, has yet been obtained in sufficient quantity for chemical identification. If the ethyl ester is in fact present, it may well be an artefact resulting from esterification of trigonelline by solvent ethanol. The acetylcholine-like components could contribute to the irritant effect of the smoke. THC in a dose of 3 mg/kg was found, in the cat under chloralose, to affect neither chorda-stimulated salivary secretion nor the bradycardia in response to vagal stimulation, and it is therefore probable that the atropine-like material, rather than THC, chiefly causes the salivary and heart rate effects of taking cannabis. But because THC can reduce acetylcholine output from the parasympathetic nerve plexus of the intestine (see below), this depression of transmitter release may potentiate the atropinic action.

The chief active principles of cannabis have long been known to be insoluble in water and strongly lipophilic. The octanol : water partition ratio of THC was too high to be measured, but exceeded 500 : 1 (comparable with trichloroethylene and higher than most barbiturates). A saturated solution of THC in saline contains about 5 μg/ml. To obtain crude cannabis, THC or propyl-THC in saline solution, they were mixed with 0·5 to 2 times their weight of 'Tween 80.' Both cannabis and the pure substances act as anti-frothing agents, but none will reduce the surface-tension-lowering activity of the Tween solutions. Because we still do not know which of the pharmacologically active fractions are absorbed when cannabis is smoked or ingested, experiments were made with crude cannabis as well as with THC and propyl-THC.

Hypothermic Action

Miras[3] reported that cannabis lowers the body temperature of rats. This response, tested at an environmental temperature of 20° C, was confirmed in mice and found to be related to the dose. Intraperitoneally, 200 mg/kg produced a peak fall of 1° C. Intravenously, 12 mg/kg was ineffective, and 25 mg/kg produced in six mice a mean peak fall of 2·5° C, with recovery over a period of 30–40 min; the effect increased with increasing dosage in both magnitude (to 8° C) and duration (over 8 h). There may be peripheral factors contributing to the hypothermia, but the principal action of cannabis seems to be central. We have confirmed the observation[4] that it is effective intracerebrally. Further, it is considerably more effective by this route than when given intravenously; a dose of 25 mg/kg injected into the cerebral ventricles produced a hypothermia lasting over 8 h, with a peak fall of 6° C. It was already clear[4] that the intracerebral route was more effective than the intraperitoneal route in producing hypothermia; but this might have been the result of loss of drug during passage through the liver during absorption of the intraperitoneal dose *via* the portal vessels. The fate of any of the intracerebral injection that reaches the circulation should, however, be comparable with that of intravenously given drug. THC itself has now been shown[5] to produce hypothermia in mice when given intraperitoneally.

Because hypothermia itself both changes animal behaviour and alters physiological and pharmacological responses, so that some of the actions attributed to cannabis could have been secondary to the body temperature change, we have felt it necessary to repeat some of the previous work in conditions where significant hypothermia will not occur. All the experiments that follow, therefore, on catalepsy, analgesia and prolongation of sleeping time, were made with the mice in an environment at 30° C, known to be their thermal neutral zone (ref. 6 and unpublished work of R. G. P.).

Catalepsy

The cataleptic effect of cannabis[7] in the mouse has been developed into an objective assay, based on the proportion of time spent motionless when straddling a wire ring. Assays were four to nine point with seven to nine replications, suitably randomized. Control tests were made with 'Tween 80' solutions. Intraperitoneal or subcutaneous injections were used. All experiments were made at 30° C. Crude cannabis elicits detectable cataleptic effects at 200 mg/kg intraperitoneally or 100 mg/kg subcutaneously, and nearly maximal effect at about 500 mg/kg with duration greater than 4 h. THC was found to be active at 5 mg/kg; propyl-THC was found to be 4·8 times less active. The THC content could account for the bulk of the cataleptic effect, but comparison of the time course of action of crude cannabis with that of THC and propyl-THC suggests that propyl-THC contributes significantly to the earlier part of the response, THC itself acting relatively slowly.

If repeated daily subcutaneous doses of 100 mg/kg crude cannabis are made, the level of catalepsy achieved increases up to 8 days, indicating a cumulative effect. The recovery from the daily dose seemed faster at the end of the period than at the start. The results were reminiscent of the fat-soluble barbiturates, with which both cumulation and tolerance occur.

The cataleptic response was studied because it was an animal response which could be related to certain aspects of the human experience with cannabis. The dose required to produce the effect in mice, whether of pure THC or in terms of the THC content of the resinous residue (2·4 per cent), is roughly ten times the human effective dose when expressed per unit weight. If one compares, for a range of centrally active drugs, the doses per kilogram used to produce the characteristic actions of the drugs in mice or rats with the corresponding doses per kilogram of the pharmacopoeia, the mouse/man ratio ranges from about 2 to 100, and with some drugs the total dose for a rat is comparable with the total dose used in man. This arises, at least in part, from the more rapid turnover of drug in small animals, and is also influenced

by the route of administration and the intensity of the effect studied. So far as the doses found necessary in the experiments in this paper are concerned, therefore, there is no reason to doubt the relevance of the observations, *mutatis mutandis*, to human experience.

Analgesia

To test for analgesic action, the response to intraperitoneal injection of phenylbenzoquinone[8] was used, cannabis or other drugs being given subcutaneously; all experiments were done at an environmental temperature of 30° C. Cannabis had a threshold effect at 100 mg/kg and a marked action at 200 mg/kg. Using the same technique, 0·4 mg/kg morphine and 1·2 mg/kg chlorpromazine were approximately equivalent to 140 mg/kg cannabis resin. The results are comparable with those obtained with cannabis[9] and THC[8-11] by a variety of methods without temperature control.

We have confirmed the observation of Loewe[12] that cannabis prolongs pentobarbitone sleeping time in mice even if the experiment is made in an environmental temperature of 30° C. The effect is just detectable at 50 mg/kg intraperitoneally and increases with dose; with 200 mg/kg cannabis intraperitoneally the mean sleeping time in a group of nine mice given 50 mg/kg pentobarbitone 30 min later increased to 85 min, from a control value of 34. This prolongation was detectable 3 h after cannabis administration, but sleeping times tested 24 and 48 h after cannabis were normal, and no sign of induced enzyme activity after a single dose of cannabis was found.

It was possible that cannabis, while not itself overtly hypnotic, might potentiate barbiturate hypnosis by its own central action. A control experiment with ether which is not metabolized was therefore made. With subcutaneous injection of cannabis or 'Tween 80' control, and intraperitoneal injection of ether in olive oil, cannabis was found not to prolong ether sleeping time in a dose which prolonged pentobarbitone sleeping time. The evidence is therefore strengthened that cannabis interferes with microsomal degradation of barbiturates.

Toxicity

A preliminary study was made of the lethality of cannabis injected each day intraperitoneally at various dose levels. These experiments were made at the normal room temperature of 20° C. A single dose of 5,000 mg/kg killed all of five mice; 1,000 mg/kg killed all of four mice in 4 days; 500 mg/kg killed two of five mice in 45 days; 100 mg/kg caused no deaths in 50 days. These results imply either cumulation of the drug itself, or summation of its effects. The cause of death has not yet been analysed; but it may be noted that in cats under chloralose, THC at 2 mg/kg substantially depresses both respiration and blood pressure.

Teratogenicity in rats and hamsters[13,14] and foetal resorptions in mice[15] have been described at dose levels of 4·2–100 mg/kg daily at the appropriate stage of pregnancy.

Muscarinic and atropinic actions by water-soluble factors have been described above. The three fat-soluble fractions were tested on the guinea-pig ileum stimulated transmurally or with acetylcholine. THC, in a concentration of 1 μg/ml. upwards, was found to depress the twitch response to transmural stimulation but not the response to acetylcholine (which was sometimes potentiated). It must therefore depress acetylcholine output by the nerve endings; in this way it resembles morphine[16], catecholamines[17] and anaesthetics[18], but it differs from them in its much slower onset of effect and very delayed recovery, still incomplete after many hours. Propyl-THC has a similar action, with some initial stimulant effect, but recovery from its effects was much more rapid, and it was less active than THC. The third (uncharacterized) fraction depressed both the twitch and the response to acetylcholine and resembled a nonspecific depressant such as occurs among higher alcohols.

The fractions were also tested on the guinea-pig vas deferens stimulated by noradrenaline, by electrical stimulation with brief trains of shocks, or by stronger field stimulation with brief shocks, the latter being particularly useful in preliminary screening tests. THC and propyl-THC had no consistent effect on the response of the vas to noradrenaline. THC slightly reduced the response to field stimulation by trains of shocks. The response to strong single shocks was greatly potentiated by both THC and propyl-THC; THC was approximately three times more potent, and considerably slower in action. This response is insensitive to tetrodotoxin, and so presumably reflects a direct response of the smooth muscle to the electric field. Cocaine and some other local anaesthetics produce a similar potentiation.

Accumulation in Fat?

Cannabis thus contains, apart from THC, at least five other pharmacologically active components, three water-soluble and two fat-soluble, one of which has been identified as the propyl homologue of THC. In the water-soluble fraction, atropinic activity has been found which may be responsible for the effects of cannabis on salivary secretion and heart rate in man. This effect may be potentiated by the ability of THC to depress acetylcholine output from parasympathetic nerves.

Cannabis resin given to mice lowers body temperature by up to 8° C. Because hypothermia of this degree could change the animal's responses significantly, tests for the action of cannabis in producing catalepsy, analgesia and prolongation of barbiturate sleeping time were repeated, with the mice in their thermal neutral zone, to prevent body temperature changes. In these conditions cannabis still produces analgesia and catalepsy, and still prolongs pentobarbitone sleeping time. It was also shown that the sleeping time caused by a non-metabolized anaesthetic (ether) was not prolonged. Doses required for these effects ranged from 25 to 200 mg/kg according to route of injection. In preliminary toxicity tests the LD_{50} for a single dose lay between 1,000 and 5,000 mg/kg; for doses repeated daily it lay between 500 and 1,000 mg/kg.

Finally, in view of the report from a university survey[19] that of 153 students who had taken cannabis, 72 per cent took it daily or more often, attention should be paid to the fat-solubility of certain of the active principles of cannabis, their prolonged action, and the liability to cumulation.

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Creep and Friction of Ice

by
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The creep of polycrystalline ice in uniaxial compression has been studied over a very wide range of strain rates. The creep is far too slow to explain the friction of ice observed at very low sliding speeds, but experiments with single crystals show that sliding produces recrystallization at the interface, the ice presenting an orientation favourable to easy glide in directions tangential to the interface.

BARNES[1,2] has described experiments carried out in our laboratory on the indentation of ice using spherical and conical indenters. At temperatures above about $-3°$ C the indentation pressure was sufficient to produce appreciable pressure melting. With colder ice, although the indentation pressure was higher, pressure melting was no longer possible and the indentation process was essentially one of plastic flow. The hardness could be correlated with the yield stress of the ice in exactly the same way as for metals[3]. In particular it was found that the indentation hardness decreased with loading time due to the creep of the ice. Indeed, an analysis correlating the high temperature creep of metals, oxides and carbides with their hardness behaviour was found to be applicable to the hardness and creep properties of ice at temperatures above $-50°$ C.

Creep of Ice

Recently we have extended that study of the creep properties of ice to a much wider range of strain rates and temperatures, although in here we describe only the behaviour of ice above about $-10°$ C. The ice was polycrystalline (grain size about 1 mm) and was prepared from distilled water. A cylindrical specimen was subjected to compression between hard platens which were pressed together under a constant load. Because the total deformation was not large (≤ 5 per cent) this is very close to conditions of constant stress. The rate of approach of the platens was observed and from this the total strain determined at any time after the load was applied. The general behaviour is shown in curve II, Fig. 1. The portion OA is the instantaneous strain ε_0 which occurs when the load is first applied, AB the region of transient creep, BC the region of secondary or viscous creep and CD the region of tertiary creep. Similar characteristics are observed with metals and other solids. With metals examined under tension, tertiary creep is largely associated with the formation of intergranular fractures produced by triaxial tensile stresses. In compression tests such a mechanism can scarcely apply and the behaviour must be caused by other processes. With ice in our experiments, when the applied stress is compressive, tertiary creep is due primarily to recrystallization of the ice in an orientation favourable to easy glide[4]. This recrystallization is clearly visible in polarized light. At high stresses the secondary creep BC becomes increasingly shortened until the transient creep appears to be immediately followed by tertiary creep (curve I), whereas at low stresses the creep behaviour is still dominated by transient processes during the time available for an experiment (up to 100 days) and secondary creep is never established (curve III). The creep rate depends on the part of the creep curve chosen and so the simplest quantity to study is the minimum creep rate which can be deduced from the point of inflexion in curve I, from the secondary creep rate in curve II and from the final creep rate in curve III. Fig. 2 shows results obtained in this way for polycrystalline ice at $-2°$ and $-8°$ C over a wide range of strain rates: here the minimum observed creep rate $\dot{\varepsilon}$ is plotted against compressive stress σ on log–log ordinates. We shall show that the marked curvature at low stresses is due to the dominance of transient creep.

Over the whole range OABC the behaviour at any given stress may be accurately described by a typical creep equation of the form

$$\varepsilon = \varepsilon_0 + \alpha t^{1/3} + \dot{\varepsilon}_s t \qquad (1)$$

where ε_0 is the instantaneous strain, ε the total strain after time t, and α a suitable constant. The term $\alpha t^{1/3}$

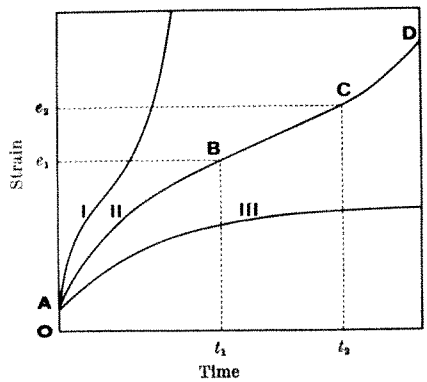


Fig. 1. The creep of ice under constant load at various levels of stress.

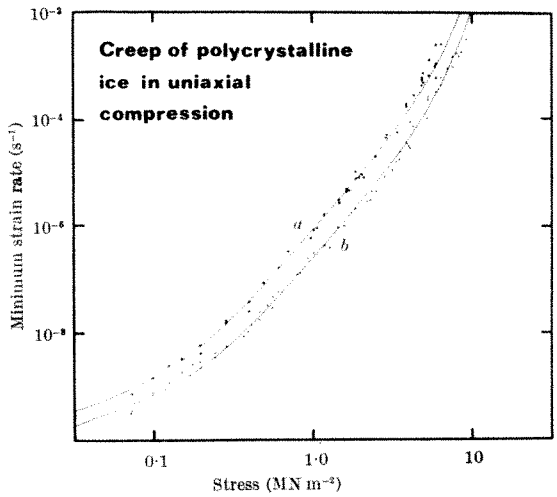


Fig. 2. The variation of the minimum creep rate $\dot{\varepsilon}$ with stress σ. *a*, $-2°$ C; *b*, $-8°$ C.

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describes the transient creep and $\dot{\varepsilon}_s t$ the secondary or viscous creep. If now we analyse the behaviour in terms of equation (1) we may extract the true secondary creep from the data even when viscous creep seems to be completely overtaken by recrystallization and tertiary creep. At very high stresses this analysis is restricted to the early portion of the creep curve where the observed points are randomly distributed about the curve derived from that equation; at large strains dynamic recrystallization enhances the creep rate and this is always greater than that predicted. Unfortunately it is not possible to analyse every creep curve in this manner because in our earliest experiments insufficient attention was paid to obtaining data at small strains when in transient creep. The results for secondary creep are shown in Fig. 3. There is now no curvature at low stresses.

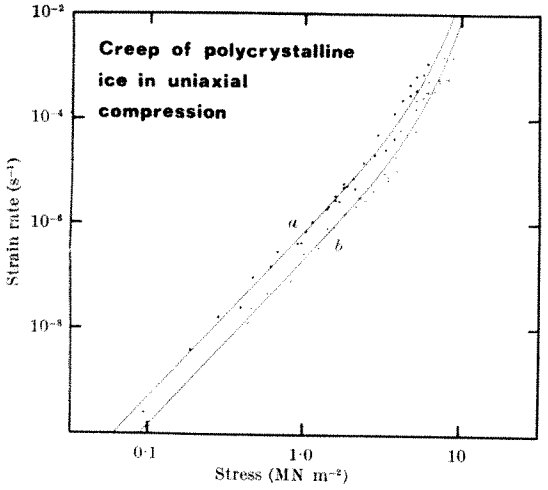


Fig. 3. The variation of the secondary creep rate $\dot{\varepsilon}_s$ with stress σ. *a*, −2° C; *b*, −8° C.

The secondary creep rate $\dot{\varepsilon}_s$ may be described by a relation of the form

$$\dot{\varepsilon}_s = A\sigma^n \exp(-Q/RT) \qquad (2)$$

over a limited range of stress where σ is the applied stress, n a constant of order 3 or 4, Q an activation energy. Clearly if $\dot{\varepsilon}_s$ were simply a function of σ^n these plots should give straight lines of slope n and the gap between such plots at different temperatures should provide a measure of the activation energy. At low stresses the slopes are indeed of order 3, and the calculated activation energy is of the order of 120 kJ mol^{-1}. At higher stresses the slope increases such that at stresses of the order of 10 MN m^{-2} the apparent slope is about 5 and Q is still about 120 kJ mol^{-1}. In fact, it turns out that expressing the viscous creep in terms of equation (2) can be misleading. Over the whole stress range a single relation of the form

$$\dot{\varepsilon}_s = A'(\sin h\alpha\sigma)^n \exp(-Q/RT) \qquad (3)$$

holds. It is only when we attempt to describe a portion of it in the form of equation (2) that n, the power of σ, appears to change. Indentation hardness measurements again show a correlation with these creep data, the stress level generally corresponding to that part of the creep curve where the value of n is of order 4–5. A fuller description of these results and a discussion of the creep processes will be published elsewhere.

Slow Speed Friction of Polycrystalline Ice

The friction of ice sliding on a flat surface has been studied using the apparatus designed by Barnes[6]; this is housed within a large refrigerator kept at temperatures constant to within 0·1° C. The sliding speed could be varied from 10^{-8} to 10^{-1} ms^{-1}. The slider which was polycrystalline ice in the form of a cone of included angle of 170° was pressed on to the lower surface under a normal load of either 32 or 78 kg. The tip wore away and after a few traversals of the lower surface the cone acquired a flat contact region (about 5–10 mm diameter) which continued to grow by creep and wear, but very much more slowly. A consequence of the large included angle is that the deformation within the ice is confined to a zone close to the interface where the shear stresses are very much greater.

Fig. 4 shows the dependence of the coefficient of friction on sliding speed at −11·7° C. It is seen that the maximum friction for ice on rock (peak to valley roughnesses of about 1·5 μm) is large (about 0·9) and approximately constant for sliding speeds between 10^{-6} and 10^{-4} ms^{-1} (region BC). Slip stick occurs at all speeds above 10^{-6} ms^{-1}. At higher speeds the coefficient of friction diminishes rapidly to a value of 0·05 or less (region CD). At lower speeds there is also a slight fall in friction (region AB). By way of contrast, when ice slides on a steel surface of comparable roughness the friction remains low over a wide speed range and the evidence suggests that this is due to poor adhesion at the interface.

Simple calculations of heat flow[6] show that for ice sliding on rock (sliding speed 10^{-2} ms^{-1}, contact zone 100 mm in diameter, frictional force 10 kg corresponding to $\mu = 0{\cdot}3$) the temperature rise of the interface is of the order of 15° C. It is sufficient to produce some surface melting and this presumably accounts for the observed drop in friction as suggested by Bowden and Hughes[7] many years ago. With ice sliding on a metal surface the corresponding effect would only be expected at sliding speeds of 10^{-1} ms^{-1} or more.

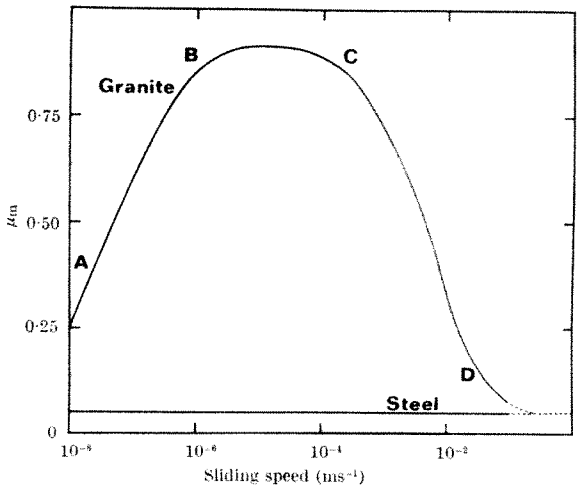


Fig. 4. The friction of ice at −11·7° C on granite and on steel. Each curve is derived from approximately fifty individual measurements. The experimental scatter is ±10 per cent. The granite was a coarse-grained quartz–feldspar–biotite–muscovite granite from the Correnie quarries, Alford, Aberdeenshire.

The most interesting feature of the friction of ice on rock is the region AB (Fig. 4). There is other evidence to show that there is strong interfacial adhesion between the ice and the rock so that shearing apparently occurs within the ice itself. We should therefore be able to explain the tangential force necessary to produce sliding in terms of the creep properties of ice itself. If, however, the creep characteristics of ice described in Fig. 3 are used, it is found that the observed tangential stresses are too small and shear would have to occur over an unrealistic thickness of ice of the order of 10^{-2} m. This problem was first recognized by Barnes in his preliminary friction measurements. For this reason he suggested the presence of a

viscous liquid film at the interface*. This seems unacceptable for temperatures much below 0° C. An alternative is to take into account the effect of the combined normal and tangential stresses in calculating the creep rate at the interface. We assume that about four-fifths of the normal contact pressure is in the form of a hydrostatic component[10] so that only about one-fifth of the pressure plays its part as a shear stress in facilitating creep When μ is small this deviatoric component of the normal load appears to the power $(n-1)$ in the combined stress creep equation so that it does, in fact, greatly increase the calculated tangential creep rate. It would, however, also be accompanied by marked creep in the vertical direction whereas this is not observed. One way of explaining away this difficulty is to assume that contact occurs only at discrete asperities continuously reformed during sliding so that the true stresses are higher. This assumption is unattractive, for if the cone is deliberately worn to give a larger geometric area of contact the friction over the region AB increases roughly in the same proportion.

Most of these difficulties are resolved if experiments are carried out with an ice slider made of a single crystal of ice.

Friction of Single Crystals of Ice

A single crystal of ice was cut into conical form with its c axis normal to the cone axis and therefore parallel to the direction of sliding. This was slid over a granite surface. After a few repeated traversals a flat was worn on the cone as with the polycrystalline specimen. The frictional behaviour was the same as that shown by polycrystalline specimens. If, however, the ice specimen was examined in polarized light, it was found that the surface layers of the ice had recrystallized to a depth of about 0·2 mm and that all the surface crystallites were oriented with the c axis normal to the sliding interface, that is all the crystallites were in the direction of easy glide relative to the interface. The situation resembles that of tertiary creep shown in Fig. 1. It is not possible from our creep data to quantify the creep behaviour in this regime. Our own measurements and those of other workers[4,11] indicate, however, that creep in this direction is about 100 times easier than when ice is in other orientations. These results suggest that in those experiments where there is good adhesion between the ice and the substrate the friction at low sliding speeds is determined by the force required to produce creep within a very thin layer of ice close to the interface: as a result of recrystallization this layer develops an orientation favourable to easy shear tangential to the surface. This layer is also unfavourably oriented for creep in a direction normal to the interface so the normal stress does not produce appreciable creep in this direction.

These results have a direct bearing on the creep of glaciers. They also suggest that similar processes may be involved in the sliding, at high temperatures and for protracted periods, of hexagonal metals. Evidence for this is provided by the experiments of Buckley[12].

We thank the director of the Scott Polar Research Institute, Cambridge, for the use of the cold laboratories for the creep experiments, and Mr M. Badcock for help with polishing the granite surface. One of us (J. C. F. W.) was a recipient of a Natural Environment Research Council grant.

* An important paper on the adhesive properties of ice by Jellinek[8,9] seemed to support this hypothesis. He measured the adhesive strength in shear and in tension of thin disks of ice frozen between metal or polymeric platens. The adhesion in shear was far less than in tension and he attributed this to the presence of a thin liquid-like amorphous layer with properties intermediate between those of ice and water. If such a film is present it is easy to show that viscous forces resisting shear will be far smaller than those resisting tension. However, his results can be explained much more simply without invoking the liquid film if the contribution of friction between the platen and the ice is considered. If Y_0 is the unlaxial plastic yield stress of bulk ice in frictionless tension or compression experiments the yield stress Y, both in tension and compression, for ice between rigid platens will be given by

$$Y = Y_0(1+\mu a/h) \quad (4)$$

where μ is the coefficient of friction at the platens, and a/h the ratio of the specimen width to height. Jellinek used disk shaped specimens for which a/h varied from about 1 to 20 and the observed tensile yield stress for steel platens was found to lie between 1 and 7 MN m^{-2}. His data fit equation (4) extremely well. A simple derivation of this equation together with a more detailed discussion is to be found in ref. 10; pp. 37–40. It is true that a transition layer a few molecules thick may well exist at temperatures closer to 0° C and we do not wish to dispute this. Our main point is that our low-speed friction results may be explained without invoking the presence of such a film.

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First Results from the Selective Chopper Radiometer on Nimbus 4

by

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This article describes the temperature profiles through the atmosphere that are being obtained from the radiometer on Nimbus 4.

Ways in which radiometric measurements from satellites can give information about the atmosphere have recently attracted a great deal of attention. In 1959 Kaplan[1] suggested that temperature could be inferred from the emission from the ν_2 vibration of carbon dioxide centred at a wavelength of 15 μm in the infrared. These measurements were first carried out on the Nimbus 3 satellite launched in April 1969, which included a 7-channel grating spectrometer and a Michelson interferometer. Nimbus 3 showed clearly that the temperature structure in the atmosphere up to 30 km altitude can be measured with an accuracy such that the error in the mean temperature of layers 200 mbar thick is less than 2° C in clear conditions[2,3].

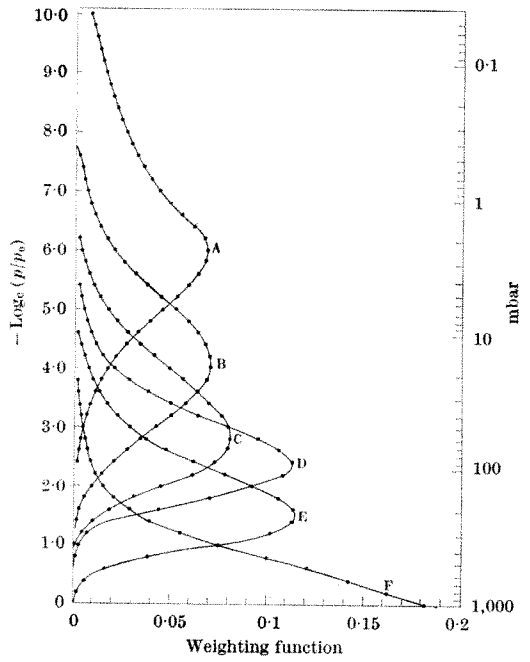


Fig. 1. Weighting functions for the six channels plotted against pressure as the vertical coordinate.

The development work on the selective chopper radiometer, which is a vertical temperature sounder for the Nimbus 4 satellite, began in 1961. An original feature is the simultaneous observation by interference techniques of a large number of individual lines in the CO_2 spectrum first considered by Houghton[4] and Smith[5], and finally achieved by selective chopping using CO_2 filled cells[6]. The instrument combines "coarse" resolution by interference filters (from 3·5 to 10 cm^{-1} bandwidth) with "fine" resolution within the bandwidth achieved by selective chopping or absorption by a cell of CO_2 (ref. 7).

The use of the CO_2 cells in absorption (channels C, D, E and F) selects wavelengths at which the absorption coefficient of CO_2 is relatively constant within each channel and has the effect of improving the vertical resolution of the instrument. The "selectively chopped" channels (A and B) pick out the line centres and allow radiation to be received from considerably higher altitudes than is otherwise possible. A second advantage is that this method of selection of a uniform absorption coefficient enables sufficient energy to be collected for adequate radiometric accuracy. In fact the integrated spectral bandwidth of the radiometer is rather greater than that of spectrometers. The design freedom thus created is used to make a radiometer of convenient size for use on satellites. The instrument is fully described by Houghton and Smith[8] and Abel *et al.*[9].

For an infinitely deep atmosphere which is horizontally stratified, the intensity of radiation energy I emitted vertically upwards in one of the spectral channels of the radiometer may be written

$$I = \int B(T)\, K(y)\, dy \qquad (1)$$

where $B(T)$ is the Planck function appropriate to the wavelength of the interval and T is the atmospheric temperature at the level where the pressure is p. The quantity I when in units of energy per unit time per unit spectral interval per unit area per unit solid angle is known as the radiance. The parameter $y = -\log p$ is chosen as the dependent variable for reasons of convenience. The weighting function $K(y)$ is dependent on the characteristics of carbon dioxide in the spectral interval appropriate to a particular channel. For the purposes of numerical computation, equation (1) may be written as

$$I = \sum_i B(T_i)\, K(y_i)\, \Delta y$$

The values of $K(y_1)$ for the six channels are plotted in Fig. 1 where the discrete values at different levels belonging to a given channel have been joined together.

The Nimbus 4 satellite was launched on April 8, 1970, into a polar Sun-synchronous orbit of about 1,100 km altitude. The selective chopper radiometer was switched on at the beginning of the sixth orbit and has worked

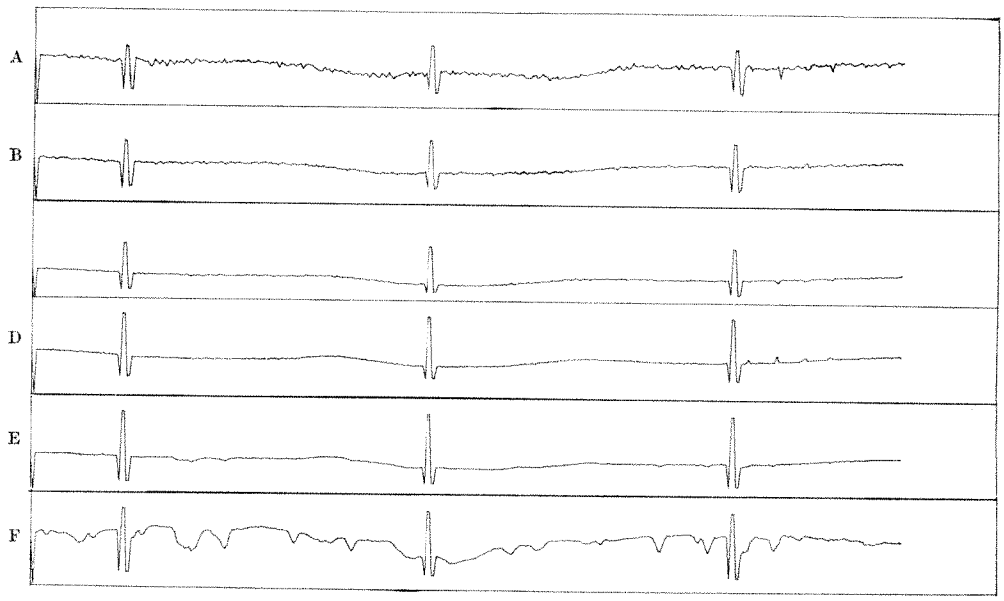


Fig. 2. Plot of signals received (averaged over 16 s) from the six channels for one complete orbit on June 22, 1970. The channel numbers are shown on the left. Calibration sequences occur three times during the orbit. In these sequences the largest signal is from a black body at instrument temperature (about 290 K) and the smallest signal corresponds to zero radiation from space.

completely satisfactorily from the start. Data are transmitted in near real time over a data link from Washington DC to Oxford and Reading. Initial processing is carried out on the data immediately they are received so that the results are plotted within about one day of the measurements being made. Figs. 2 to 6 show various presentations of the results which are being carried out on a routine basis.

Fig. 2 shows the signals from the six radiometer channels for one orbit. Fig. 3 shows a temperature profile computed from the six radiances using the inversion method described by Rodgers[10], compared with a radiosonde and a rocketsonde launched almost at the same time as the passage of Nimbus 4 over Wallops Island. In this comparison, which is for a clear atmosphere, no adjustment of the weighting function $K(y)$ has been made in order to have a good match with the radiosonde or the rocketsonde. The weighting functions appropriate to the different channels have been deduced from laboratory measurements combined with calculations based on the carbon dioxide spectrum. It can be seen that there seems to be satisfactory agreement apart from the region of channel B where systematic differences of about 5° C between the rocketsonde temperatures and those deduced from the radiances have been noticed.

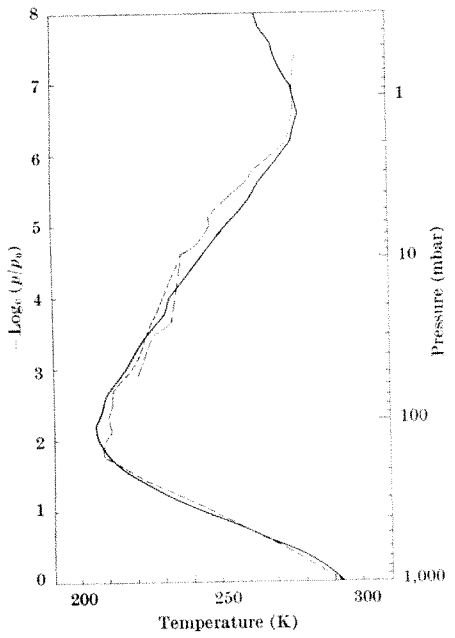


Fig. 3. Comparison between temperature profile deduced from the radiances (full line) over Wallops Island June 17, 1970, at 1644 Z, radiosonde from Wallops Island June 17, 1970, at 0000 Z and rocketsonde from Wallops Island June 17, 1970, at 1630 Z.

Fig. 4 shows a mean meridional profile of the atmospheric temperature in which the radiances inverted as described by Rodgers[10] are averaged over latitude circles for a period of one day, and plotted out as a cross-section. In Fig. 5 the radiances in channels A and B are plotted for one particular day. The orbits over which data were collected are shown in Fig. 6. The upper stratosphere over the north pole is warm at this time of year with a small gradient of temperature from the warm pole to the colder equator. The upper stratosphere near the south pole is cold. The most interesting feature of the maps is that whereas the isopleths for channel B are nearly circular and circumpolar, for channel A the coldest region is not at the pole and there is considerable structure in the pattern of isopleths. Substantial changes occur in this pattern with time which are reflected to only a small extent in channel B. Instrumental causes for these changes can be ruled out because overlapping observations

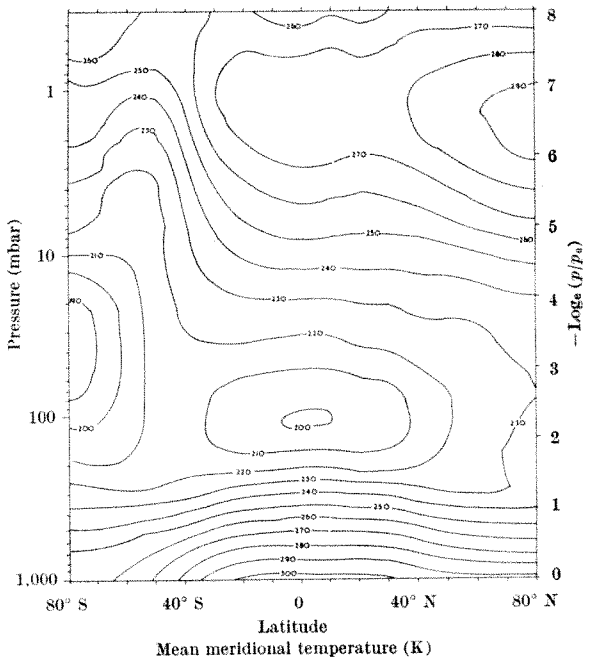


Fig. 4. Mean cross-section of the atmosphere from 80° N to 80° S for June 22, 1907.

from different orbits are usually consistent and day to day changes can be followed in a sensible way.

The vertical resolution of the radiometer is clearly demonstrated by examining the signals from channels E and F in Fig. 2. The irregular dips in channel F (centred at 5 km height (see Fig. 1)) arise when the radiometer views cloud radiating essentially as a black body at a temperature depending on the height of the cloud top. In the next highest channel E (centre 10 km) very little of these effects can be seen showing that this channel is not sensitive to radiation at the level of most cloud tops. Estimates have been made from data from a number of orbits of the proportion of time that the signals in different channels are noticeably affected by cloud. These estimates are about 50 per cent for the ground channel (G)*, about 40 per cent for channel F (centred at 5 km) and only about 3 per cent for channel E (centred at 10 km).

Table 1. ACCURACY OF TEMPERATURE MEASUREMENT

Channel	A	B	C	D	E	F	G
Representative viewing temperature (K)	250	240	230	220	240	270–210	300–220
Viewing time for 1 K r.m.s. noise (s)	60	25	7	3	1	1	1
No. of independent measurements per orbit	100	250	900	2,400	6,400	6,400	6,400

Finally, Table 1 summarizes the observed accuracy of the temperature measurements by showing the integration times necessary to achieve an r.m.s. noise temperatures of 1 K.

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* This channel, which is in the atmospheric window near 11 μm, can be switched in instead of channel C on command.

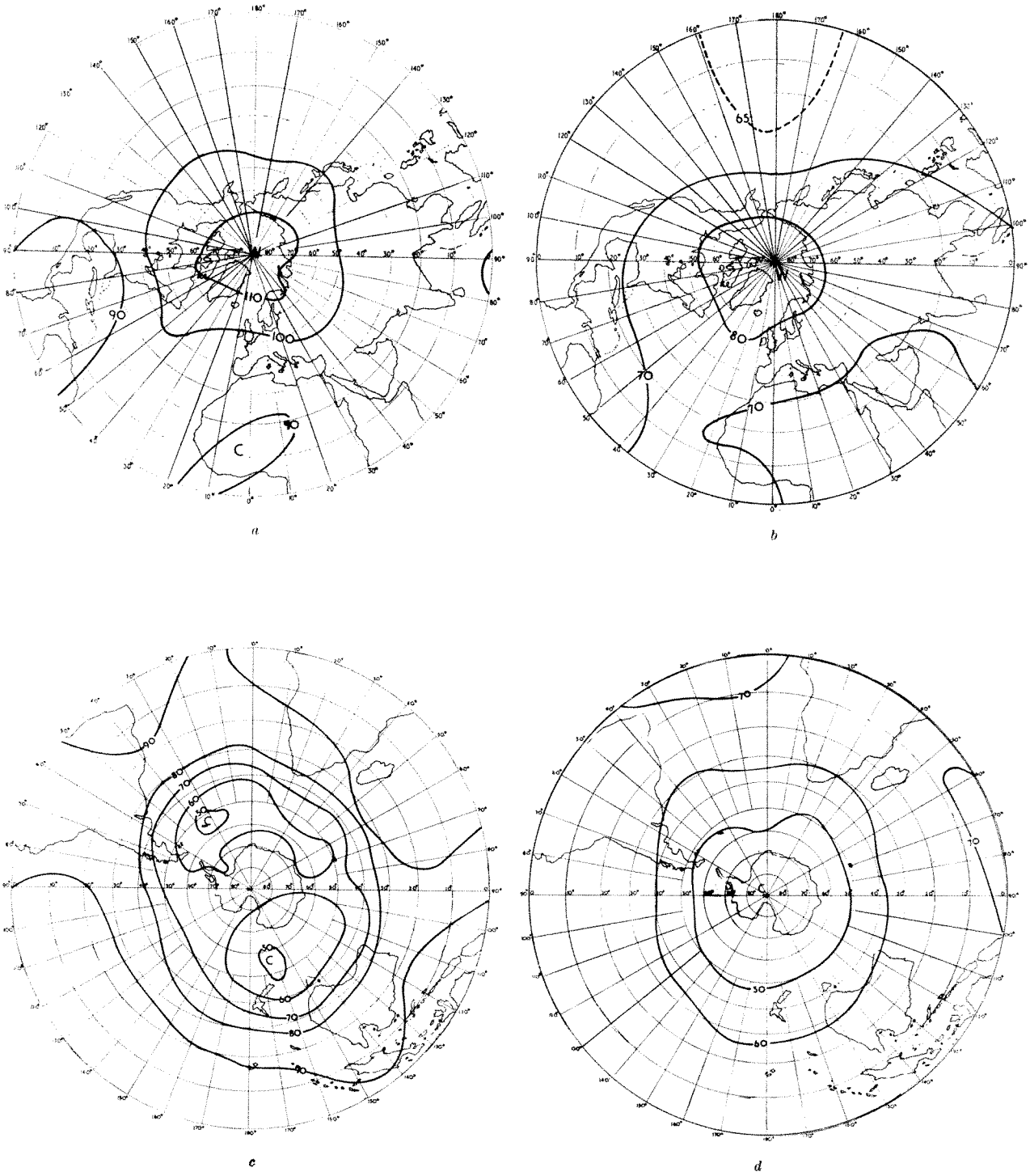
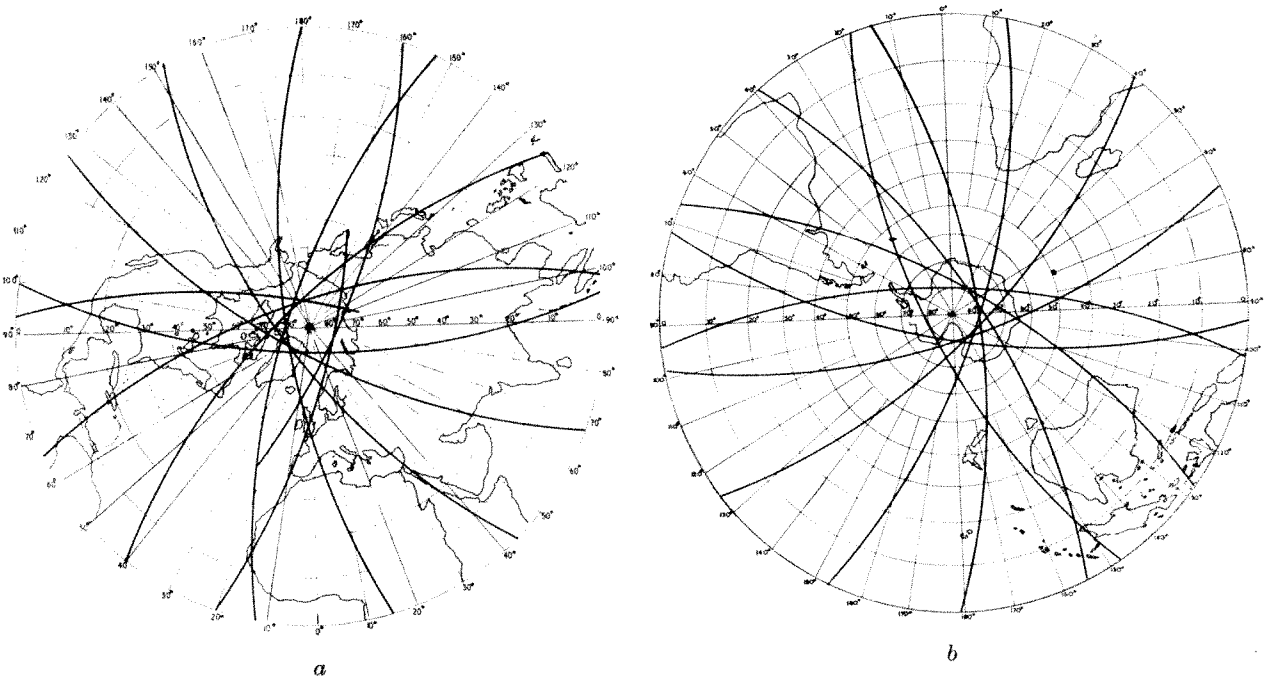


Fig. 5. Maps showing isopleths of radiances in channels A and B. The units of radiance are mW m^{-2} s^{-1}. The equivalent temperatures of a black body which would give the same radiance are:

Radiance	Equivalent temperature (K)
50	224·7
60	235·5
70	243·6
80	251·9
90	259·8
100	267·8
110	274·2

W and C show warm and cold areas respectively. All the maps are for June 22, 1970. *a*, N hemisphere channel A; *b*, N hemisphere channel B; *c*, S hemisphere channel A; *d*, S hemisphere channel B.



a *b*

Fig. 6. Maps showing the orbits from which data for the maps in Fig. 5 were obtained. *a*, N hemisphere; *b*, S hemisphere.

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Thermal Emission Spectra of the Earth and Atmosphere from the Nimbus 4 Michelson Interferometer Experiment

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Profiles of atmospheric temperature, humidity and ozone can be recovered from the thermal emission spectra in the interval 400 to 1,500 cm^{-1} obtained from the Nimbus 4 satellite. The spectra can also be used to study surface restrahlen effects and other geophysical and meteorological phenomena.

THE meteorological research satellite Nimbus 4, launched on April 8, 1970, into a circular polar orbit of 1,100 km altitude, carried, among other instruments, a Michelson interferometer similar to the one placed in orbit by Nimbus 3 approximately a year earlier[1]. The infrared emission spectra of the Earth and its atmosphere recorded by Nimbus 4 are better than the earlier results. Instrumental improvements include (1) a higher spectral resolution equivalent to 2·8 cm^{-1} compared with 5 cm^{-1}, (2) a narrower field of view of 5° compared with 8°, and (3) a lower noise equivalent radiance, particularly noticeable at the higher wave number end of the spectrum. The upper wave number limit was chosen at 1,500 cm^{-1} (6·7 μm), in part to permit the higher spectral resolution without a change in the data interface to the Nimbus spacecraft. The lower limit of 400 cm^{-1} (25 μm) for the recorded spectral range is the same as on Nimbus 3.

The scientific objectives of this experiment are similar to those of the earlier IRIS experiment, that is, the vertical soundings of atmospheric temperatures, humidities and ozone concentrations. The recording of the complete spectrum between 400 and 1,500 cm^{-1} has also permitted other scientific investigations, studies of surface conditions[2] for example. In the first IRIS experiment, the demonstration of the principles of atmospheric soundings of temperature and composition was emphasized. Application of the large body of data to circulation studies and other meteorological tasks is now beginning. The higher spectral and spatial resolution and better signal-to-noise ratio of the Nimbus 4 interferometer will permit considerable refinements in these techniques.

Fig. 1 shows three spectra selected from the Nimbus 4 data to illustrate their quality and usefulness. Included are the coldest spectrum and the warmest spectrum

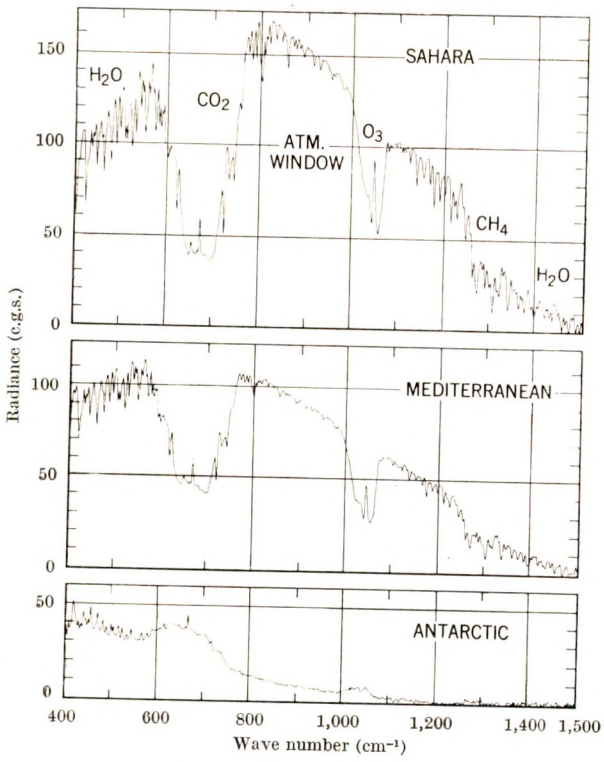


Fig. 1. Selected spectra obtained with the Michelson interferometer flown on the Nimbus 4 satellite. The three spectra shown here, obtained over the Sahara Desert, the Mediterranean and the Antarctic, are apodized, resulting in a resolution equivalent to 2·8 cm⁻¹. Atmospheric constituents responsible for the various absorption features are indicated in the upper spectrum.

recorded on orbit 29 (April 10, 1970), along with a mid-latitude case taken over the Mediterranean. Each spectrum is computed from a single interferogram recorded by the instrument within a 13-second interval. The instrumental technique used and the on-board calibration procedures are discussed elsewhere[3].

The spectra of Fig. 1 were processed using an apodization or smoothing function to reduce the side lobes of the instrument function, and the same spectra are shown in Fig. 2 without smoothing. The spectral resolution in this case is almost twice as high, corresponding to about 1·5 cm⁻¹. When the data are processed in this way, the instrument function has more pronounced side lobes, so the apodized spectrum is preferred for a radiometric analysis such as that required in obtaining atmospheric temperature profiles. For line identification, however, the higher resolution of the unapodized spectrum has a distinct advantage.

The warmest spectrum of the three shown in Figs. 1 and 2 was recorded over the Sahara Desert. The field of view of the instrument covering a circular area 100 km in diameter has been superimposed on a television picture (Fig. 3) taken by the Nimbus Image Dissector Camera System at the same time. The data show the features of CO_2, H_2O, O_3 and CH_4 as indicated in Fig. 1. The effects of the residual ray phenomenon (restrahlen) originating from the SiO_2 in the surface result in brightness temperatures approximately 10 K lower in the window region on the high wave number side of the 1,042 cm⁻¹ (9·6 μm) ozone band than those on the low wave number side. The maximum brightness temperature of 320 K (47° C) in the atmospheric window is consistent with a poor heat conducting material at the surface, such as dry sand.

The Mediterranean spectrum was recorded shortly thereafter. The maximum brightness temperature of 285 K (12° C) is close to the water surface temperature. The coolest spectrum recorded over the Antarctic plateau indicates a surface temperature of 190 K (−83° C). The CO_2, O_3 and H_2O features appear in emission, indicating a warmer atmosphere above a colder surface. The inferred surface temperature is extremely low,

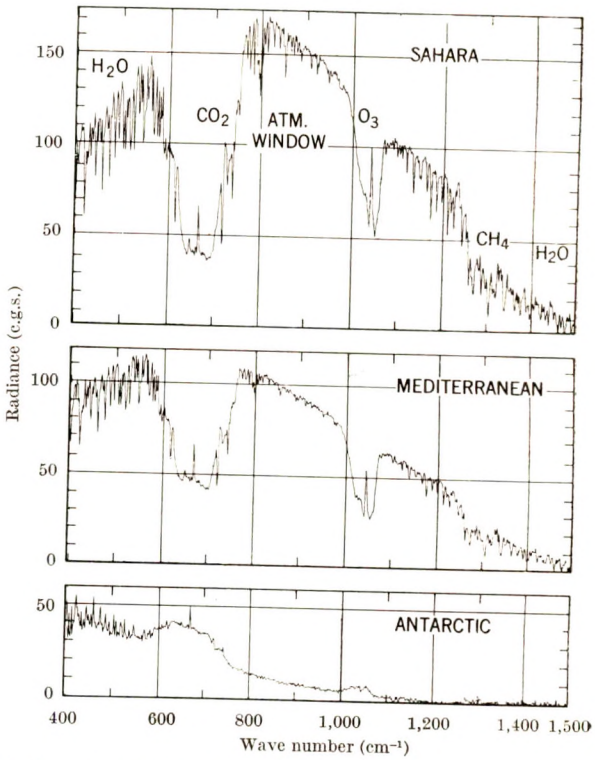


Fig. 2. The same spectra as shown in Fig. 1 are given here in their unapodized form with a resolution equivalent to 1·5 cm⁻¹.

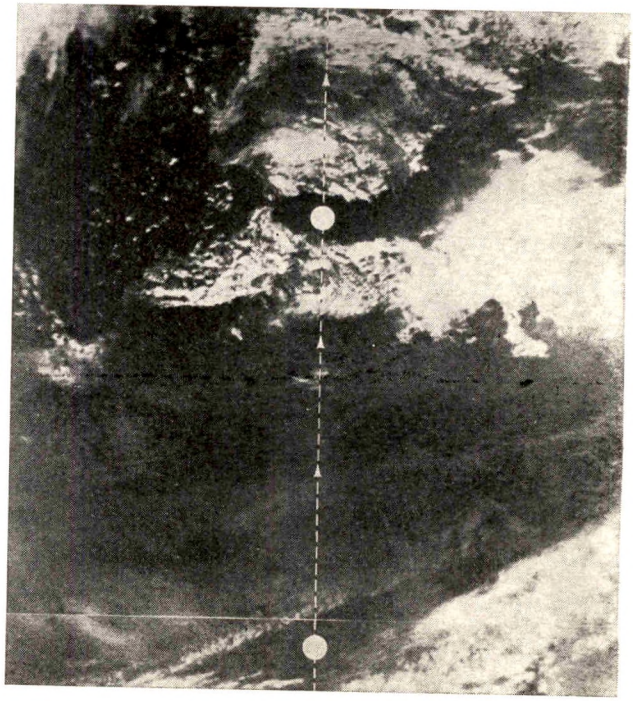


Fig. 3. Television picture obtained from the Nimbus 4 satellite. The fields of view of the Michelson interferometer, corresponding to the Sahara and Mediterranean spectra, given in Figs. 1 and 2, are indicated by the circles, and the subsatellite path is indicated by the broken line.

but shelter temperatures of −80° C along with strong surface inversions have been recorded at Plateau Station located in the same general region.

Estimates of temperature and humidity profiles obtained from the spectrum taken over the Mediterranean

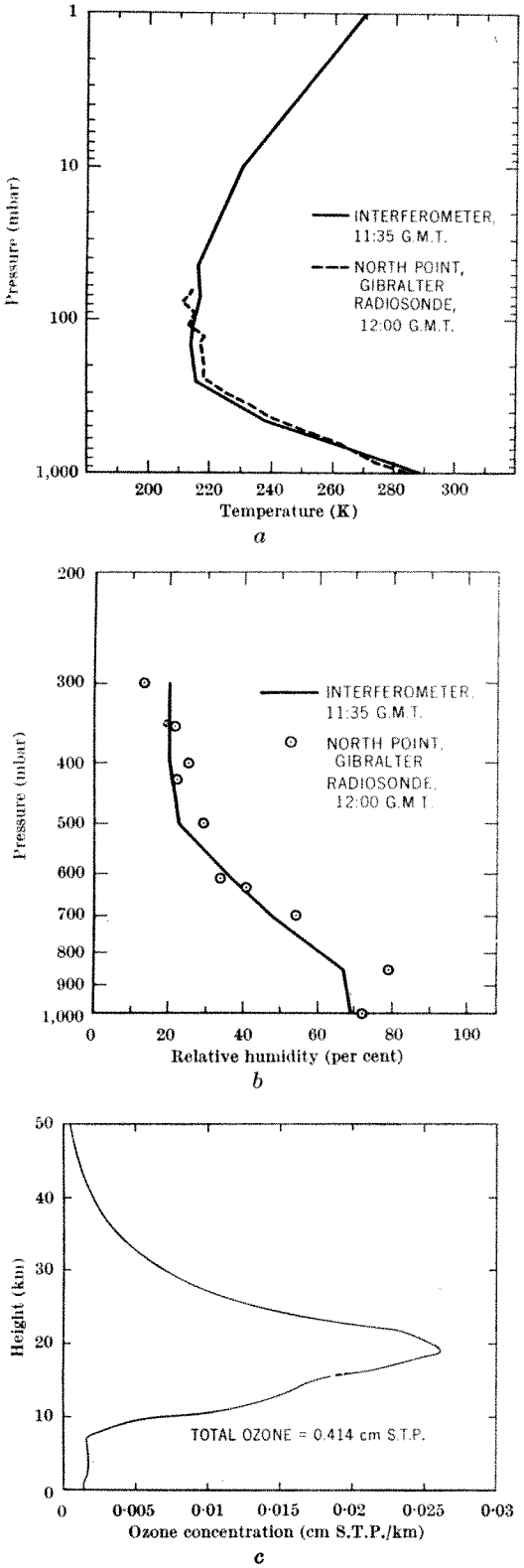


Fig. 4. Vertical profiles of atmospheric parameters obtained from the Mediterranean spectrum shown in Fig. 1. The profiles include (*a*) temperature, (*b*) relative humidity, and (*c*) ozone. Measurements of temperature and humidity obtained from a nearby radiosonde are shown for comparison.

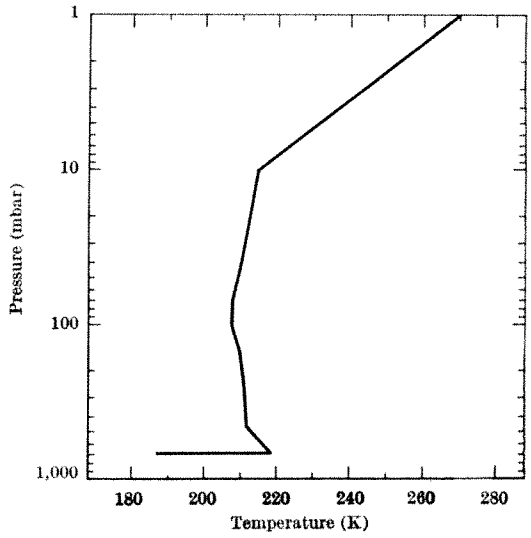


Fig. 5. Vertical atmospheric temperature profile obtained from the Antarctic spectrum shown in Fig. 1. A climatological value of 700 mbar was taken as the surface pressure. The actual thickness of the surface inversion layer could not be resolved with the temperature estimation technique, and the inversion appears as a discontinuity at the bottom of the profile.

are shown in Figs. 4*a* and *b*, respectively, where they are compared with data from a radiosonde launched from Gibraltar. The ozone profile inferred from the same spectrum is shown in Fig. 4c. The techniques used for obtaining the temperature and humidity profiles have been discussed by Conrath *et al.*[2] and the method for calculating ozone profiles by Prabhakara *et al.*[4]. Because of the interesting behaviour of the Antarctic spectrum, an estimate of the temperature profile for this case has been made (Fig. 5) even though no radiosonde data are available for comparison. The profile can be anticipated intuitively from an examination of the spectrum (Figs. 1 and 2). The warmer upper atmosphere is associated with the Q-branch of the CO_2 band at 667 cm^{-1} where the highest radiances in the band occur, while the approximately isothermal portion of the profile is associated with the more transparent portions of the CO_2 band and the stronger lines of the water vapour rotation band. The cold surface temperature results in the relatively low radiances found in the window region and between the water vapour lines.

These preliminary results show that the interferometer experiment on Nimbus 4 is providing high quality infrared spectra of the Earth and atmosphere. The low noise equivalent radiance, high spectral resolution, and broad spectral coverage make the data ideally suited for studies of atmospheric radiative transfer as well as the analysis and development of remote sensing techniques. The experiment also provides data for many geophysical and meteorological investigations. Temperature and humidity profiles derived from the spectra on a global basis will be useful in numerical modelling experiments of the atmospheric circulation.

We thank the many members of the Nimbus Project and the group at Texas Instruments Inc. who were responsible for the construction of the instrument. Dr C. Prabhakara provided the ozone profile estimate. We also thank Drs V. V. Salomonson, I. Revah, V. G. Kunde, and B. Schlachman for help and Mr G. Wolford for computer programming.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Precise Radio Source Positions: A Comparison of Two Independent Lists

MEASUREMENTS of radio source positions to an intended accuracy of 1 arc second or better have recently been carried out independently at the National Radio Astron- cm), although the actual methods which were use Both sets of observations were done by means of interferometry at a nominal frequency of 2,695 MHz (λ 11·1 cm), although the actual methods which were employed differ radically. The positions measured for the twenty-eight sources common to the two lists are generally in excellent agreement, as will be shown. Papers describing the methods used, and containing full lists of measured positions, will be published separately elsewhere (ref. 1 and unpublished work of Adgie *et al.*).

The measurements at the National Radio Astronomy Observatory were made with the three-element interferometer[2]. The position of each source was measured independently on at least two of the element spacings 16,200λ, 18,900λ, 21,600λ or 24,300λ. The technique was novel in that it yielded an absolute measurement of the source declinations, in no way dependent on previously determined coordinates for any radio source or on accurate previous knowledge of the geometry of the instrument. It also gave an absolute measurement of the right ascensions apart from an unknown zero-point which was fixed by requiring that the measured right ascensions agree in the mean with the optically determined values for the identified sources in the list (unpublished work of Kristian and Sandage). The NRAO programme dealt with forty-six sources, of which forty have known optical counterparts.

The measurements at the Royal Radar Establishment were made with the RRE two-element interferometer[3], with an element spacing of about 5,800λ along a north–south line. Each source was observed 4 h before and 4 h after upper culmination. Over a range of declinations from 0° to 60° this gives two sets of fringes which are close to position angles of ±45° and have almost the maximum resolution of 36 arc s. This arrangement has the great advantage that the measurement accuracy is almost independent of declination. No attempt was made to measure the baseline parameters directly, the system being calibrated with the optical positions of thirty-six identified sources[4–7]. The measurement of declination required the determination of three baseline constants and relied on having a good distribution of sources throughout the full declination range. For the right ascensions, only one constant was required; this fixed the zero point of the right ascensions, as in the NRAO measurements. But the optical right ascensions used by NRAO were taken from a new set of optical positions (Kristian and Sandage, unpublished) and were independent of the positions used by RRE. The RRE programme dealt with 178 sources.

There are twenty-eight sources common to the two lists, and their measured positions, with the differences between them and the estimated uncertainties, are given in Table 1. Fig. 1 is a cross-plot of the distribution of the position differences in the two coordinates. These position differences are closely grouped with the exception of the measurement for 3C 230. This source is undoubtedly confused somewhat by NRAO 340 (PKS 0950+00), so that its displacement from the group is not surprising.

The remaining twenty-seven position differences were allocated weights inversely proportional to the squares of their estimated uncertainties. Table 2 gives the resulting weighted mean position difference, together with the standard errors as calculated from the observed position residuals. The standard errors for one position difference are close to 0·5 arc s in each coordinate. This indicates

Table 1. RADIO SOURCE POSITIONS AND POSITION DIFFERENCES

Source	Right ascension 1950.0 NRAO	RRE	NRAO–RRE	Declination 1950.0 NRAO	RRE	NRAO–RRE
PKS 0106+01	01 h 06 m 04·50 s ±0·05 s	04·52 s ±0·03 s	−0·02 s ±0·06 s	+01° 19′ 00·3″±1·0″	00·9″±0·5″	−0·6″±1·1″
3C 48	01 h 34 m 49·82 s ±0·04 s	49·86 s ±0·04 s	−0·04 s ±0·06 s	+32° 54′ 20·4″±0·6″	20·8″±0·5″	−0·4″±0·8″
PKS 0229+13	02 h 29 m 02·53 s ±0·05 s	02·53 s ±0·04 s	0·00 s ±0·06 s	+13° 09′ 40·9″±1·0″	40·9″±0·6″	0·0″±1·2″
CTA 21	03 h 16 m 09·12 s ±0·04 s	09·21 s ±0·03 s	−0·09 s ±0·05 s	+16° 17′ 40·3″±0·7″	41·1″±0·5″	−0·8″±0·9″
NRAO 140	03 h 33 m 22·38 s ±0·04 s	22·42 s ±0·04 s	−0·04 s ±0·06 s	+32° 08′ 36·8″±0·6″	36·7″±0·5″	+0·1″±0·8″
3C 119	04 h 29 m 07·88 s ±0·05 s	07·94 s ±0·04 s	−0·06 s ±0·06 s	+41° 32′ 08·6″±0·7″	08·6″±0·5″	0·0″±0·9″
3C 120	04 h 30 m 31·60 s ±0·04 s	31·57 s ±0·03 s	+0·03 s ±0·05 s	+05° 14′ 59·5″±0·8″	60·1″±0·5″	−0·6″±0·9″
NRAO 190	04 h 40 m 05·25 s ±0·04 s	05·29 s ±0·03 s	−0·04 s ±0·05 s	−00° 23′ 20·1″±0·8″	21·0″±0·5″	+0·9″±0·9″
3C 138	05 h 18 m 16·50 s ±0·05 s	16·56 s ±0·03 s	−0·06 s ±0·06 s	+16° 35′ 27·2″±0·8″	27·0″±0·5″	+0·2″±0·9″
3C 147	05 h 38 m 43·52 s ±0·04 s	43·49 s ±0·05 s	+0·03 s ±0·06 s	+49° 49′ 42·4″±0·6″	43·1″±0·5″	−0·7″±0·8″
3C 191	08 h 02 m 03·78 s ±0·08 s	03·79 s ±0·04 s	−0·01 s ±0·09 s	+10° 23′ 56·5″±1·6″	57·5″±0·6″	−1·0″±1·7″
3C 230	09 h 49 m 25·05 s ±0·06 s	24·97 s ±0·04 s	+0·08 s ±0·07 s	+00° 12′ 38·4″±1·3″	40·5″±0·6″	−2·1″±1·4″
PKS 1055+01	10 h 55 m 55·30 s ±0·07 s	55·35 s ±0·03 s	−0·05 s ±0·08 s	+01° 50′ 02·8″±1·6″	03·2″±0·5″	−0·4″±1·7″
PKS 1116+12	11 h 16 m 20·74 s ±0·06 s	20·81 s ±0·03 s	−0·07 s ±0·07 s	+12° 51′ 06·6″±1·2″	06·8″±0·5″	−0·2″±1·3″
3C 256	11 h 18 m 04·09 s ±0·05 s	04·12 s ±0·05 s	−0·03 s ±0·07 s	+23° 44′ 21·4″±0·7″	21·4″±0·7″	0·0″±1·0″
PKS 1148–00	11 h 48 m 10·10 s ±0·05 s	10·15 s ±0·03 s	−0·05 s ±0·06 s	−00° 07′ 12·9″±0·9″	12·9″±0·5″	0·0″±1·0″
NRAO 389	11 h 53 m 44·11 s ±0·05 s	44·13 s ±0·04 s	−0·02 s ±0·06 s	+31° 44′ 46·4″±0·8″	46·4″±0·5″	0·0″±0·9″
3C 287	13 h 28 m 15·91 s ±0·05 s	15·96 s ±0·04 s	−0·05 s ±0·06 s	+25° 24′ 37·0″±0·8″	37·5″±0·5″	−0·5″±0·9″
3C 286	13 h 28 m 49·66 s ±0·04 s	49·72 s ±0·04 s	−0·06 s ±0·06 s	+30° 45′ 58·3″±0·7″	58·4″±0·5″	−0·1″±0·9″
3C 298	14 h 16 m 38·80 s ±0·05 s	38·84 s ±0·03 s	−0·04 s ±0·06 s	+06° 42′ 19·7″±1·0″	20·7″±0·5″	−1·0″±1·1″
3C 345	16 h 41 m 17·57 s ±0·04 s	17·64 s ±0·04 s	−0·07 s ±0·06 s	+39° 54′ 10·6″±0·6″	10·6″±0·5″	0·0″±0·8″
3C 380	18 h 48 m 13·47 s ±0·04 s	13·54 s ±0·05 s	−0·07 s ±0·06 s	+48° 42′ 41·0″±0·7″	40·5″±0·5″	+0·5″±0·9″
3C 418	20 h 37 m 07·41 s ±0·06 s	07·45 s ±0·05 s	−0·04 s ±0·08 s	+51° 08′ 36·0″±0·8″	36·4″±0·5″	−0·4″±0·9″
PKS 2127+04	21 h 28 m 02·58 s ±0·05 s	02·66 s ±0·05 s	−0·08 s ±0·07 s	+04° 49′ 04·1″±0·8″	04·0″±0·7″	+0·1″±1·1″
PKS 2145+06	21 h 45 m 36·05 s ±0·06 s	36·16 s ±0·05 s	−0·11 s ±0·08 s	+06° 43′ 40·4″±1·3″	39·9″±0·7″	+0·5″±1·5″
CTA 102	22 h 30 m 07·77 s ±0·04 s	07·83 s ±0·03 s	−0·06 s ±0·05 s	+11° 28′ 22·8″±0·7″	23·1″±0·5″	−0·3″±0·9″
3C 454	22 h 49 m 07·68 s ±0·04 s	07·77 s ±0·05 s	−0·09 s ±0·06 s	+18° 32′ 44·2″±0·7″	43·6″±0·7″	+0·6″±1·0″
3C 454·3	22 h 51 m 29·50 s ±0·04 s	29·54 s ±0·03 s	−0·04 s ±0·05 s	+15° 52′ 53·7″±0·7″	54·9″±0·5″	−1·2″±0·9″

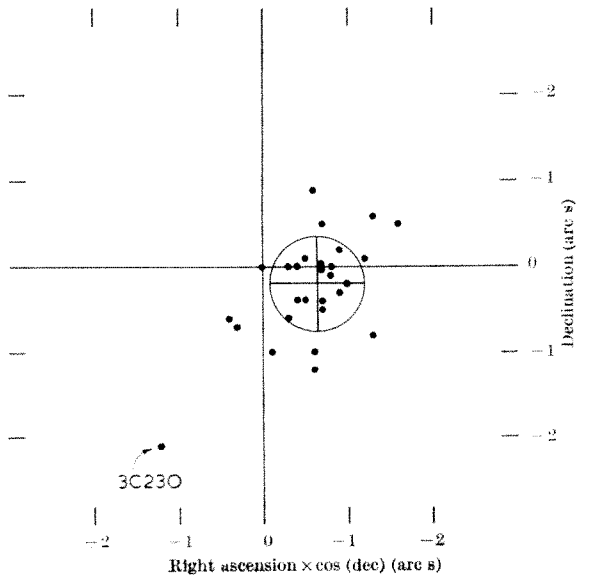


Fig. 1. Radio position differences NRAO–RRE. The circle (diameter 1·1 arc s) is the median circle of a two-dimensional Gaussian distribution having a standard error of 0·5 arcs in each coordinate.

that the random errors in each catalogue are about 0·35 arc s in each coordinate (about 0·025 s in RA).

The standard errors calculated from the estimated uncertainties given in Table 1 are about twice those given by the observed residuals. This is not surprising, for the uncertainties quoted for each catalogue are intended to include some allowance for systematic as well as for random errors.

Table 2. MEAN POSITION DIFFERENCES AND STANDARD ERRORS

	R.A.	R.A. cos (dec)	Dec
Weighted mean difference (NRAO–RRE)	−0·044 s ±0·006 s	−0·58″ ±0·09″	−0·19″ ±0·10″
Standard error of one position difference of average weight	0·034 s	0·46″	0·50″

The mean difference in declination is just less than twice its standard error, and can hardly be considered significantly different from zero. The close grouping of the two sets of measurements in declination is an important result, as it shows that measurements based on a sufficient number of optical calibrators (RRE) can give satisfactory agreement with absolute measurements (NRAO).

In the measurement of right ascensions the radio catalogues are on the same footing; they both give absolute R.A. differences but require optical calibrators to fix the R.A. zero point. The small standard error in the R.A. differences between catalogues indicates good agreement by NRAO and RRE in the measurement of relative right ascensions. The catalogues nevertheless differ significantly in zero point, by −0·044 s in R.A. The cause of this difference is not clear. The standard error of the mean R.A. difference is only 0·006 s, and this suggests that most of the discrepancy must arise from differences among the optical positions used to fix the zero point. Further consideration of such differences lies outside the scope of this letter, which is intended to draw attention to the existence of radio position lists having standard errors significantly better than 1 arc s.

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Seasonal Changes in the Ionosphere

MARKED changes occur in the ionosphere during the year. In most studies statistical methods have been used because of the superimposed day to day fluctuation usually dividing the year into summer, winter and equinoctial periods. This type of examination, however, tends to mask the fundamental changes particularly at the equinoxes.

An important feature is that in the southern hemisphere the seasonal change is abrupt in the Sept–Oct equinox and more gradual in March–April. These changes appeared consistently during investigations in Australia of travelling ionospheric disturbances as a reversal of the N–S component of travel of TID's in late September or early October[1].

The effect showed up again in satellite observations. I demonstrated the simultaneous occurrence of ionization changes deduced from satellite records of total ionization and from ionosonde records, and changes in direction of TID's, in September 1958 (ref. 2). The occurrence of a magnetic storm at this time was noted and further observations indicated that a magnetic storm could trigger off the change. This has since been confirmed by Titheridge[3] in New Zealand from observations on a geostationary satellite, which showed that the seasonal change involved a major change in total ion content at all times of the day, and was initiated by a magnetic storm.

A comprehensive set of travelling disturbance data was available covering a period of twelve years, and a closer examination of that data was made.

The data consisted of records from a three-point system for observing the movements of travelling ionospheric disturbances near Sydney (lat. 34° S, long. 150° E). The period examined was from April 1948 to October 1959, covering a complete sunspot cycle. For the present investigation the deduced directions of horizontal movement of all disturbances were plotted for each day of the two month periods March–April and September–October in each year. These plots showed clearly that the seasonal change in direction was not a smooth transition but there were fluctuations between summer and winter directions lasting for a few weeks. This pattern was particularly obvious in the Sept–Oct period.

When the daily K_p indices were plotted it was found that the changes to the new direction were consistently associated with marked rises in K_p and temporary reversions after the main change were associated with low values of K_p.

In all years there were sufficient data to assess the significant changes. The features extracted for each

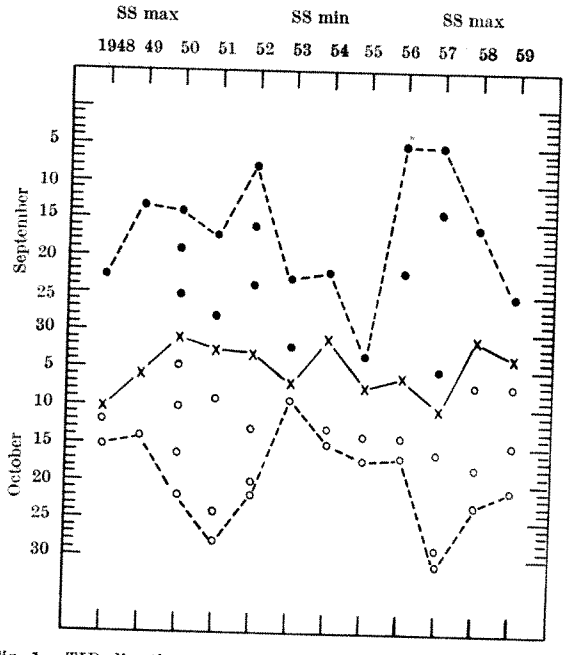


Fig. 1. TID direction changes, September–October. ●, Temporary change; ×, main change; ○, temporary reversions.

year were the temporary changes to the new direction, the main change after which the new direction predominates, subsequent reversions to the old direction and the final change to the new direction after the last temporary reversion, which is clearly defined in the September–October plots. These features are summarized in Fig. 1. Although there may be earlier temporary changes, the main changes all take place within the first ten days of October, the actual day apparently being decided by a magnetic increase. There are some subsequent temporary reversions but the final changes to summer directions all occur by the end of October. The variation in day of occurrence during the sunspot cycle is small but suggests a half-cycle variation and this seems more strongly indicated in the final change dates and on the time spread and number of temporary changes. The change on September 17, 1958 (ref. 2), was actually a preliminary one before the main change.

The transition shown by Titheridge in 1965 began on September 27 and the total change occupied about 25 days. The particular storm which initiated it could therefore correspond with a temporary change of direction of TID's. The total change period of 25 days seems to agree well with the average period when disturbance directions were susceptible to magnetic control (Fig. 1).

To confirm that the effect was essentially an equinoctial one a check of magnetic indices and disturbances was made for the other months of the years involved.

During the winter months, May to August, major changes of direction were rare. In the falling years of the sunspot cycle (1949–51) none were recorded. From 1952 to 1955 the rate of occurrence was about one per year associated with high K_p values. In 1956 there were three cases. 1957–58 were years of unprecedented sunspot activity and the summer influence extended into May and June, but in July and August winter directions were steady.

It seems clear, therefore, that the Sept–Oct equinox is a time of instability in the ionosphere and it is then particularly susceptible to fluctuations in the magnetic field which result in changes in the distribution of ionization and corresponding changes in the direction of travel of disturbances.

It seems that the magnetic fluctuations affect chiefly the north–south component of movement of disturbances and the directional changes are most marked in the Sept–Oct equinox when the north–south component predominates. At this time magnetic storms are known to have effects similar to the seasonal change and can thus have a triggering effect making the change seem quite abrupt.

In the March–April equinox the seasonal change in the direction of movement is more gradual, apparently for two reasons; magnetic disturbances tend to retard the seasonal change and the east–west component of movement is then predominant so that changes in the north–south component have less effect in terms of a direction change.

This letter aims only at presenting factual information and the results offer wide scope for theoretical interpretation. Duncan[4] provides a good basis by comparing the effects of seasonal changes and magnetic storms. The possibilities are obvious for extending the investigation in time, in height and in latitude using other types of data already available.

It is particularly desirable to have comparative information from the northern hemisphere. For current investigations geostationary satellite data would be most convenient, but for correlation with information for the earlier years described here ionosonde and earlier satellite records could be used.

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Upper Bound to the Density Jump at the Boundary of the Earth's Inner Core

The Earth has an inner core about the size of the Moon. Convincing observations of the seismic waves PKiKP, reflected at steep angles from the inner core boundary (ICB), were announced by Engdahl *et al.* (annual meeting of the Seismological Society of America), using the Large Aperture Seismic Array (LASA) in Montana (epicentral distances $\Delta < 40°$). These identifications agree with expected travel times and wave slownesses[1] ($p = \mathrm{d}t/\mathrm{d}\Delta$), and pPKiKP minus PKiKP time lags.

This extraordinary discovery confirms the earlier identified[2] PKiKP waves, reflected at grazing angles from the ICB ($105° < \Delta < 110°$). It establishes once and for all that the ICB (radius $r \sim 1{,}216$ km) is sharp[3,4] relative to seismic longitudinal waves with periods of order 1 s. It also opens the way for the direct calculation, by purely seismological means, of the density contrast between the material of the outer (fluid) core and of the inner core.

Here we derive tentatively (partly to demonstrate the method involved) the first upper bound on this estimate, using measured amplitudes of PKiKP and PcP phases recorded at LASA. (The PcP phase is reflected at the sharp boundary between the mantle and outer core (MCB), at a radius of about 3,479 km.) The observed phases, shown in Fig. 1, were generated by the underground explosion Faultless (magnitude about 6·0) in Nevada ($\Delta \sim 10°{\cdot}9$). The directly measured amplitude ratio, $\mathcal{A}$ = amp PKiKP/amp PcP, should be a reliable estimator for two reasons. First, the phases (which have similar appearances) are recorded by the same seismometers and with identical processing; no instrumental or crustal adjustments are needed. Second, the downgoing PKiKP

and PcP rays leave the explosive source at almost the same angles of emergence ($e \approx 89°$, 87°, respectively). Consequently, no nodal surfaces of source radiation complicate the energy partition and we may assume that the wave amplitudes in the downgoing PKiKP and PcP rays are essentially equal.

The identified phases in Fig. 1 have residuals of only a few seconds against the appropriate standard travel time tables. Further, processing with the LASA array indicates slownesses of about 0·5 and 1·2 s deg^{-1} for the recorded PKiKP and PcP waves. These raw values are close to the expected values of $p = 0{\cdot}3$ and $p = 1{\cdot}0$ s deg^{-1}, respectively. Measurements of $\mathcal{A}$ from four independently processed groups of signals from different subclusters of the array yield a mean of $\mathcal{A} = 0{\cdot}38$ with a variation of about 5 per cent.

The raw value for $\mathcal{A}$ must, however, be adjusted for the relative geometrical spreading τ_{ig}, of the two waves, as well as the relative frictional attenuation τ_{iQ}. Given the P velocity distribution down to the ICB, the value of τ_{ig} can, in principle, be determined exactly. By theory, for the same source and receiver, apart from a factor 1/4 involving the angles of incidence

$$\tau_{ig}^2 = \left| \left(\frac{dp}{d\Delta}\right)_{PKiKP} \Big/ \left(\frac{dp}{d\Delta}\right)_{PcP} \right| \qquad (1)$$

For steep reflexions, the seismic rays are approximately straight lines. In this case, we easily derive

$$\frac{dp}{d\Delta} = \frac{1}{2} p \cot \frac{\Delta}{2} - \frac{1}{t} p^2 \qquad (2)$$

where Δ is the epicentral distance and t is the travel time of the phase in question. From equation (2), $\tau_{ig} = 0{\cdot}47$ for $\Delta = 10°{\cdot}9$. In principle, τ_{ig} can be checked,using equation (1), from the curvature of the empirical travel time curves; in practice, uncertainties in measuring curvature are high. Present standard tables suggest $\tau_{ig} \approx 0{\cdot}5$–$0{\cdot}6$.

The PKiKP waves reflected from the inner core have additional frictional attenuation compared with the PcP waves. The former travel a further distance of more than 3,500 km through the liquid outer core. (Special effects due to the outer boundary and physical state of the 440 km thick transition shell[4] around the inner core are ignored throughout this argument.) Suppose the attenuation of the wave amplitude varies like

$$\tau_{iQ} = \exp(-\pi t / Q\tau) \qquad (3)$$

where τ is the period, and Q a dimensionless attenuation factor.

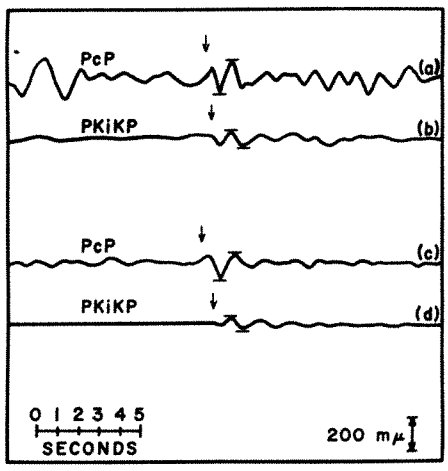


Fig. 1. Seismograms at LASA, Montana ($\Delta = 10°{\cdot}9$) from the underground nuclear explosion Faultless in Nevada on January 19, 1968. The traces are: *a* and *b*, signal sum of 25 seismometers in the C_4 cluster; *c* and *d*, velocity-filtered sum from seismometers across complete array. Arrows show phase onsets, horizontal bars show amplitudes measured.

In the liquid outer core, the value Q_c for P waves of period 1 s is probably in excess of 10,000. Seismic measurements indicate that Q values for P waves increase to about 1,500 near the base of the solid mantle. The purely radial free oscillation of the Earth, ${}_0S_0$, persists for weeks after great earthquakes, favouring a mean Q value greater than 10,000 for compressional vibrations of the whole Earth. More direct values for Q_c can be obtained from amplitude measurements of the family of seismic waves PKP, PKKP, PKKKP, and so on, although this work has not yet been done. These waves are multiply reflected from the underside of the MCB; PKKP has two legs in the outer core, and so on. Such waves with three and four legs are often recorded under appropriate observational conditions. Engdahl[5] has reported observing multiple reflexions up to PKKKKKP. Rough calculation suggests that these observations require $Q_c > 10{,}000$. From equation (3), this value gives $\tau_{iQ} \geq 0{\cdot}85$.

The required density ratio d_I at the ICB arises as follows. Let subscripts 0, 1, 2, 3, 4 refer to the terrestrial surface, the top and bottom of the MCB and ICB, respectively. Let e and f be the angles at which P and S waves emerge at a boundary.

Consider the ray system generated by an incident P wave at the ICB in terms of the P and S potential functions[6] φ and ψ; incident and reflected waves in the liquid core may be represented by

$$\begin{cases} \varphi_3 = A_3 \exp\{i\varkappa(z \tan e_3 + x - ct)\} \\ \qquad\qquad + C_3 \exp\{i\varkappa(-z \tan e_3 + x - ct)\} \\ \psi_3 = 0 \end{cases} \qquad (4)$$

where $2\pi/\varkappa$ is the wavelength, and z is along the radius towards the centre. Similarly, the potentials for refracted P and SV waves in the inner core (assumed solid) are

$$\begin{cases} \varphi_4 = D_4 \exp\{i\varkappa(z \tan e_4 + x - ct)\} \\ \psi_4 = B_4 \exp\{i\varkappa(z \tan f_4 + x - ct)\} \end{cases} \qquad (5)$$

Three boundary conditions apply: zero tangential stress (to a close approximation) and continuity of normal displacement and normal stress. The resulting three equations in the unknown amplitudes A_3, C_3, D_4, B_4, are

$$\begin{bmatrix} \tan^2 f_4 - 1 & 0 & 2 \tan e_4 \\ 1 & -\tan e_3 & -\tan e_4 \\ 2\beta_4^2 \tan f_4 & d_I \alpha_3^2 \sec^2 e_3 & -\beta_4^2(\tan^2 f_4 - 1) \end{bmatrix} \begin{bmatrix} B_4/A_3 \\ C_3/A_3 \\ D_4/A_3 \end{bmatrix} = \begin{bmatrix} 0 \\ -\tan e_3 \\ -d_I \alpha_3^2 \sec^2 e_3 \end{bmatrix} \qquad (6)$$

The third equation contains the required $d_I (= \rho_3/\rho_4)$ at the ICB. Equation (6) together with appropriate triads from MCB continuity conditions may be solved simultaneously for $\mathcal{A}$ as a function of adopted values for d_I and β_4, the (unknown) shear velocity in the inner core.

From recent seismological studies[7–10] the values adopted as most likely, in usual units, for density ρ, P velocity α, S velocity β at MCB and ICB are: $\rho_1 = 5{\cdot}51$, $\alpha_1 = 13{\cdot}20$, $\beta_1 = 6{\cdot}99$; $\rho_2 = 9{\cdot}70$, $\alpha_2 = 7{\cdot}99$; $\rho_3 = 12{\cdot}35$, $\alpha_3 = 10{\cdot}31$, $\alpha_4 = 11{\cdot}23$. The reflexion coefficient for steep PcP rays at the MCB is roughly proportional to a term $|\rho_2\alpha_2 - \rho_1\alpha_1|$ which is quite sensitive to the uncertainties of the particular combination of densities and velocities which have been estimated for the MCB. The adopted values give a high (but not maximum) value to this term. The choice is

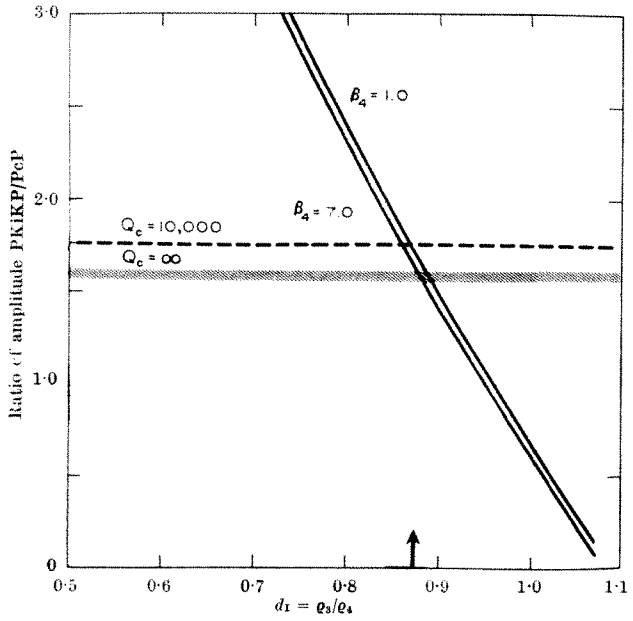


Fig. 2. The variation of the amplitude ratio PKiKP/PcP at the Earth's surface for $\Delta = 10°{\cdot}9$ as a function of d_I, the density ratio at the inner core boundary.

consistent with seeking a lower bound to d_I at ICB (and hence a high reflexion coefficient for PKiKP there).

The results of the computation are shown in Fig. 2 for two representative cases, $\beta_4 = 1{\cdot}0$ and $7{\cdot}0$. The small offset between the two curves is a result of the small proportion of wave energy converted to SV waves in the inner core at steep angles of incidence. Ignorance of the actual β_4 values does not, therefore, much affect the uncertainty in the lower bound to d_I. (Derr[9] inferred $\beta_4 \approx 2{\cdot}2$ km s^{-1} from observations of terrestrial free oscillations.)

The broad horizontal line in Fig. 2 represents the observed value of the amplitude $\mathcal{A}$ adjusted for the complete geometrical factor 0·24 only ($Q_c = \infty$). The parallel broken line corresponds to $\mathcal{A}$ after allowance for the frictional attenuation $Q_c \approx 10{,}000$.

From the region of intersection of the curves we deduce that a fairly extreme minimum value of d_I is 0·86; a more likely minimum is perhaps 0·875. For the plausible value[9–11] of $\rho_3 = 12{\cdot}35$, these ratios suggest a maximum density jump at the ICB of less than 1·8 g cm^{-3}. The relatively steep slope to the curves in Fig. 2 strengthens the overall argument by demoting the various uncertainties associated with the adjusted observed estimate of $\mathcal{A}$.

It is quite remarkable that seismological evidence alone can establish that, 1,200 km from the Earth's centre, the density does not exceed 14·25 g cm^{-3}. (The pressure at this radius is $3{\cdot}3 \times 10^{12}$ dyne cm^{-2}.) Only a few years ago, central densities in the Earth as high as 18 g cm^{-3} were seriously considered. Uncertainties in the present bound need to be checked from many additional observations of recorded PcP and PKiKP amplitudes. (The ratio of the amplitudes of PKPPKP and PKiKP, also recorded on the same seismogram, should provide an additional equation of condition.)

The result $\rho_4 < 14{\cdot}25$ is in close agreement with recent core models of Bullen[10], and also with Birch's conclusions[12], based partly on the results of shock wave experiments on iron, that the Earth's central density is close to 13 g cm^{-3}. The principles involved in making the present estimate do not depend, of course, on assumptions concerning the chemical composition of the core. If the bound reported here can be confirmed, important inferences on the composition of the whole core follow.

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Eustatic Sea Level Changes in the Arafura Sea

The lowest eustatic sea level during the past 35,000 years has until recently been estimated to be -130 m[1]. Preliminary results of recent marine geological research in the Arafura Sea substantiate the evidence[2] for a worldwide eustatic lowering of sea level of more than 130 m between 21,000 and 14,000 yr BP. Observations in the Arafura sea also support contemporary discoveries[3,4], which resulted from the introduction of submersibles and narrow beam echosounders, of submarine terraces down to 200 m. These deep terraces reflect a much lower sea level earlier in the Pleistocene. Radiometric dating of a shallow-water reef coral, recovered from a terrace at -200 m during descent in the Japanese research submersible Yomiuri, gave an age greater than or equal to 170,000 yr BP.

The morphology of the Arafura is that of a submerged subaerially eroded landsurface[5], with a fluvial pattern of channels and drowned reefs near the edge of the shelf[6]. Near the Australian coast the old subaerial[2] topography has been covered by fine grained marine sediments. Depths on the shelf are generally between 50 and 80 m and the edge of the shelf is usually between 120 and 200 m. The southern part of the shelf has undergone very little movement since the late Pliocene[7]. During 1969, the Bureau of Mineral Resources carried out a marine geological survey of the Arafura shelf and covered most of the area by dredge sampling, gravity coring, shallow continuous seismic profiling and echosounder profiling. In addition, three traverses across submarine terraces were undertaken (Fig. 1) in the Japanese submersible Yomiuri.

A number of terraces and nickpoints representing low sea level stands have been observed in echosounder and continuous seismic profiles at depths of 64 m, 90 m, 130 m, 170–175 m and at about 200 m. The first three of these terraces correspond closely to those reported on other parts of the continental shelf of Australia such as the Sahul Shelf[7] and the Great Barrier Reef[8]. A shallow-water fossiliferous algal rock recovered by dredge from a submerged reef between 130 m and 175 m (station 1231, Fig. 1) gave a radiocarbon date of $18{,}700 \pm 350$ yr BP (Table 1). This rock is older than the shell material obtained from a 130 m surface on the Sahul Shelf[7] ($16{,}910 \pm 500$ yr BP), and it is possible that the rock came from a depth significantly greater than 130 m. The terrace between 170 and 175 has not yet been dated. A platy

fossiliferous beachrock, which was collected during dive III at a depth of 150 m (Fig. 1), may correspond to a terrace at similar depth off the Great Barrier Reef[2], apparently formed 13,600 to 17,000 yr BP.

The still deeper terrace at −200 m observed in the seismic and echosounder profiles was investigated by submersible (dive II, Fig. 1). A traverse across the feature revealed a mud-filled channel with a maximum depth of 210 m at the base of a scarp 20 m high. Samples of rock from the base of this scarp at 200 m depth were collected with the mechanical arm of the submersible. The rocks consisted of a mixture of shallow-water reef coral, abraded mollusc and foraminiferal fragments, bryozoans, and algal fragments, cemented loosely by calcite. A high proportion of void space and the presence of material affected by wave action suggest a littoral origin. Radiocarbon dating gave an age greater than 30,000 yr BP for this material (Table 1). Further dating by H. H. Veeh of the Australian National University using the uranium series method on unrecrystallized coral in one of the samples indicated an age of 170,000 yr or more. This shows that the 200 m terrace was formed before the last glacial lowering of sea level, possibly during one of the previous glacials. A similar suggestion has been made by Dill[3] for the deep sea terraces discovered on the continental slope off California and around most of Australia. Thus there is reason to accept a lowering of sea level greater than the 130 m earlier postulated[1], during the late Würm Glacial to about −175 m[2], but the deeper terraces in the Arafura Sea and those discovered by Dill[3] between depths of 182 m and 214 m are older than this, and may have been formed by sea level lowering during one of the previous glacials. As earlier demonstrated by Donn *et al.*[9] the maximum eustatic lowering of sea level took place during the Riss glacial; this glacial could be the period during which the deeper terraces originated.

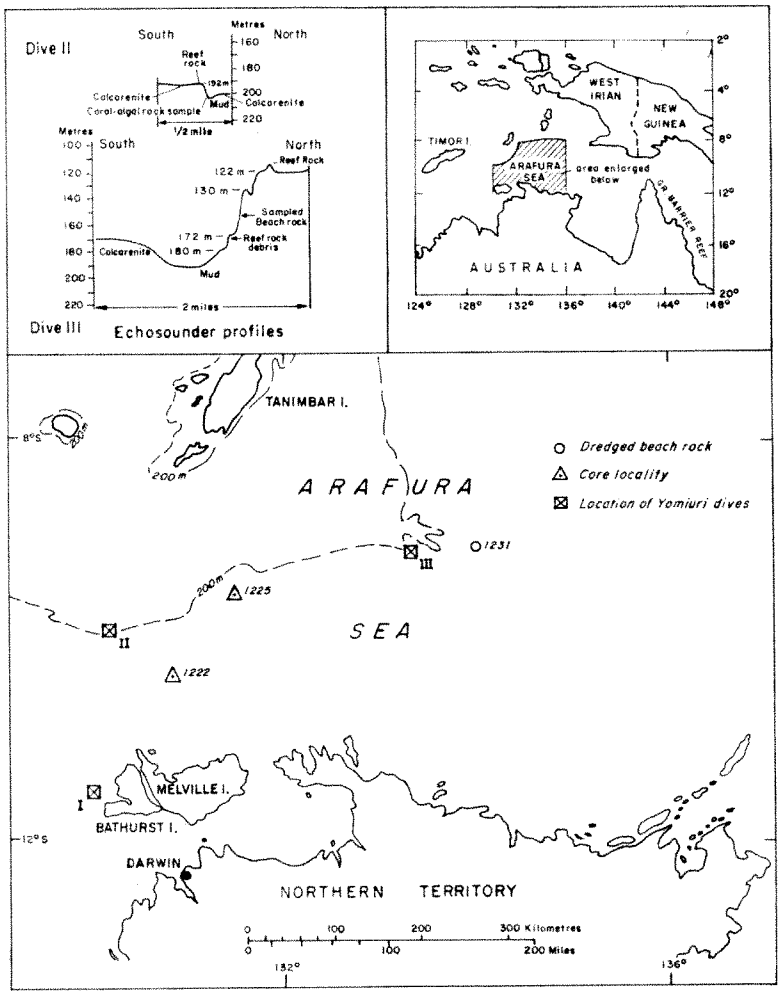


Fig. 1. Locality map of dated samples and submersible descents with echosounding profiles in the vicinity of dives II and III.

Two further dates of material from short cores are available. Core No. 1225 (Fig. 1), water depth 117 m, was stopped by a shelly horizon 125 cm below the surface. Abraded shells from the bottom of this core gave an age of older than 30,000 yr BP by the radiocarbon method (Table 1). Core No. 1222, water depth 99 m, struck wood 130 cm below the surface, which gave a radiocarbon date of 14,500 ± 700 yr BP (Table 1). These two dates point to marine transgressions at around 14,000 yr BP and before 30,000 yr BP; the earlier event is subject to serious doubt because the worn shell material may not be a littoral deposit and, even if it is, the shells may significantly antedate the transgression. The wood appeared to occur in a fossil soil, but here again the possibility that it is derived must be borne in mind.

The history of sea level changes in the Arafura Sea agrees with that of other areas in Australia[2,8], the east coast of America and the south-east Caribbean Sea[10]. Dates from recently collected material in the Arafura Sea indicate a eustatic lowering of sea level to about −200 m before 170,000 yr BP. This was followed by a transgression before 30,000 yr BP, and a subsequent lowering of sea level to −175 m after 20,000 yr BP. A rise in sea level to its present position eventuated soon after 14,500 yr BP.

Table 1. RADIOMETRIC DATES OF ARAFURA SEA SAMPLES

Station No.	Location	Depth (m)	Age BP (years)	Material
1,231	9 07′ S 133 52′ E	130–175	18,700 ± 350	Beach rock
Dive II	9 55′ S 130 03′ E	210–200	⩾ 170,000	Coral*
1,225	9 44′ S 131 0′ E	117	> 30,000	Shells, base of 125 cm core
1,222	10 20′ S 130 46′ E	99	14,500 ± 700	Wood, base of 130 cm core

Radiocarbon dates were supplied by the Department of Nuclear and Radiation Chemistry, University of New South Wales, NSW, Australia.

* Dating by H. H. Veeh of the Australian National University.

The uranium/thorium date was supplied by Dr H. H. Veeh of the Australian National University. Dr H. A. Jones and Mr D. S. Trail of the Bureau of Mineral Resources collaborated in the collection of field data and in discussion of the results. The collection of some of the samples was made possible through the assistance of the Yomiuri Newspaper Company, who put the submersible at our disposal.

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Plane Bed Thresholds of Fine Grained Sediments

WHEN the flow of fluid over a plane bed of loose grains is increased from zero, a condition is reached where a few grains move. This is known as the threshold of grain movement. This report describes the determination of the threshold under flows of water and oil for beds of mineral grains of uniform size with diameters between 20 and 200 μm.

Shields[1] proposed that the tangential fluid stress at the boundary τ_0 responsible for grain movement at the threshold should be combined with the weight, per unit area of the bed, of the particles forming its topmost layer. This gives a non-dimensional entrainment function

$$\theta_0 = \frac{\tau_0}{(\sigma - \rho)gD}$$

also known as the dimensionless fluid stress[2], σ and ρ being the densities of grain and fluid, g the gravitational acceleration and D the particle diameter. The boundary Reynolds number is defined as U_*D/ν where $U_* = (\tau_0/\rho)^{\frac{1}{2}}$ and ν is the kinematic viscosity of the fluid. If the threshold values of U_* and θ were labelled U_t and θ_t, Shields argued on dimensional grounds that θ_t should be a function of U_tD/ν only.

This has been confirmed several times[3-6]. It has been established that θ_t is nearly constant, at about 0·05, for a wide range of diameters greater than 200 μm.

200 μm is a critical diameter for plane beds of geologically important minerals. Above this diameter such beds are hydrodynamically rough at the threshold, below it they are smooth[7,8]. There is every reason to suspect that the value of θ_t for finer grains will be greater than the value of 0·05 found for coarser material because when the bed is hydrodynamically smooth a laminar sublayer is established in the fluid immediately above it. Short-lived velocity fluctuations that can perhaps quadruple the instantaneous force on exposed particles for a given mean stress[9-11] are much reduced. Bagnold has argued[2] that θ_t should not, however, exceed 0·4 for cohesionless grains.

Little has been published about the thresholds of plane beds of material under 200 μm diameter. Hjulström's[12] work on river sediments is often quoted as showing that the absolute value of stress necessary for erosion increases as the size decreases below this value. It appears that θ_t increases to a value greater than 10 for $D = 10$ μm. Rees[6] reports a value $0{\cdot}1 < \theta_t < 0{\cdot}4$ for a clean 10 μm silt.

New threshold determinations have been made in a recirculating flume 6 m long and 30 cm wide (Fig. 1), equipped with an orifice plate flow gauge[13] for discharge measurement and a micrometer point gauge for measuring water heights and hence surface slopes. Depth of flow and bed surface slope were measured with the microscope used for threshold observations. Thresholds were determined by observing through a microscope the first movement of a plane sediment bed as the fluid stress over it was increased.

Observations were made using closely sized fractions of quartz sands and silts, obtained by sieving and by air and water elutriation of natural materials. Particular care was taken to remove very fine grained material. The sphericity and angle of repose of each sediment grade were estimated microscopically. Other measurements were made using spherical lead glass "ballotini", crushed silica and crushed plastic of about 2 mm diameter.

Two fluids were used, tap water and Mentor 28 oil ($\nu = 0{\cdot}08$ Stokes). All water flows were turbulent, but most of those with oil seemed laminar. Values of bottom stress in good agreement were estimated using du Boys's equation $\tau_0 = \rho g h \beta$ where h is the depth of flow and β its surface slope, and by using established engineering formulae. Corrections were made for accelerations and for side wall effects.

Sediment beds in water were prepared for threshold determination by depositing the material grain by grain from water moving not quite strongly enough to cause erosion. In the oil experiments, beds had to be prepared by raking.

The threshold stress of each bed was measured about ten times with different depths of water. The range of depths was limited by the apparatus to 2–7 cm. Table 1 gives the mean values of θ_t. Fig. 2 shows the results plotted, together with those of other workers, on a diagram of θ_t against U_*D/ν. The broken line is an extrapolation made by Shields.

The results add further confirmation to Shields's general argument although they do not agree with his extrapolation. Within the experimental scatter a single curve relates measurements made with a thousand-fold range in the boundary Reynolds number by means of a hundred-fold range of submerged density and an eight-fold range of fluid viscosity. θ_t increases for low values of the Reynolds number, U_*D/ν to a value approaching the theoretical maximum of 0·4 suggested by Bagnold.

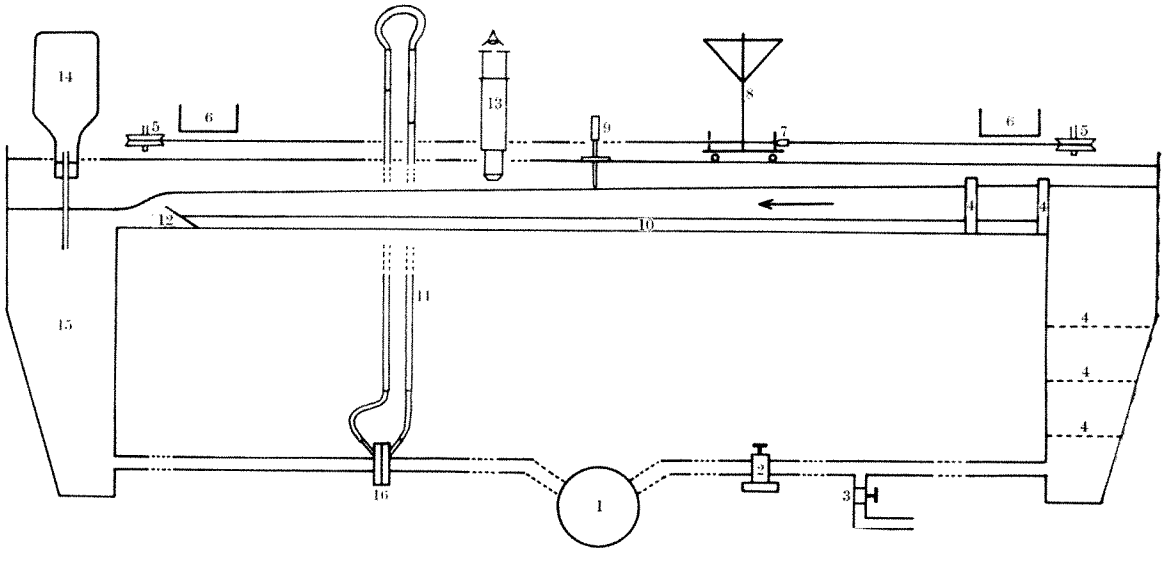


Fig. 1. The flume and apparatus. 1, Centrifugal pump; 2, flow-rate valve; 3, outflow top; 4, baffle plates; 5, pulleys; 6, collecting boxes; 7, messenger; 8, sand hopper; 9, micrometer screw gauge; 10, sediment bed; 11, differential manometer; 12, weir; 13, travelling microscope; 14, silt hopper; 15, fluid; 16, flow gauge.

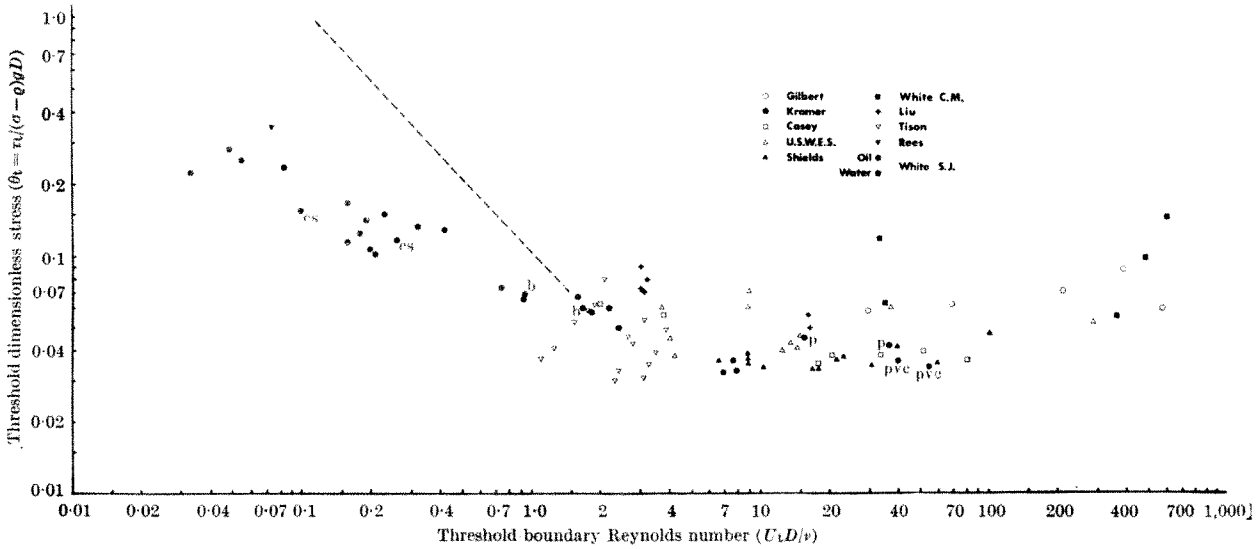


Fig. 2. The threshold of sediment movement as a function of the boundary Reynolds number. b, Ballotini; cs, crushed silica; p, polystyrene; pvc, Welvic P.V.C.

It was thought that it might be possible to detect a correlation between θ_t and the physical properties of the sediments used. Multiple regression analyses showed no correlation between θ_t and such sediment properties as sphericity, sorting and angle of repose. Neither was any relationship found between θ_t and the depth of flow or the surface slope of the fluid.

Table 1. MEAN VALUES FROM THE EXPERIMENTS

Material	Median diameter, D (mm)	Mean dimensionless threshold stress, θ_t Du Boys's equation	Engineering formulae
		Tap water	
Polystyrene	2·2	0·053±0·006	0·037±0·007
Welvic P.V.C.	2·0	0·037±0·002	0·040±0·005
Natural sediment	0·170	0·055±0·003	0·060±0·003
Natural sediment	0·153	0·058±0·002	0·061±0·003
Lead-glass ballotini	0·137	0·055±0·001	0·066±0·003
Natural sediment	0·133	0·071±0·002	0·078±0·003
Natural sediment	0·093	0·066±0·002	0·068±0·002
Lead-glass ballotini	0·088	0·073±0·003	0·066±0·003
Natural sediment	0·077	0·073±0·002	0·074±0·003
Natural sediment	0·044	0·125±0·004	0·131±0·003
Crushed silica	0·033	0·112±0·004	0·123±0·005
Natural sediment	0·030	0·102±0·003	0·098±0·007
Natural sediment	0·029	0·103±0·006	0·109±0·003
Natural sediment	0·028	0·146±0·004	0·153±0·004
Natural sediment	0·024	0·110±0·003	0·118±0·005
Crushed silica	0·016	0·151±0·007	0·154±0·010
		Mentor oil	
Polystyrene	2·2	0·037±0·005	0·034±0·002
Welvic P.V.C.	2·0	0·034±0·002	0·034±0·001
Natural sediment	0·133	0·132±0·003	0·132±0·003
Natural sediment	0·093	0·122±0·009	0·125±0·007
Lead-glass ballotini	0·088	0·143±0·006	0·139±0·003
Natural sediment	0·077	0·166±0·008	0·167±0·009
Natural sediment	0·046	0·218±0·014	0·248±0·022
Natural sediment	0·033	0·254±0·015	0·248±0·011
Natural sediment	0·030	0·288±0·016	0·269±0·023
Natural sediment	0·025	0·219±0·006	0·218±0·012

Evidence was sought for "ageing" of the bed during each series of experiments. The bed laid down initially by deposition or by raking might be more or less resistant to erosion than a bed the surface of which had been modified by exposure to stresses at or near to the threshold. No correlation was found between measured threshold and the order of the experiments, however, and it was concluded that no significant ageing took place.

This investigation is not complete because it deals only with plane bed thresholds of sediments that are as near as possible free of the cohesive material that is a frequent component of their size equivalents in nature.

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Silurian Fossils from New Zealand

IN spite of its relatively small land area, New Zealand contains a remarkably complete fossil record. Up to the present the Carboniferous and the Silurian have been the only two Phanerozoic geological periods not known to be represented by fossils. The apparent absence of Silurian fossils has been considered strange because upper Ordovician and lower Devonian fossils are present in sedimentary sequences in north-west Nelson, South Island, which do not seem to be interrupted by regional unconformity, and which therefore presumably contained sediments of Silurian age[1]. The conclusive identification of Silurian fossils from north-west Nelson is therefore of considerable interest.

A useful summary of the history of geological endeavours in New Zealand is provided by Waterhouse[2]. In particular he pointed out that a Silurian age had, in the past, been wrongly assigned to several of the principal shelly faunas and rock groups of the South Island. For instance, the Haupiri Group of north-west Nelson was considered Silurian on stratigraphic grounds until Benson recorded[3] Cambrian trilobites. Similarly the Baton River fauna[4] of north-west Nelson was considered Silurian[5] until Shirley

showed[6] the fauna to be Lower Devonian (see also ref. 7). Grindley *et al.* considered[8] the Silurian to be represented in Nelson by unfossiliferous argillites (Wangapeka Formation of ref. 1).

Credit for discovering the first Silurian fossils goes to Professor Harold Wellman who collected in 1960 a boulder containing fragmental brachiopods from Dry Stream at the southern end of Takaka Valley, north-west Nelson. Should the Wangapeka Valley *Notoconchidium* fauna (see later) prove to be Silurian, this credit would go to Dr L. R. Grange for collections made in 1923–24. Subsequently Cooper[9], following up Wellman's reconnaissance, collected a number of deformed fragmental brachiopod moulds from quartzites and sandstones of the Hailes Knob Quartzite near Hailes Knob, in the same district. The collections on which this report is based were made by the authors with M. R. Johnston and D. N. Williams during February 24–26, 1969, and are chiefly from new localities in the same formation.

The Hailes Knob Quartzite (about 196 m thick) is composed principally of metaquartzite and quartz sandstone and outcrops as a small (about 5 square km) synclinal inlier along the crest of the Pikikiruna Range. The Hailes Knob Quartzite is underlain conformably by Mount Arthur Marble from which upper Ordovician conodonts are known[10]; overlying beds are not preserved. Identifiable fossils are confined to the uppermost beds. Collections are dominated by brachiopods and are principally from leached gritty sandstones. Although restricted in variety, the brachiopod faunas include material best referred to the Silurian pentameroid, *Conchidium*[11], as well as dalmanelloids and strophomenoids; indeterminate tetracoral, trilobite and crinoid remains also occur. The age of the fauna is late Wenlockian or Ludlovian. Thus the Ordovician–Silurian boundary probably lies near the top of the Mount Arthur Marble at Hailes Knob. It should be noted that further south, beds above similar limestones are Upper Ordovician[12].

A second fauna, from quartz sandstones of the Wangapeka Valley, 40 km to the south, has been re-examined and found to contain the brachiopod *Notoconchidium*[13,14]. Other fossils include *Pleurodictyum* (now definitely known from the Silurian of Australia[15–17]) and *Molongia*; the single poorly preserved brachiopod specimen referred[13] to *Schizophoria* is now thought to be more probably a strophedontid. It has recently been suggested[13] that *Notoconchidium*, previously thought to be Lower Devonian in age in Victoria, Australia[15], is confined to the Ludlovian, suggesting a Silurian age for the Wangapeka Valley fauna. But Devonian age has recently been demonstrated[19] for upper beds of the Yass (NSW, Australia) sequence (see also ref. 20); from this a probably Gedinnian age can be inferred for *Notoconchidium*-bearing strata at Heathcote (Victoria, Australia). Thus the age of the *Notoconchidium* beds in New Zealand is uncertain.

Grindley mapped[1] the fossiliferous quartz sandstones of the Wangapeka Valley as Ellis Formation, but he was unaware of the Hailes Knob Quartzite; strata mapped as Ellis Formation elsewhere have so far proved unfossiliferous. Near Baton River, the Baton Formation lies unconformably on older rocks[4], apparently excluding the Ellis Formation. Detailed mapping, collecting and faunal studies between Takaka Valley and Wangapeka Valley are required to elucidate the relationships of Ordovician to Devonian sediments in that region of north-west Nelson. Accurate determination of the age of the New Zealand *Notoconchidium* fauna may have important implications for some East Australian Siluro–Devonian sequences.

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Disposal of Waste Material in Tectonic Sinks

GEOPHYSICAL phenomena such as deep earthquakes seem to mark zones where the material of the Earth's surface is being drawn into the mantle. The processes concerned take place at geological rates, and it may be estimated that surface material takes a million years to reach a depth of 25 km. Nevertheless, the process is often locally concentrated, so that subsidence is accompanied by grossly intensified accumulation of sediment where it should be possible to dispose of unwanted material permanently and in unlimited quantity.

Global production of industrial and domestic waste continually increases. A single nation produces more than 1 million tons per day of solid waste[1] and further regions are becoming industrialized so that worldwide production will increase without limit. The principal difficulty lies not in the sequestration of highly toxic material but in disposing of industrial and domestic refuse with low toxicity but large volume.

The solutions so far proposed have been but palliative[2]. Organic waste may be treated for use as fertilizer so that re-cycling is almost complete, but as technology progresses, solid waste containing metals and plastic is becoming more diversified and its elements more tightly bound, putting further beyond reach its sorting and re-use. Incineration does no more than re-distribute waste matter in the form of solid residue and atmospheric pollution. Energy considerations make it difficult to consider seriously proposals to inject unwanted material into orbit, or to dispose of it by nuclear burning.

At present the most widespread practice is to discard waste locally in makeshift fashion, under such appellations as "land fill". This solution is reaching its limits



Fig. 1. Location of sea-floor trenches and gravity anomalies associated with zones of subduction in the crust and mantle. The locus of surface-consumption on the floor of the trenches, shown in detail in Fig. 2, is too restricted to be visible at this scale. The fleches are plotted on the plate which is underthrusting, or being overthrust by, its neighbour.

simply because of a shortage of space. In some regions—San Francisco, for instance—the situation is such that train services have been organized to deposit refuse hundreds of kilometres from its source.

The practice entitled "ocean dumping", in effect the disposal of waste matter by sinking it at sea, offers no more prospect of being a solution than would be random "continental dumping". Yet restricted localities on the sea-floor are the site of downwarping and rapid sedimentary burial connected with events taking place in the Earth's interior. The processes responsible have not yet been elucidated, but the most plausible explanation is that an outer layer of the mantle is subject to circulatory convection.

Material is being added to the floor of the world ocean from below, in such a fashion that a process of spreading takes place outward from the axis of the ocean ridges[3,4]. Spreading takes place at a rate of between 3 and 9 cm per year, varying with locality. On a conservative assumption that the average spread rate is 5 cm/yr, that the ridge system is 40,000 km in length, and that a crust only 30 km thick takes part in this process, 60 km^3 of new crust is being added to the Earth's surface each year. As the planetary volume is changing less than this amount if at all, a corresponding volume of "old" crust must somewhere be replaced in the planetary interior. The sea-floor magnetic pattern indicates that the replacement process is not being effected by a general thickening of the crust, but is concentrated in zones of subduction (Fig. 1). These are marked by deep-seated seismicity, negative gravity anomalies, and tectonic trenches or foredeeps. In these localities crustal material is being drawn into the mantle.

On the basis of the magnetic evidence, more than 2,000 km^2 is being added to the Earth's surface each millennium and an equivalent area destroyed. It is not conceivable that surface destruction can take place without engulfment of material which has been deposited on the surface. Engulfment also takes place in concentrated fashion, in this case in the foredeep or trench fronting the zones of subduction.

A view common within the geological fraternity is that the tectonic trenches form where plate-like segments of the crust impinge on one another, resulting in thrusting of one plate beneath its neighbour. The subsurface form of some trenches, indicative of their deformation mode, is shown in Fig. 2. This is based on acoustic investigations with the restriction that detail is revealed only in the uppermost layers. In consequence it has not been possible to determine whether subduction takes place principally by subsidence and enfolding of the sea-floor, by its being thrust beneath the continental margin, or by a combination of both processes.

The convective process is slow, so that on the average an element of the Earth's surface must be exposed for a period in years equal to the surface area of the Earth divided by the area annually destroyed—$2{\cdot}6 \times 10^8$ years on the basis of earlier reckoning. In reality, however, depositional re-cycling and growth of the cratons result in gross departures from the average.

At points within sedimentary sinks the burial process is at its most rapid, because there the sediment from part of a continent may be deposited within a small area. Within a zone of 12 km^2 at the debouchment of the Fraser, for example, a river of intermediate size draining part of British Columbia, sediment deposited annually is in excess of 30 cm in thickness, ranging up to 6 m/yr in the foreset beds off the mouth of the river[8,9]. Conversely, almost no sediment has been found on the floor of the trench flanking western South America where the climate is arid[10].

The burial of surface material is therefore concentrated locally for two reasons: (1) the surface trace of subduction zones forms less than 1 per cent of the Earth's surface; (2) burial rate is multiplied a million or more times near the mouth of a major river. In places these factors operate together. The result is that in sedimentary sinks located within zones of subduction deposited material is buried within a few months and will never be seen again.

Depositional sinks of this nature are unique, for only here may material be said to disappear, ultimately into the planetary interior, without effort on our part. The

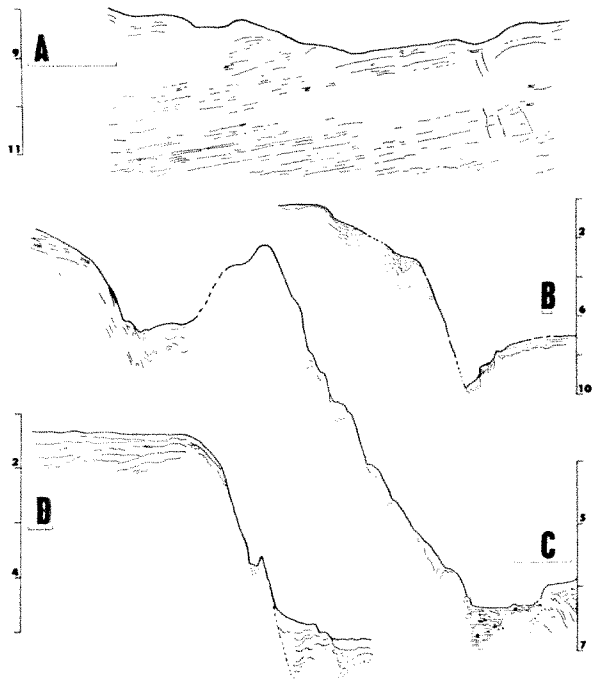


Fig. 2. Sediment accumulation and deformation in representative tectonic trenches, shown by acoustic profiling. Locations are shown in Fig. 1. In each profile the scale bar below the letter represents a horizontal distance of 10 km. The digits describe the two-way travel time in seconds. The topography of the trench is determined by the rate of sedimentation in relation to the rate of subsidence. In *A* (south-east extremity of Puerto Rico Trench; line drawing from profile published by Chase and Bunce[5], so far as possible omitting off-line events and multiples), the floor of the Atlantic on the right is underthrusting or being overthrust by the Caribbean crustal section, left; sediments are available only from nearby islands. A common feature in the tectonic trenches is the presence of one or more benches playing the part of sediment traps, as in *B* (Japan Trench, after Lugwig *et al.*[6]). Slumping and turbidity currents play a large part in sedimentation filling these and the trench floor (*C*, Middle Americas Trench, after Ross and Shor[7]), leaving the steeper slopes thinly covered with sediment in an unstable condition. In areas of heavier sedimentation or slower subsidence (*D*, Middle Americas Trench[7]), the topographic outline of the trench is obscured by infill.

refuse production of the globe, for example, would be buried as fast as it is produced if it were deposited in compacted form at the foot of the foreset beds of such rivers as the Fraser. The delta of the Fraser is located within narrow waters and is not suited for the disposal of waste, nor is its locality known with certainty to represent a zone of subduction. Deposition by this river, however, must be exceeded by that in many less completely surveyed.

We should perhaps regard such sedimentary sinks as a resource to be examined and utilized. To do so would require locating and mapping zones of intense deposition: investigation of the behaviour of waste material as it becomes deeply buried; and an examination of the economics of transporting material to sinks distant from its source. All these measures are feasible.

Demarcating present-day depositional sinks would require efforts similar to surveys already in progress. On the periphery of the sediment fan off-lying a major river, sedimentation is concentrated at one point or other for millennia at a time. The controlling factors are the development of subaqueous valleys canalizing density currents[11] and the slumping of accumulated masses. Within the trenches the slopes rarely show great thicknesses of sediment (Fig. 2), because this is carried by slumping to the trench floor or to sediment traps formed by the flank benches. Profiles suggest that the flank accumulations are temporary; disposition into the mantle takes place finally at the trench bottom (Fig. 2, *A* and *C*).

The behaviour of waste material as it becomes buried is important as regards the effect on marine life of the expulsion of water from sediments containing waste matter, including the common metals. The effects would presumably be minuscule compared with those resulting from present-day run-off from unburied refuse. Effluvia from the volcanic processes connected with the emplacement annually of 60 km³ of crust along the axis of the ocean ridge system must be immeasurably greater. It would be instructive in this regard to examine the effect on marine life of the emplacement of the metal-bearing brines in the Red Sea[12].

The cost of the investigations named would not be inconsiderable. In feasibility studies the cost of compressing waste material and its transportation to distant regions must also be taken into account. Facilities have already been operated at which municipal refuse is compacted to the stage at which its density is greater than 1·1 g/cm³. Its texture becomes that of building blocks. To judge by the experience of the petroleum industry, barge transportation of compressed refuse across an ocean would cost less than its piecemeal transportation 100 km by road or train. The organization of bulk transportation would bring into play the economies of mass production, as against the extraordinarily inefficient means of disposal at present available. These cost one nation £1,875 million ($4·5 billion) per annum[1], and cannot be made effective.

In an age in which waste material is mass-produced, only a mass-production disposal technique can be successful. The tectonic sinks are apparently so capacious as to take care of any conceivable quantity of material. The principal benefit of this form of disposal would be that its effect on the environment would not be cumulative. The ultimate effect to be expected would be the discovery of a distinctive, and perhaps, puzzling, suite of metamorphic rocks.

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Absorption Spectrum of Water Vapour in the Region of 23 cm^{-1} at Low Temperatures

The absorption of submillimetre radiation by the Earth's atmosphere is dominated by the pure rotation spectrum of the water molecule. If we are looking at submillimetre wave spectra for evidence of the presence in the atmosphere of species other than monomeric water, then regions in the gaps between the more intense lines of this molecule are of particular importance. One of these gaps where a

number of measurements have been made is in the vicinity of 23 cm^{-1} (435 μm wavelength). Here the absorption as measured by recording solar radiation coming through a slant path in the atmosphere is too great to be explained by considering only monomeric water vapour lines and magnetic dipole oxygen lines[1]. It is therefore of interest to investigate the cause of the additional absorption by making laboratory measurements.

Observations which use the Sun as a source necessarily involve absorption paths that are, in part, at lower temperatures than are usual at sea level and below the temperatures at which laboratory investigations have so far been made, so we have extended the latter to a lower temperature. An additional incentive to do this work comes from an interest in the hypothesis that the anomaly might be explained if we included in our considerations absorption by multimeric water molecules. We believe that the results give positive evidence in favour of their existence.

Fig. 1 shows spectra of a fifty metre path of water vapour at a pressure of 0·4 torr (approximately 5×10^{-5} MN/m^2) with an overpressure of two atmospheres of argon (approximately 0·2 MN/m^2). The observations were made in a multipass absorption cell and results are given for gas temperatures of 248 K and 298 K. From these it can be seen that the spectrum recorded at the lower temperature shows features in the vicinity of 23 cm^{-1} which are significantly stronger than in the spectra recorded at the higher temperature. These cannot be attributed to monomeric water pure rotation lines, of which the positions are indicated. We also show the energy level of the lower state of the transition for the monomer lines and from these values we can understand why some of them, for example that at 30·5 cm^{-1}, have such a marked decrease in intensity at the lower temperature. In contrast, several features, including the one singled out at 23 cm^{-1}, increase at the lower gas temperature.

The 23 cm^{-1} feature may be identified with an observation in the same region in earlier laboratory results. Here there was a slight indication of absorption in a 28 metre path of water vapour at a pressure of 4 torr and a temperature of 273 K[2]. The fact that the feature is now so much stronger brings out the importance of using an overpressure to broaden the lines. We also mention an unassigned feature at 22·9 cm^{-1} observed in a path of 180 m at a pressure of 15 torr and a temperature of 293 K[3].

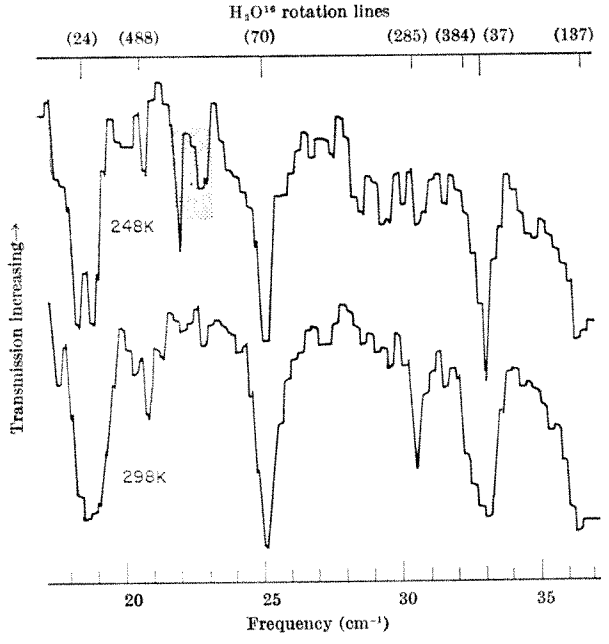


Fig. 1. Transmission of a mixture of water vapour and argon at two temperatures. The numbers in parenthesis beside the marks indicating the frequencies of the pure rotation water lines give the energies in cm^{-1} of the lower states of the lines. Path length, 50 m; water vapour pressure, 0·4 torr; argon pressure, 1,400 torr; spectral resolution, 0·5 cm^{-1}

It seems to be most unlikely that a stable complex between argon and water could be formed at the pressures we used and that it would have a spectral feature resembling something observed in the atmosphere. We submit therefore that these spectra are a further indication of the existence of multimers of water in the vapour phase, of which the dimer seems to be the most likely species[4].

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Low Threshold Laser Action of a Rare Earth Chelate in Liquid and Solid Host Media

Several authors have reported on the fluorescence line-spectra exhibited by certain rare-earth chelates, and suggested their relevance in the search for new laser systems[1–4]. Both europium and terbium chelates have been used in pulsed liquid–lasers operating at room temperature with input energy thresholds in the range of 200 to 2,000 J. The europium system operated on the 612 nm (orange) line of the chelate $Eu(BTA)_4Py$ (BTA = benzoyl trifluoroacetone, Py = pyrrolidine), whereas the terbium system, based on $Tb(TFA)_3$ (TFA = trifluoroacetylacetone) gave an output in the green region at 547 nm (refs. 5–7).

Transparent organic polymers, such as poly(methyl methacrylate) (PMMA), polystyrene, polycarbonates and acrylonitrile-based copolymers, could in principle be used as host media for laser-active chelates. It has been reported that $Eu(TTA)_3$ and $Tb(TTA)_3$ (TTA = thenoyl trifluoroacetone) have exhibited stimulated-fluorescence phenomena in very thin PMMA fibres cooled to 77 K (refs. 8–10). The technique used in the preparation of fibres, however, cannot readily be adapted to the manufacture of chelate-doped polymer rods. Also, I find that the method commonly used for preparing massive specimens of PMMA (and some other polymers) where a solution of the additive (in this case chelate) in monomer is treated with benzoyl peroxide to initiate the polymerization reaction cannot be used here, because the chelates are oxidized to non-fluorescent products in these conditions. Accordingly, I have devised a technique in which gamma radiation is used to initiate the polymerization. This gives polymer specimens of good optical quality, with little or no degradation of the chelate additive[11].

Having unsuccessfully tested a number of specially prepared europium and terbium chelate-doped polymer rods for stimulated fluorescence and other effects, I have recently investigated a neodymium-containing chelate using both PMMA and an organic liquid as host media. I had assumed, in view of the low-threshold capabilities of established neodymium systems (Nd in glass, selenium oxychloride and so on), that the neodymium chelate would be more compatible with the low-power pumping cavity used in this work (800 J maximum).

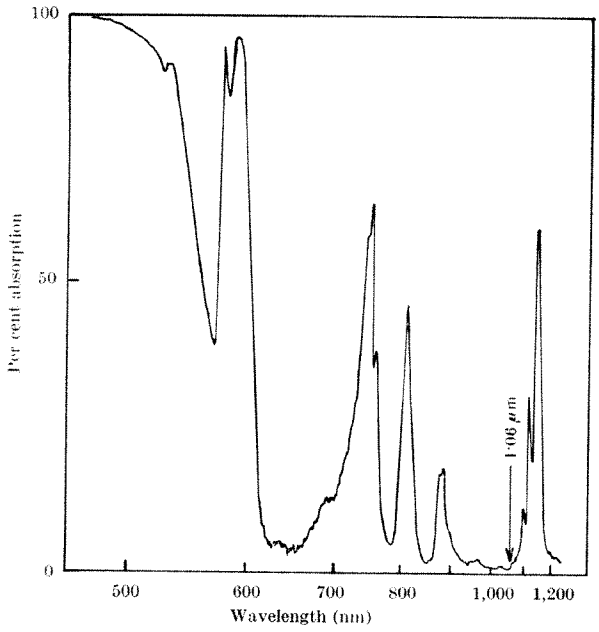


Fig. 1. Absorption spectrum of 10 mmole $l.^{-1}$ solution of "didymium" chelate in 1: 9 acrylonitrile/CCl_4 solvent. 10 cm path-length cell.

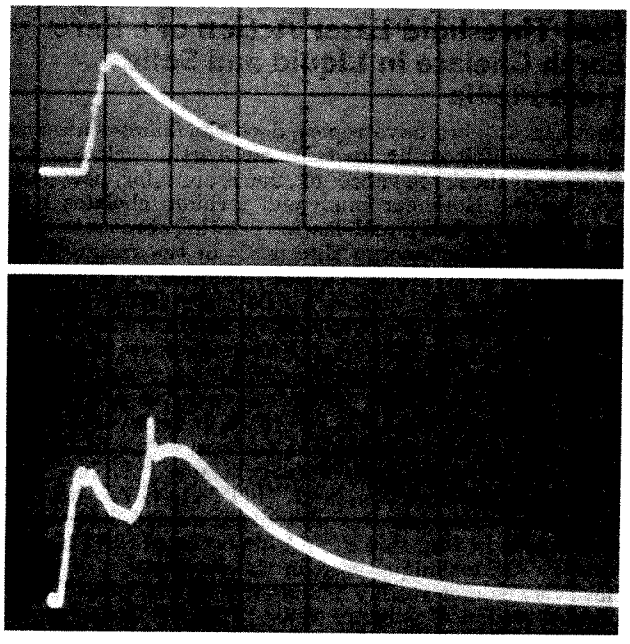


Fig. 2. Upper trace, flash pulse only at 200 J input. Lower trace, fluorescence pulse from chelate solution at 200 J. Horizontal sweep 100 μs/cm.

The chelate was prepared by complexing "didymium" chloride (presumed to contain about 70 per cent $NdCl_3$) dissolved in alcohol with TTA in the presence of pyrrolidine, using proportions of the reactants which would favour the formation of the tetra-chelated product $(Nd(TTA)_4Py$ (didymium chloride : TTA : Py; 1 : 4 : 4 molar). Initially, trials were carried out using a 10 mmole/l. solution of the chelate in 1 : 9 acrylonitrile/carbon tetrachloride solvent, having first established that this particular solvent is transparent in the region of the neodymium laser wavelength (1·06 μm) and that the absorption spectrum of the resulting solution exhibits a "window" in this region (Fig. 1). Samples of the solution, maintained at room temperature and held in glass cells of various dimensions, were excited by means of a 75 mm arc-length flash tube using a spherical-mirror cavity similar to that described by Samelson *et al.*[5]. The fluorescence pulses were detected by a fast rise-time silicon photodiode and displayed on a 50 MHz bandwidth oscilloscope. In the early experiments extraneous light from the flash tube was filtered out using an infrared pass filter, but this was later found to be unnecessary.

The fluorescence pulses obtained from the neodymium chelate solution exhibited irregularities, which I attribute to stimulated fluorescence and laser–oscillation effects when the pumping flash intensity exceeded a critical threshold value. Also, the shape of the time-resolved irregularities depended on the degree of optical feedback present in the glass cell used to contain the solution. Using a minimum feedback system, in the form of a 12 cm path-length × 7 mm internal diameter cell fitted with flat non-metallized end windows, the pulse exhibited a secondary (stimulated) peak occurring about 100 μs after the pumping flash peak. On increasing the feedback by adding an external fully aluminized 10 cm focal-length concave mirror the secondary peak showed superimposed "spikes", which I attribute to heavily damped relaxation-oscillations (Fig. 2). On adding a second (partially transmitting) mirror at the output end of the cell, however, the second peak and spikes could no longer be observed, possibly as a result of the generation of spurious laser modes falling outside the acceptance cone of the photodiode. Finally, well defined relaxation oscillations were observed using an 11 cm cell of 8 mm inner diameter having approximately hemispherical non-metallized ends, with a pumping threshold of about 120 J (Fig. 3). The oscillations in the latter case were probably associated with complex total internal reflexion modes, and it seems probable that the threshold level would be considerably reduced in a properly designed cell capable of axial-mode operation.

The secondary-peak phenomenon (Fig. 2) was again observed using 8 cm × 8 mm PMMA rods containing 20 mmoles/l. of the "didymium" chelate, but the

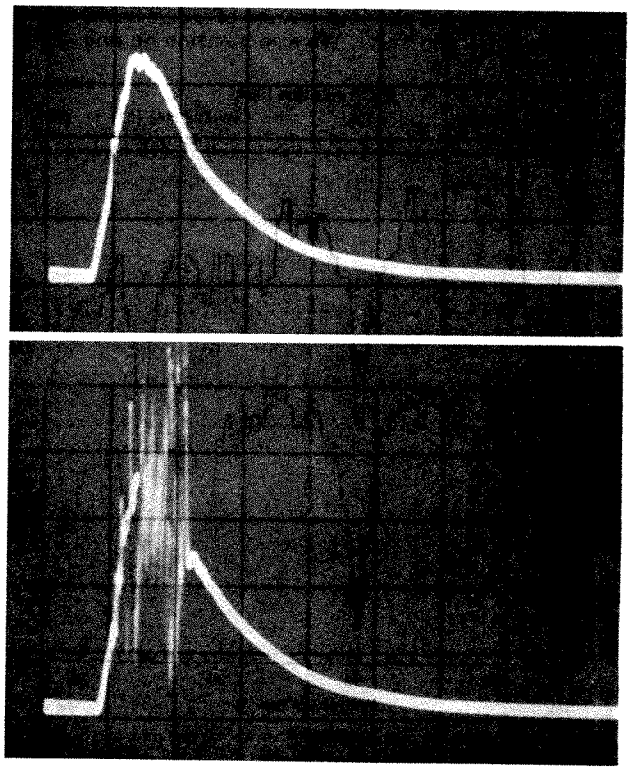


Fig. 3. Fluorescence pulses. Upper trace, 110 J input. Lower trace, 120 J input (threshold). Horizontal sweep 50 μs/cm.

threshold in this case was about 200 J and spiking phenomena were not so pronounced. PMMA is not the best polymer host for this particular chelate, however, because it absorbs in the 1·06 μm region. The losses in an 8 cm rod would amount to about 70 per cent per double-pass. It is possible that some of the acrylonitrile based copolymers would be more suitable.

The liquid laser system described here is probably comparable as regards operating temperature (300 K) and threshold with the inorganic neodymium systems ($Nd/SeOCl_2$, $Nd/POCl_3$) already in use, but has the advantage that the solvent is non-corrosive (although moderately toxic) and is therefore more adaptable to continuous-flow equipment. Also, the utilization of pumping energy is probably unusually efficient, for energy can be transferred to the Nd^{3+} ion both directly (Nd^{3+} ion absorption band, 570–610 nm, Fig. 1) and via the chelate ligands (450–580 nm band, Fig. 1). This being so, it is possible that a continuous-wave laser could be developed. The successful demonstration of stimulated processes in the neodymium chelate/PMMA system suggests that further research on the use of polymers as hosts may be productive.

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BIOLOGICAL SCIENCES

Selective Constraints on Amino-acid Substitutions during the Evolution of Proteins

RECENTLY, several workers have suggested that during the evolution of proteins most amino-acid substitutions (in the sense of mutations that have spread to fixation) have been neutral in selective value[1–4]. These views have not received unanimous support[5,6]. It seems appropriate, therefore, to examine the pattern of substitutions in order to detect any possible influences of natural selection. I here analyse the relationship between the frequencies of particular substitutions and the chemical properties of the amino-acids concerned in them.

For our purpose we need an objective classification of the chemical properties of amino-acids. Fortunately, this has been provided by Sneath[7], who has constructed a "numerical taxonomy" of amino-acids. On the basis of 134 chemical characters he drew up a matrix (of $D = 1 - S_{SM}$[8]) in which every amino-acid was compared with every other, and each comparison was represented by a value of D indicating the degree of chemical dissimilarity between them.

Data on the relative frequencies of amino-acid substitutions can be obtained from the compilations of Dayhoff (ref. 9: Fig. 9–3, page 76). She tabulates the numbers of substitutions, between particular pairs of amino-acids, that have been observed during the evolution of a "representative" series of proteins (cytochrome *c*, haemoglobin, myoglobin, virus coat proteins, chymotrypsinogen, glyceraldehyde-3-phosphate dehydrogenase, clupeine, insulin and ferredoxin). In my analysis these numbers have been corrected both for the frequencies of amino-acids (given in Fig. 9–6 of ref. 9, page 78) and for the different probabilities of mutational changes between amino-acids imposed by the genetic code.

The calculation of a corrected number proceeds as follows. I consider only those substitutions that can result from a single nucleotide replacement (that is, only those proceeding from one-step mutations). Let N_{AB} represent the number of substitutions between amino-acid A and amino-acid B recorded by Dayoff (note that this represents the number of substitutions A→B plus the number of substitutions B→A). Let the frequency of A in the proteins considered be a and the frequency of B, b. Let the relative probability of the mutation A→B, according to the genetic code (assuming equal frequencies of synonymous codons and excluding nonsense codons from the analysis), be p_{AB}, and the probability of the mutation B→A be p_{BA}. These probabilities may be unequal because different amino-acids are coded by different numbers of synonymous codons. The corrected number of substitutions is then

$$R_{AB} = \frac{N_{AB}}{ap_{AB} + bp_{BA}}$$

When N_{AB} is zero, it is arbitrarily given a value of 0·50 in order to avoid loss of information. The value of R_{AB} is then likely to be an overestimate, the error being in the right direction. A corrected number has been calculated in this manner for each of the 75 one-step mutations permitted by the genetic code. Each number is proportional to the average rate of evolutionary substitution between a particular pair of amino-acids.

Fig. 1 is a scatter-diagram showing the relationship between the corrected numbers of substitutions (R) and the values of D obtained by Sneath[7]. Each spot represents a pair of amino-acids. There is an obvious negative correlation, indicating that substitutions which involve relatively small chemical changes are very much more frequent than those involving relatively large changes. This relationship can be caused only by constraints imposed by natural selection.

There is a difficulty in testing the significance of the correlation because the spots are not statistically independent of one another. It can be overcome by allowing 18 degrees of freedom (for the 20 amino-acids) rather than 73 (for the 75 spots). In spite of this restriction, the correlation is still highly significant (using logarithms of R; $r = -0{\cdot}652$, $P \ll 0{\cdot}01$). Because the true number of degrees of freedom is certainly greater than 20, the significance is probably well below 0·1 per cent. The correlation is not an artefact arising from the combination of heterogeneous data. If the proteins are treated individually, they give similar results (unpublished).

The observation that rates of substitution are dependent on the magnitude of chemical differences between the amino-acids is not a new one. Zuckerkandl and Pauling[10] have drawn the distinction between "conservative" substitutions producing little chemical change and "radical" substitutions producing gross chemical change. They have pointed out that the former are the more common. The advantage of the present method, however, is that it allows the relationship to be quantified. It shows that the degrees of "conservatism" and the rates of substitution are quite precisely related, and it confirms the view of Zuckerkandl and Pauling that there is a continuous, but wide, spectrum of different rates.

This is important because it invalidates several later, and less critical, studies of protein evolution. For example, King and Jukes[1] have argued that most substitutions are selectively neutral. For several proteins they have plotted the number of changes per site against the number of

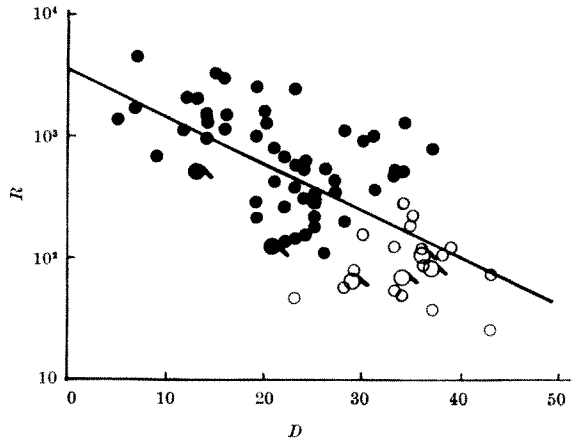


Fig. 1. The relationship between corrected number of substitutions (R) and chemical difference (D)[7] for the 75 amino-acid interchanges permitted (in one mutational step) by the genetic code. The values of R are derived from a "representative" series of proteins (see text). Open circles represent substitutions that were not observed in the proteins examined. To avoid loss of information the number of substitutions in each of these cases was assumed to be 0·50, so that their values of R are likely to be over-estimates. Large circles with tails represent substitutions involving cysteine. The negative correlation is highly significant ($r = -0{\cdot}652$, $P \ll 0{\cdot}01$, using logarithms of rates). The sloping line shows the regression of R on D.

sites having the specified number of changes. After making allowance for a (somewhat arbitrary) number of "invariant" sites, they conclude that the remainder fit the Poisson distribution. They regard this apparent correspondence as evidence that most of the substitutions occur randomly. The Poisson distribution applies to events that have a low but constant probability. The analysis set out above, however, demonstrates that the probabilities of substitutions vary greatly in relation to the chemical properties of the amino-acids. We must conclude that the apparent correspondence reported by King and Jukes is an accident. This criticism also applies to the work of Fitch and Margoliash[11,12], who have based their arguments about "concomitantly varying codons" on an apparent correspondence with the Poisson (although in this case certain "hypervariable" sites were also excluded).

The relationships demonstrated in Fig. 1 do not by themselves eliminate the possibility that most substitutions are selectively neutral. It is conceivable, though unlikely[5], that the selective constraints indicated by the figure merely ensure that the probability of a neutral substitution is very much reduced when the chemical differences are great. Taking this view, which corresponds to those of King and Jukes[1] and Kimura[2], one is forced to conclude that if most substitutions are selectively neutral, most mutations are not. In Fig. 1 the average (geometric mean) rate of substitution is 362 (95 per cent confidence limits, 207 and 630, assuming 18 degrees of freedom). The maximum rate, which according to the argument just stated should be the rate of neutral substitution ($D = 0$), is about 3,320 (95 per cent confidence limits, 940 and 11,830, assuming 18 degrees of freedom). The average rate is thus only about one-ninth of the "neutral" rate (95 per cent confidence limits, 1/3 and 1/29, assuming 18 degrees of freedom). We must conclude that at least 85–90 per cent of the mutations causing a change of amino-acid are, on the average, selectively disadvantageous. This result is incompatible with Kimura's own argument, for he has deduced that more than 50 per cent of such mutations are neutral[4].

King and Jukes[1] have estimated that 5–10 per cent of mutations are neutral. At first sight my own calculations might seem to support them, but they do not. Fig. 1 takes into account only one kind of selective constraint, and there are many others. There are, for instance, limitations imposed by the kind of protein in which the substitutions occur[6], by the position of the amino-acid in the protein[10,13], and by the type of organism in which the protein is foun When all such factors are taken into account it seen likely that our estimate of the average proportion "neutral" mutations will be nearer 0·1 per cent than per cent.

Zuckerkandl and Pauling[10] have remarked that "a parently chemists and protein molecules do not share tl same opinions regarding the definition of the most pron inent properties of a residue". The opinions of tl protein molecule are made manifest in the pattern evolutionary substitutions. We have to make chemic sense of them and the method of analysis promises make this possible.

Sneath's classification is based on 134 characters, of which are given equal weight. From the evolutiona point of view, however, it is clear that the individu chemical properties of the amino-acids are not all equal importance. It is therefore possible to modify tl classification by weighting characters so as to maximi the relation between R and D. The weights of individu characters would then be a direct measure of the importance to the function of the protein.

Substitutions involving cysteine seem to depa systematically from the regression line given in Fig. (the cysteine pairs are shown as large circles with tails If the chemical characters of cysteine are weighted reduce the departure from the regression, the ability form disulphide bonds is given a high value. In oth words we could, without previous knowledge of prote chemistry, have predicted the importance of the disulphi bond.

The method still has to be developed, but some interes ing results can be expected, particularly if the greate possible number of chemical properties can be incorporate into the classification. It is satisfying that the studer of evolution can perhaps contribute to solving th problems of the chemist.

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F(ab')$_2$-like Fragments from Severely Burned Patients provide a New Serum Immunoglobin Component

INTEREST in immunoglobulin fragments in serum centre around their antigenic sites which stimulate aut antibody[1], and the insight they provide into the met

bolism of immunoglobulins. They also have the potential to alter host immune defence mechanisms. For example, the $F(ab')_2$ fragment prepared *in vitro* from IgG is less effective than intact IgG in protecting animals injected with pathogens[2], in aiding phagocytosis[3,4] and in clearing bacteria from the blood stream[2,5]. Also, $F(ab')_2$ fragments are non-cytotoxic in the presence of complement[6,7] and enhance the growth of tumours[8,9]. Because severely burned patients have larger than normal concentrations of proteolytic enzymes[10] and immunoglobulins in their serum[11–13], we regarded them as potential models for *in vivo* degradation of immunoglobulin, and examined serum from forty patients with 3–70 per cent body surface area full-thickness burns for immunoglobulin fragments. The following report identifies for the first time a serum $F(ab')_2$-like fragment in severely burned patients.

Immunoelectrophoretic[14] patterns of sera developed with rabbit anti-whole human serum prepared in our laboratory showed a new precipitin arc (Fig. 1*a*). Antisera to IgG, IgA, IgM_1 and IgM_2 (Immunology Inc.) were also used, and only anti-IgG produced the new arc (Fig. 1*b*) as well as its characteristic arc. This indicated that the new serum component was related to IgG; hereafter in this report it is designated $F(ab')_2$-burn.

Both the time interval after the burn and the severity of the burn determined whether $F(ab')_2$-burn was present. Its characteristic arc was seen in patterns of sera obtained only on days 6 to 16 after the burn. When patients were grouped according to percentage body area covered with full-thickness burn, the proportion of each group with serum $F(ab')_2$-burn was as follows: >39 per cent, 11/11; 18–39 per cent, 13/20; <18 per cent, 0/9.

$F(ab')_2$-burn was isolated from serum. The precipitate obtained with 55 per cent saturation $NH_4)_2SO_4$ was dialysed against *p*H 8·0 Tris/HCl buffer (Sigma) and distilled water, placed on a column of 'Sephadex G-200' (Pharmacia) and fractions were collected during 0·1 M stepwise addition of 0·1 M–0·5 M Tris/HCl buffer. The IgG peak from burned patients' serum had a large trailing shoulder in contrast to the IgG peak from normal serum. The trailing shoulder was fractionated using a column of 'Sephadex G-150' which produced separation of IgG and $F(ab')_2$-burn. No $F(ab')_2$-burn fragment was obtained when IgG from normal serum was fractionated using 'Sephadex G-150' in the same way.

Ultracentrifugation of the isolated $F(ab')_2$-burn in 10–40 per cent sucrose gradients, at 27,500 r.p.m. for 24 h. suggested that it was similar in size to $F(ab')_2$ fragments. For further comparison, therefore, $F(ab')_2$ fragments were prepared from IgG by pepsin degradation[15,16] at *p*H 3·5 for 48 h. using 50 mg of IgG/mg of pepsin (Worthington), and 0·05 N HCl and NaOH to adjust the *p*H. $F(ab')_2$ was isolated by 55 per cent saturation $NH_4)_2SO_4$ and 'Sephadex G-150' as before.

Immunoelectrophoresis of $F(ab')_2$ gave a precipitin arc identical to the $F(ab')_2$-burn arc in serum when developed with anti-$F(ab')_2$ prepared in our laboratory (Fig. 1c). When patterns of serum from burned patients were developed with anti-Fc, the IgG arc was still present, but the $F(ab')_2$-burn arc was not (Fig. 1*d*). This indicated that $F(ab')_2$-burn did not have Fc antigenic determinants, which is also characteristic of $F(ab')_2$. The IgG arc developed with anti-Fc also showed anodal splitting, which suggested the presence of a free Fc-like fragment. When IgG was mixed with either $F(ab')_2$-burn or $F(ab')_2$ and developed with anti-IgG, the pattern was similar to the burned patient's serum shown in Fig. 1*b*. Additional patterns developed with antisera to kappa or lambda chains (Melpar Inc.) also had the characteristic $F(ab')_2$-burn arc indicating the presence of these light chains in $F(ab')_2$-burn.

Ultracentrifugation in a model E Spinco ultracentrifuge provided further evidence for the similarity between $F(ab')_2$-burn and $F(ab')_2$. Each fragment migrated in a single homogeneous peak, 10 mg/ml. 0·05 M NaCl, 59,780 r.p.m., and corrected $s_{20,w}$ values[17] were 5·23 for $F(ab')_2$-burn and 5·17 for $F(ab')_2$.

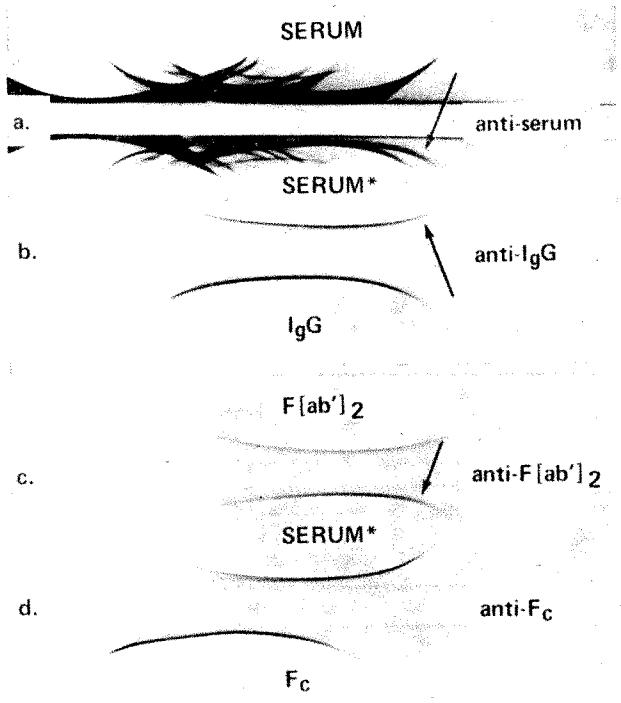


Fig. 1. Immunoelectrophoretic patterns with an arc (arrow) characteristic of an $F(ab')_2$-like component in serum* from a patient 8 days after a severe burn. *a*, Anti-human serum demonstrates the new component in the patient's serum* on day 8 (lower) but not in serum on day 20 (upper); *b*, anti-IgG shows the $F(ab')_2$-like component in serum* (upper) cathodal to the faint IgG which is compared with an isolated IgG (lower); *c*, anti-$F(ab')_2$ shows no IgG arc with the $F(ab')_2$-like component in serum* (lower) similar to an isolated $F(ab')_2$ (upper); *d*, anti-Fc detects the IgG but not the $F(ab')_2$-like component in serum* (upper). The cathode is to the right.

$F(ab')_2$-burn and $F(ab')_2$ were reduced[18] with cysteine and ultracentrifuged in sucrose density gradients. The Fab'-burn and Fab' reduction products were approximately half the size of the original molecules as indicated by their sedimentation at the rate of a 3·5 S Fc control[19]. These results further indicated the similarity of $F(ab')_2$ and $F(ab')_2$-burn.

Therapy in the University of Michigan Burn Unit probably contributes to formation of $F(ab')_2$-burn. Patients are actively immunized with *Pseudomonas aeruginosa* vaccine[20,21] (prepared at the unit) shortly after admission. Passive immunization with 1/4–1 U of plasma from immunized human donors usually begins after 1–4 days. The increased level of immunoglobulins, plus the increased proteolytic activity, probably produces enough $F(ab')_2$-burn to be detectable in serum, even though the half-life of $F(ab')_2$ is only 10–14 h[22].

Isolated $F(ab')_2$-burn possessed the antibody activity characteristic of $F(ab')_2$. Tube agglutination (Table 1) showed that the anti-*Pseudomonas* titre of $F(ab')_2$-burn isolated from serum of a burned patient was similar to titres of the whole serum, IgG isolated from the serum, and $F(ab')_2$ prepared from the IgG. This patient had received only active immunization, and $F(ab')_2$-burn was therefore derived from endogenous IgG. Whether $F(ab')_2$-burn results from an abnormal mode of catabolism or an accelerated physiological process is not known. According to current concepts of immunoglobulin metabolism, it is unlikely to be a synthetic product. It is not C-reactive protein. Patients did not receive $F(ab')_2$-burn by passive immunization, for its characteristic arc was not detected in immunoelectrophoretic patterns of four therapeutic plasmas.

It is unlikely that $F(ab')_2$-burn was produced *in vitro* during or after bleeding. Sera were collected and immunoelectrophoresis was begun within 1 h of bleeding, and $F(ab')_2$-burn was absent in sera from lightly or severely burned patients before 6 or after 16 days. As an additional control, the proteolytic enzyme inhibitor 'Trasylol' (Bayer AG), which inhibited *in vitro* degradation of sera from burned patients[23], was placed in needles, syringes and tubes used to collect blood from patients with an 80 per cent full-thickness burn and a 40 per cent variable-thickness burn. The new immunoelectrophoretic arc was still present even though the patients' blood did not clot. We could reproduce the arc artificially but only after a minimum of ten cycles of freezing and thawing of serum or IgG. Similar *in vitro* degradation of immunoglobulin during storage has been reported by others[24,25]. Immunoelectrophoretic artefacts were prevented by avoiding antigen or antibody excess, as determined in preliminary titrations. Any questionable pattern was developed at least twice to rule out errors in technique. The new immunoelectrophoretic arc was not found in thirty fresh sera of randomly selected control patients with a wide variety of diseases. It has been pictured in other human sera and globulin preparations[26–29], but additional characteristics were not correlated. The characteristic arc in burned patients' serum has been correlated with similar arcs in immunoelectrophoretic patterns of serum from patients with multiple myeloma and of urine from patients with systemic lupus erythematosus[30].

Table 1. ANTIBODY ACTIVITY OF AN $F(ab')_2$-LIKE SERUM IMMUNOGLOBULIN FROM A BURNED PATIENT

Serum or globulin	Titre 2*	4	8	16	32	64	128	256	512
Burned patient's									
$F(ab')_2$ like immunoglobulin	+	+	+	+	+	+	+	+	0
Serum	+	+	+	+	+	+	+	+	+
IgG	+	+	+	+	+	+	+	+	+
$F(ab')_2$	+	+	+	+	+	0	0	0	0
Non-burned patient's									
Serum	+	+	0	0	0	0	0	0	0
IgG	+	+	0	0	0	0	0	0	0
$F(ab')_2$	0	0	0	0	0	0	0	0	0

The agglutinin titre of the serum immunoglobulin is compared with that of: whole serum, the serum's IgG, and $F(ab')_2$ prepared from the IgG, of burned and non-burned patients.
The burned patient was injected with *Pseudomonas aeruginosa* vaccine; the control non-burned patient was not. The vaccine was used as antigen in determining titres.

* Undiluted serum or 10 mg of globulin/ml. of 0·85 per cent NaCl.

This report therefore provides strong evidence that the serum of severely burned patients contains an immunoglobulin component comparable with the enzymatically produced $F(ab')_2$ fragment. It is characterized by a specific cathodal arc in immunoelectrophoretic patterns of serum, and it must be differentiated from a similar fragment which may be produced *in vitro*.

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F_K-*lac*, an Episome with Unusual Properties found in a Wild Strain of a *Klebsiella* Species

F-PRIMES, episomes consisting of an F-factor joined to a fragment of the bacterial chromosome, arise spontaneously in Hfr males of *Escherichia coli*, and are easily selected because of their infective properties[1]. It is therefore surprising that only two such episomes have been reported from natural sources: F_0-*lac* found in a strain of *Salmonella typhosa*[2] and P-*lac* found in *Proteus* species from a single hospital[3,4]. Both of these episomes were recognized because their lactose-negative hosts had become lactose-positive (Lac^+), and both are highly infective and show mutual interference with F-factors of *E. coli*, that is the presence of one excludes the other.

We describe here a third F-*lac* of natural origin possessing some unexpected properties. It was discovered in a normally Lac^+ *Klebsiella* species, carries an active repressor gene of the type possessed by *fi*$^+$ R-factors, and shows mutual exclusion with F in *E. coli*, but is completely unable to promote conjugation.

The host strain (V9A), a *Klebsiella* provisionally identified as a member of *K. aerogenes*, came from faeces sampled from a wild vole population (*Microtus agrestis* L.) in a long-established Scottish Forest (area C-1a of the Carron Valley vole population[5]). This source was examined in a search for Enterobacteriaceae from environments with no known history of antibiotic treatment. *Klebsiella* V9A is resistant to two antibiotics, tetracycline (Tc) at 40

μg/ml. and ampicillin at 20 μg/ml., and is Lac+ like other *Klebsiella*.

V9A gave no transfer of resistance to *E. coli* in overnight matings, but transfer of Tc-r was achieved with the help of either an F-type or an I-type transfer factor, previously introduced into the donor. In typical experiments (Table 1) the derepressed I sex factor transferred itself with high efficiency, while F-*gal* was transferred some 2,000 times less frequently in comparable tests. Both sex factors transferred Tc at about 0·4 per cent of the rate of sex factor transfer. This and other experiments show that Tc resistance is caused by a plasmid gene. Ampicillin resistance was not transferred, but indirect evidence suggests that it is also extra-chromosomal. Work on these resistance genes will be published elsewhere.

Table 1. TRANSFER OF SEX FACTORS AND TETRACYCLINE RESISTANCE FROM *Klebsiella* STRAIN V9A TO *E. coli* K12

Sex factor introduced into *Klebsiella*	Mating time (h)	Transfers per K12 recipient of: Sex factor	Tc-r	Transfer ratio Tc/sex factor (per cent)
None	16	–	$< 10^{-9}$	–
F-*gal*	3½	5×10^{-3}	2×10^{-7}	0·4
I*drd* Kan	3½	2×10^{-1}	7×10^{-4}	0·3

For the first mating, fully grown broth cultures of donor and recipient were diluted 10^{-3} into broth and incubated overnight at 37° C. For the other two matings, log phase donor cells at about 2×10^{9}/ml. and a fully grown recipient culture were diluted together 10^{-1} into broth and incubated 3·5 h without aeration. Various dilutions were then spread on selective plates to measure transfer and recipient cell frequencies. Streptomycin was used to counter-select against the donor strain. The sex factors were F-8 (F-*gal*)[1], in a Gal- mutant of V9A, and R144 *drd*, a derepressed mutant of a *coli* I-type R-factor[8], conferring resistance to kanamycin (Kan), supplied by Dr Elinor Meynell.

The F-*lac* particle in V9A was discovered by a happy chance, when MacConkey agar containing tetracycline and streptomycin was used to select Tc-r recipients in a cross between V9A carrying F-gal and a Lac- Gal- streptomycin-resistant strain of *E. coli* K12. To our astonishment, Lac+ as well as Lac- colonies were obtained. Both types were found to be Tc-r recipient colonies, but the sex factor F-*gal* was only present when Lac+ was not, suggesting that the two particles show mutual exclusion (that is, they cannot coexist) in the recipient strain, although they must have been present together in the *Klebsiella* donor. We ruled out the possibility that this Lac+ particle might have been introduced into V9A along with F-*gal* from its original (Lac+) K12 donor, by starting with another sample of V9A and infecting it with F-*gal* from a Lac- K12 strain. The new V9A/F-*gal* was able to transmit Lac+ in the same way as the previous V9A/F-*gal* strain.

Table 2. EFFECT OF RESIDENT Lac+ PARTICLE ON ABILITY OF K12 TO ACCEPT F-*his*

Episome in K12 recipient strain	Per cent of recipients infected with F-*his* in one hour Test 1	Test 2	Lac state of infected recipients
None	65	19	–
Lac+ from V9A	4	0·4	all Lac-
Relative efficiency	6 per cent	2 per cent	

Log phase donor cells (K12/F-*his*) at 2×10^{8}/ml. and a fully grown recipient culture were diluted together 10^{-1} into warm broth and incubated 1 h at 37° C without aeration. Dilutions were then spread on selective plates to measure transfer and recipient cell frequencies. Independent Lac+ infected recipient lines were used in the two tests, and twenty His+ clones from each were tested for the retention of Lac+. Relative efficiency of transfer is measured as the ratio of the proportion of His+ recipients in the Lac+ mating to the proportion in the Lac- mating, calculated as a percentage (for example, $4 \times 100/65$ gives 6 per cent).

In further matings of V9A/F-*gal* to K12 strains we were able to select recipient K12 clones which had received only the Lac+ particle. Such clones were resistant to F-specific phage (f2) and were unable to transfer Lac+ further. The recipient strain was also His-, and its ability to accept an F-prime (F-*his*) when carrying the Lac particle from V9A was compared with that of the parent (Lac-) strain by mating to a suitable K12/F-*his* donor strain under standard conditions. Table 2 shows that the presence of the *Klebsiella* Lac+ particle in the recipient reduces the frequency of *F-his* acceptance to a few per cent of the normal frequency (6 per cent and 2 per cent in the two tests), while those originally Lac+ recipients in which F-*his* becomes established lose the Lac+ particle. They also become sensitive to F-specific phage. This experiment clearly establishes the occurrence of mutual exclusion between the Lac+ particle and an F-prime in K12.

Finally, two derepressed mutants of an R-factor R1, derived by Dr Elinor Meynell, were transferred into V9A, and the F-specific phage sensitivity of infected cells and their efficiency of transfer of the sex factor into K12 were compared with those of the *Klebsiella* infected with F-*gal* (Table 3). R1 is of *fi*+ type[7], that is it carries a gene *i* the product of which represses activity of its own sex factor and of F-factors, strongly reducing the efficiency of transfer.

Table 3. BEHAVIOUR OF F-*gal* AND DEREPRESSED *fi*+ R-FACTORS IN *Klebsiella* V9A

Introduced sex factor	Sensitivity to F-specific phage	Efficiency of transfer of sex factor to K12
F-*gal*	–	Low
R1 *drd* 19 (o^+i^-)	–	Low
R1 *drd* 16 ($o^c i^+$)	+	**High**

F-*gal* (F-8) was introduced into a Gal- mutant of V9A, the R1 mutants direct into V9A. R1 *drd* 19 has a sex factor which is repressible (o^+) but makes no repressor; R1 *drd* 16 makes repressor (i^+) but is not repressible (o^c)[8]. Both R1 mutants were received from Dr Elinor Meynell. Efficiency of transfer was measured by mating for 3½ h as in Table 1 and then streaking loopfuls of the mating mixture on plates which allow only recipients infected with the sex factor to grow. Each loopful was cross-streaked over a single plate in the usual way for obtaining single colonies, so that several dilutions could be observed. All plates could be unequivocally classified as showing transfer at low efficiency (a few colonies) or high efficiency (very many colonies, with confluent growth where the loopful was first spread). Sensitivity to F-specific phage f2 was measured by spotting phage on to an overlay containing cells in soft nutrient agar (+ indicates sensitivity).

Of the two R1 mutants, *drd* 19 has been shown to produce no repressor (i^-) but to be susceptible to repressor substance (o^+) while *drd* 16 does produce repressor (i^+) but is not repressible (o^c); both R1 mutants promote their own transfer with high efficiency in K12 strains[8]. *Klebsiella* carrying F-*gal* or the repressible R1 *drd* 19 are resistant to phage f2 and transfer the infective agent with low efficiency, while clones carrying the non-repressible R1 *drd* 16 are sensitive to f2 and transfer the R1 with high efficiency. This experiment only proves that V9A carries a gene producing sex factor repressor. When the *Klebsiella* infected with an R1 mutant is mated to a Lac- K12, however, recipients carrying R1 *drd* 19 are found to be f2-sensitive and efficient donors in further matings unless they have also received the *Klebsiella* Lac+ particle, in which case they are resistant to f2 and can only transfer R1 further at low efficiency. Recipients carrying R1 *drd* 16, on the other hand, are f2-sensitive and are efficient donors of R1, whether or not they also carry Lac+. These results can only be explained on the hypothesis that the *Klebsiella* Lac+ particle itself contains a repressor gene the product of which represses the sex-factor activity of an F-factor or an *fi*+ R-factor. Only R1 *drd* 16, which has lost its repressor sensitivity, escapes this repression.

We conclude that *Klebsiella* strain V9A carries an extra-chromosomal Lac+ particle, to be named F_K-*lac*, which has an active *fi*+ repressor gene, shows mutual exclusion with F-factors in *E. coli* but not in *Klebsiella* strain V9A, and is quite unable to promote conjugation, presumably because it is deficient in some of the necessary genes. It may be noted that two different samples of vole faeces yielded V9A-type colonies carrying F_K-*lac* which were resistant to tetracycline and ampicillin, but no *Klebsiella* of the same general type not carrying the Lac episome have been found in our samples.

F_K-*lac* differs in several respects from F_0-*lac* and P-*lac*, particularly in its failure to promote conjugation, its possession of a repressor gene, and in the fact that its host is a lactose-positive species in which the episome has no apparent selective advantage. Possibly such episomes are more widespread among species of Entero-

bacteriaceae, in which their presence would not be easily recognized, than has been assumed.

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Effect of Surface Charge on the Steady-state Potassium Conductance of Nodal Membrane

The curve relating membrane conductance (G_m) to membrane potential (V_m) shifts along the voltage axis with changes in concentration of external divalent ions[1–4], external pH[5] and internal ionic strength[6]. These findings are in qualitative agreement with the suggestion made by Huxley[1] that the shift may correspond to changes in the electric field resulting from changes in the membrane surface potential, ψ_0. To test the validity of the suggestion experimental shifts need to be compared with calculated changes in ψ_0 when ψ_0 is affected by various

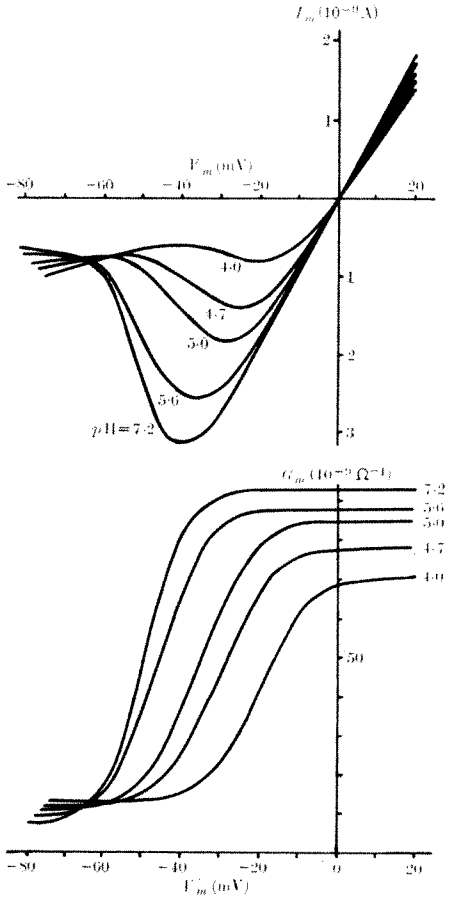


Fig. 1. Shifts of I_m–V_m curves and associated G_m–V_m curves with pH. K^+ = 100 mM; Ca^{2+} = 2 mM.

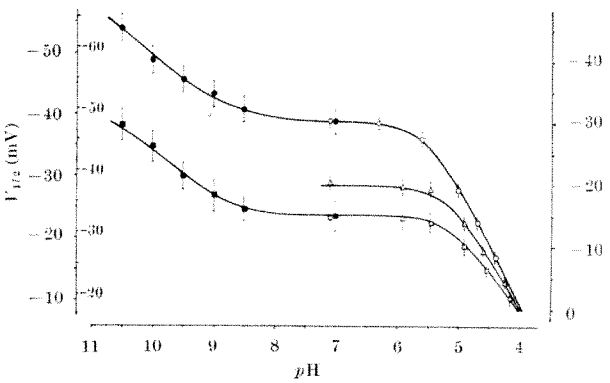


Fig. 2. Changes in $V_{1/2}$ and ψ_0 with pH. Symbols represent the mean values, with respect to nine fibres, for $V_{1/2}$. Vertical lines represent ±. Open symbols and figures on the right of the $V_{1/2}$ axis are for $V_{1/2}$ from the first set of experiments (pH 4–7); filled symbols and figures on the left of the $V_{1/2}$ axis are for $V_{1/2}$ from the second set of experiments (pH 7–10·5). ○, ●, in 2 mM Ca^{2+}; △, ▲, in 10 mM Ca^{2+}; □, ■, in 20 mM Ca^{2+}. The curves through the points which relate ψ_0 to pH were calculated by the method given in the text.

factors. While calculating ψ_0 it is essential to use the san basic assumptions concerning the charge number ar chemical nature of adsorbing groups (binding sites) the membrane surface. To this end, we studied the effe of changes in the external concentrations of H^+, Ca^{2} Mg^{2+}, Co^{2+}, Ni^{2+} and external ionic strength on stead state potassium conductance (G_m–V_m curves).

The G_m–V_m curves were calculated from steady-sta current–voltage relationship (I_m–V_m curves) obtain by the voltage-clamp method. In this method longit dinal currents along the outer surface of internodes both sides of the node under investigation were prevent by a special feedback system[7]. To obtain I_m–V_m curve a saw-tooth clamping voltage (V_m) varying at 2 mV was fed both to the input of an operational amplifi and, through a differential amplifier, to the horizont plates of a cathode-ray oscilloscope. The I_m–V_m curv thus obtained were continuous. The external potassiu concentration was 50 mM in experiments in which t ionic strength was varied; otherwise it was 100 m Solutions were kept isotonic with NaCl or Tris-H The pH was changed by the addition of small amounts biphthalate (pH 4–7) or glycine (pH 7–10·5) buffers. T ionic strength was reduced by replacing Tris-HCl wi sucrose.

Fig. 1 gives the family of I_m–V_m curves (top) and asso ated family of G_m–V_m curves (bottom) at various p values and 2 mM Ca^{2+}. At lower pH the curves shift the direction of depolarization: the V_m at which G_m half the sum of maximum and minimum, $V_{1/2}$, decreas In Fig. 2 the mean values for $V_{1/2}$ at various calciu concentrations are plotted against pH. These values we calculated from two sets of experiments: one with pH 4 and the other with pH 7–10·5. At normal [Ca^{2+}] a normal pH, $V_{1/2}$ values are scaled to the same value, for is the change in $V_{1/2}$ that matters rather than absolute values. The $V_{1/2}$-pH relationship falls into thr well pronounced regions: it has a plateau in the ran pH 8–6, rises at a pH above 8 and diminishes with a p below 6. At acid pH, Ca^{2+} has little effect on $V_{1/2}$ where at alkaline pH it has a large effect. The plateau in t $V_{1/2}$–pH relationship suggests groups of at least two typ on the membrane surface. Considering the dependen of pH effect on [Ca^{2+}] it is believed that groups of type are negatively charged and capable of binding both H and Ca^{2+} whereas the groups of type II are positive charged at pH ⩽ 7 and uncharged at pH > 7. On the ba of data presented in the literature[8] we suggested that t net surface charge is negative. Experiments with chang in ionic strength supported this suggestion: reduci ionic strength increases $V_{1/2}$ (Fig. 3), indicating an exce of net negative charge in the membrane.

While calculating ψ_0 it was assumed that the membrane phase constitutes a dielectric, or at least that its free charge density is much less than that of the solution and that there are groups of three types on the membrane surface: first negatively charged groups (at a density of σ_1) capable of binding H^+ and Ca^{2+} (I); second, positively charged groups (at a density of σ_2) at $pH \leqslant 7$ and uncharged at $pH > 7$ (II); third, negatively charged groups not capable of binding ions within a studied range of H^+ and Ca^{2+} concentration (III). The groups of type III were assumed to obtain a better quantitative agreement between experimental and theoretical relationships.

Because in our case, in solution, the concentration of univalent ions is large compared with that of divalent ions, the following relationship holds

$$\sqrt{\frac{DkT}{2\pi}}\, C_0 \cdot 2 \sin h \frac{e\psi_0}{2kT} = \sigma_B + \sigma_{ad}$$

where D is the dielectric constant of water; e, the electronic charge; k, the Boltzmann constant; T, absolute temperature; C_0 represents the total concentration of univalent ions (cations or anions) in solution per cm^3; σ_B is the charge tightly bound to membrane in our experimental conditions (with $pH < 7$, $\sigma_B = -\sigma_1 + \sigma_2 - \sigma_3$; with $pH > 7$, $\sigma_B = -\sigma_1 - \sigma_3$); σ_{ad} is the charge of the bound cations calculated according to the Langmuir formula in combination with the Boltzmann distribution law.

The shape of the ψ_0–pH and ψ_0–ionic strength curves is determined from the densities σ_1, σ_2, σ_3, the adsorption constant for Ca^{2+} (K_{Ca}) and the concentration of calcium. The adsorption constant for H^+ for the groups types I and II shifts the curves along the pH axis, and does not affect their shape. The numerical values of the parameters σ_1, σ_2, σ_3 and K_{Ca}, chosen so as to fit theoretical curves to the whole set of experimental data, are as follows: $\sigma_1 = 3{\cdot}3 \times 10^{13}$ cm^{-2}; $\sigma_2 = 2{\cdot}8 \times 10^{13}$ cm^{-2}; $\sigma_3 = 1{\cdot}9 \times 10^{13}$ cm^{-2}; $K_{Ca} = 5$ m^{-1} ($pK_{Ca} = 0{\cdot}7$). It should be emphasized that small variations of σ_1, σ_2, σ_3 and K_{Ca} from these numerical values result in marked discrepancies between ψ_0 and $V_{1/2}$.

Figs. 2 and 3 illustrate the curves obtained from the above equation with the parameters given, the values of pK_H for the groups of types I and II being 4·5 and 9·25 respectively.

A good fit of ψ_0 to $V_{1/2}$ with the same values of $\sigma_1, \sigma_2, \sigma_3$ and the same pK_{Ca}, when necessary, was observed for Ni^{2+}, Co^{2+} and Mg^{2+} with $pK_{Ni} = 1{\cdot}0$; $pK_{Ca} = 0{\cdot}9$; $pK_{Mg} = 0{\cdot}5$. It is implied that these divalent ions are bound by the groups of type I. As an example, Fig. 4 illustrates the effect of Ni^{2+} on $V_{1/2}$ (left hand ordinate) and ψ_0 (right hand ordinate) with and without Ca^{2+}.

From biochemical evidence the groups of type I, with $pK_H = 4{\cdot}5$ and pK values for divalent ions as indicated, are most likely to be carboxyl groups whereas the groups of type II, with $pK_H = 9{\cdot}25$, would be amino groups. The groups of type III might be carboxyl as well, but

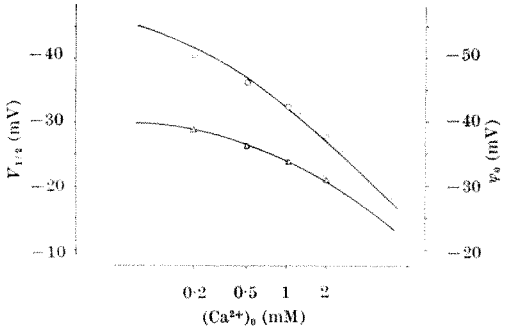


Fig. 3. Changes in $V_{1/2}$ and ψ_0 with calcium in normal (△) and 2·5 fold reduced ionic strength (○). Symbols represent the mean values, with respect to four experiments, for $V_{1/2}$. For the ψ_0–Ca^{2+} curve, see text. $K^+ = 50$ mM.

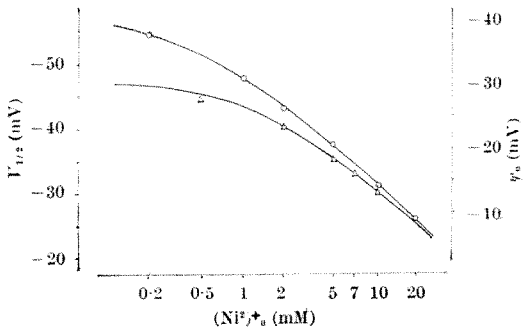


Fig. 4. Changes in $V_{1/2}$ and ψ_0 with Ni^{2+} concentration. △, In 2 mM Ca^{2+}; ○, no Ca^{2+}. Symbols represent the mean values, with respect to four fibres, for $V_{1/2}$. $K^+ = 100$ mM.

because of closely spaced positive charges, these groups have pK_H and pK_{Ca} values a unit or two lower and do not therefore participate in cation binding.

Thus the net surface charge is the sum of charges of the groups of all three types ($-\sigma_1 + \sigma_2 - \sigma_3 = -2{\cdot}4 \times 10^{13}$ electronic charges cm^{-2}) plus the charge of ions adsorbed (at pH 7 and 2 mM Ca^{2+} this charge is $+0{\cdot}7 \times 10^{13}$ electronic charges cm^{-2}) giving $-1{\cdot}7 \times 10^{13}$ electronic charges cm^{-2} in normal conditions.

Thus we may say that experimental shifts of $V_{1/2}$ and calculated changes in ψ_0 prove to be in good quantitative agreement, so that the shifts in the G_m–V_m curves produced by various factors may be actually presented as changes in membrane surface potential.

It may be helpful to correlate experimental evidence and theoretical curves. It must be emphasized, however, that by extending various experimental conditions involving shifts of $V_{1/2}$, and thus minimizing the arbitrariness in our choice of the parameters involved in the formulae for ψ_0, we substantially increase the value of this information. Thus for a quantitative agreement between ψ_0 and $V_{1/2}$ which is affected only by calcium and not by other factors[3,4], it is sufficient to assess the number of calcium binding sites as no less than 2×10^{13} cm^{-2} and pK values as no more than 1·5, the charge of each group being arbitrary. When $V_{1/2}$ is affected by several factors simultaneously, the calculation of all the curves is possible only with assumptions of the density of calcium binding sites, the charges on the sites and K_{Ca}. In this way we calculated σ_1 and pK_{Ca} as $-3{\cdot}3 \times 10^{13}$ cm^{-2} and 0·7 respectively.

The results presented may be useful for understanding the conductance–voltage relationships. Thus we may think that Ca^{2+}, or other divalent ions, are not essential in membrane conductance changes resulting from membrane potential changes, because the basic effect of calcium concentrations (namely, the shift of G_m–V_m curves along the voltage axis) is fully explained by changes in ψ_0. The surface membrane charge, resulting in ψ_0, should therefore be taken into account in any hypotheses concerning the mechanism of ionic conductance in excitable membrane.

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Initial Characterization of a Temperature Sensitive Mutant of Monkey Kidney Cell

AMONG the several temperature sensitive conditional mutants of the tetraploid cell line of African green monkey kidney (BS-C-1) is one (*ts*-2) in which simian virus 40 either fails to multiply or form tumour specific antigens (unpublished) at the temperature (39·5° C) at which cellular growth is restricted[1]. Because of the potential importance of this mutant in the study of host dependence of tumour virus multiplication, we have investigated several of its properties and have found that the mutant *ts*-2 is blocked in the incorporation of ^{3}H-thymidine in the nucleus, probably in the nucleolus.

The wild type parental cell line of BS-C-1 and the temperature sensitive mutant *ts*-2 passage 5 (clonal selection) were grown at 33° C for 3 to 4 weeks in L-15 medium[2] with 10 per cent foetal calf serum. After trypsinization, the cells (about 5×10^5/ml.) were plated in 60 mm plastic Petri dishes containing coverslips[3], at a dilution of 500 cells per plate. Replicate culture places were incubated at 33° C (pm$^+$) and 39·5 °C (pm$^-$). Every 24 h two plates from each set were taken out, the existing medium changed for fresh L-15 medium, each with 30 μCi/ml. ^{3}H-thymidine (^{3}H-TD) and 25 μCi/ml. of ^{3}H-uridine (^{3}H-UD), and the plates incubated for a further period of 4 h at the respective temperatures. [*methyl*-^{4}H]-Thymidine and generally labelled ^{3}H-uridine were obtained from the Radiochemical Centre, Amersham. Coverslips taken from culture dishes were washed in phosphate buffered saline (PBS) at *p*H 7·4, fixed in glacial acetic acid : ethanol (1 : 3), washed with 10 per cent trichloroacetic acid (TCA) and rinsed in methanol. Dried coverslips were counted directly in toluene-based scintillation fluid (efficiency 35–40 per cent) for total incorporation[4–6] of ^{3}H-TD and ^{3}H-UD in comparable conditions between the two cell lines at the pm$^+$ and pm$^-$ temperatures.

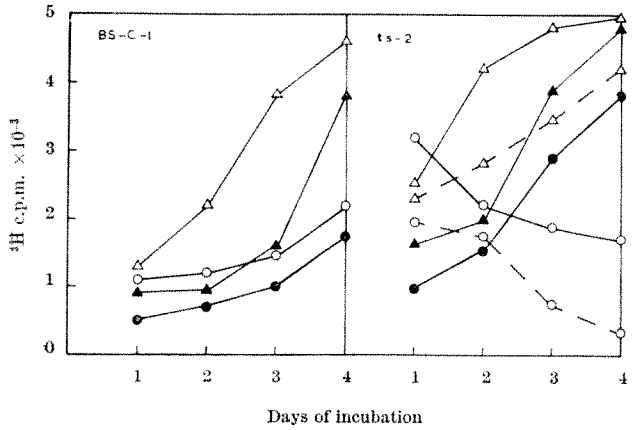


Fig. 1. Total incorporation of ^{3}H-TD (○,●) and ^{3}H-UD (△,▲) by African green monkey kidney cell line BS-C-1 and a temperature sensitive mutant clone *ts*-2, passage 5, incubated at the temperatures of 33° C (●,▲), and 39·5° C (○,△) at a cell density of approximately 5×10^5/ml. Replicate cultures were exposed every 24 h for a period of 4 h to ^{3}H-TD (30 μCi/ml.) and ^{3}H-UD (25 μCi/ml.) at respective temperatures. Discontinuous lines in the graph refer to results of experiments performed with passage 3 of the mutant clone *ts*-2. Details in text.

Results (Fig. 1) showed that during the first 4 days of incubation, the mutant *ts*-2 grew better than the parent (BS-C-1) at the pm$^+$ temperature, as indicated by higher incorporation of both ^{3}H-TD and ^{2}H-UD. In the pm$^-$ temperature, on the other hand, the BS-C-1 cell line had grown distinctly better than at 33° C, whereas the temperature sensitive mutant *ts*-2 showed relatively little increase in the incorporation of ^{3}H-TD at 39·5° C over this period. The mutant *ts*-2, however, showed at least two to three-fold more thymidine incorporation on the first day at 39·5° C than at 33° C and this gradually decreased over the next 3 days. For the corresponding period incorporation of ^{3}H-UD by the mutant *ts*-2 had remained unaffected as compared with the parent cell line (BS-C-1). The increase in the incorporation of ^{3}H-TD by the mutant *ts*-2 at the pm$^-$ temperature of 39·5° C might be attributed to the enlarging of the cell[1], caused by some unscheduled activity in the cytoplasm under conditions of absence or reduction of nuclear DNA synthesis. This is also corroborated by the results of autoradiography (Figs. 3 and 4). It should be noted (Fig. 1) that ^{3}H-TD and ^{3}H-UD incorporation by the mutant *ts*-2 at 39·5° C at an earlier passage (passage 3), though showing lower incorporation of both thymidine and uridine, also exhibited a similar trend in gradual decrease in thymidine incorporation. The difference in the rate of incorporation of ^{3}H-TD by the mutant *ts*-2 at 39·5° C between successive passages could only be attributed to leakiness of the mutant itself. It has been noted that this particular mutant (*ts*-2) showed increased leakiness with every passage, and beyond the twelfth passage it reverted considerably in its growth and metabolic activity, and was comparable with the parent BS-C-1 cell line between 33° C and 39·5° C.

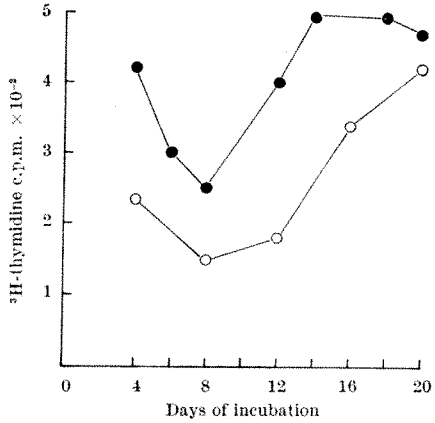


Fig. 2. Total incorporation of ^{3}H-TD by the temperature sensitive mutant clone *ts*-2, passage 5, grown at 39·5° C, shifted to 33° C for 4 h (●) following exposure to ^{3}H-TD (10 μCi/ml.). Replicate cultures used as controls (○) of *ts*-2 were exposed to ^{3}H-TD and incubated at 39·5° C for an equal length of time. Details in text.

To determine the inability of cells exposed to restrictive temperature for 20 days a temperature-shift experiment was done (Fig. 2). Replicate culture plates of the mutant cell line *ts*-2 passage 5 were incubated in conditions identical to those mentioned here, at the restrictive temperature of 39·5° C. Every 48 h culture plates were exposed to ^{3}H-thymidine (10 μCi/ml.) and incubated at the permissive temperature of 33° C for another 4 h. Replicates used as controls were incubated at 39·5° C. At least some cells remained viable for 20 days and continued to incorporate ^{3}H-TD when transferred to 33° C, though at a relatively lower rate. It should be noted that for the first 8 days the rate of ^{3}H-TD incorporation slowly decreased. This could indicate that the nature of the mutation had remained dominant in the initial stages of growth at 39·5° C, but, probably due to leakiness, the rate of ^{3}H-TD incorporation increased slightly from the eighth day onwards.

BS-C-1 and *ts*-2 (passage 5) cell lines grown at 33° C for 3–4 weeks were trypsinized, seeded overnight on coverslips in Petri dishes at 33° and 39·5° C and then exposed to 30 μCi/ml. of ^{3}H-thymidine for 48 h at the respective temperatures. Cells were then washed twice in PBS, fixed in acetic acid–ethanol (1 : 3) and the coverslips dried at room temperature. The latter were dipped

in Ilford 'L-4' liquid emulsion fluid in 1 per cent No. 1 gelatine solution and kept in the cold for 7 days before being processed. Control coverslips were run simultaneously to determine any background effect or artefacts.

The mutant *ts*-2 (clonal passage 5), although showing normal incorporation of thymidine at the pm$^+$ temperature of 33° C (Fig. 3*B*), had very little thymidine incorporation in the nucleus (and, probably, the nucleolus) at the pm$^-$ temperature of 39·5° C (Fig. 3*C*). When compared with the parent cell line BS-C-1 (Fig. 4*A*) at 39·5° C, the mutant (Fig. 4*B*) was uniformly labelled in the cytoplasm and/or nuclear membrane. A long pulse of 48 h of ^{3}H-TD was given to make sure that the reduced activity in the mutant cell line at higher temperature was not due to a low level of metabolic activity. This did not make it clear whether the incorporated thymidine was in fact in the cytoplasmic DNA or had been demethylated into uridine. Whatever the degradative product of thymidine incorporation, the temperature sensitive mutant (*ts*-2) was relatively inactive in the nucleus or nucleolus at the restrictive temperature of 39·5° C. The temperature sensitivity of *ts*-2 was probably due, therefore, to a mutation involving the nucleus.

Monolayer cell cultures of both BS-C-1 and *ts*-2 (passage 5) were grown in 20 ounce bottles for 3–4 weeks in L-15 medium at the permissive temperature of 33° C. Cells were then trypsinized and seeded at a density of 5×10^7/ml. in glass bottles and replicates incubated overnight at 33° and 39·5° C. After 24 h the culture medium was exchanged for fresh L-15 medium with 30 μCi/ml. of ^{3}H-thymidine and incubated 48 h more at the respective temperatures.

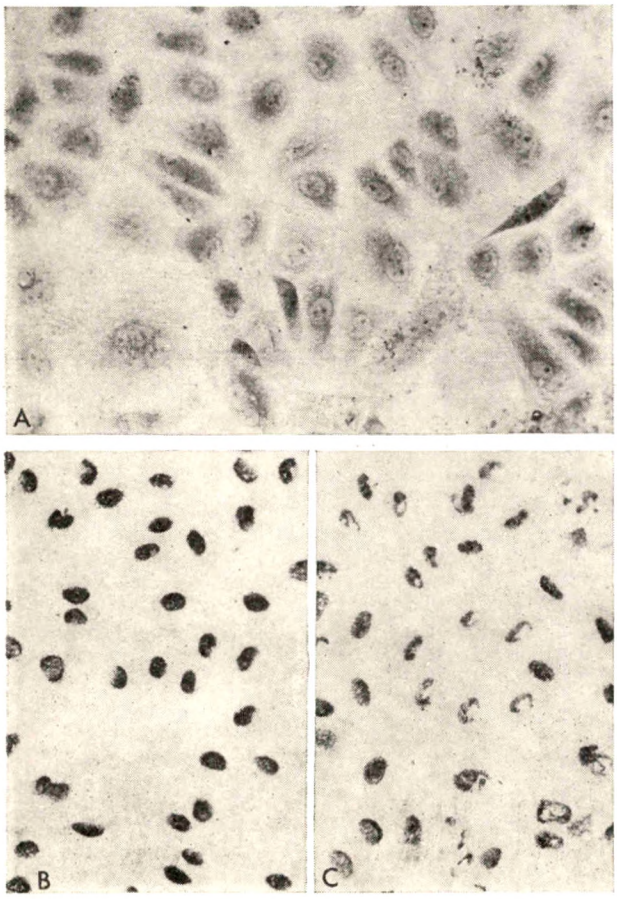


Fig. 3. *A* shows a confluent monolayer of mutant clone *ts*-2 grown at the pm$^+$ temperature of 33° C for 7 days and stained in pyronin methyl green (×180). *B* and *C* show autoradiographs (×120) of the mutant *ts*-2 at 33° C and 39·5° C respectively after exposure to ^{3}H-TD (30 μCi/ml.) for 48 h.

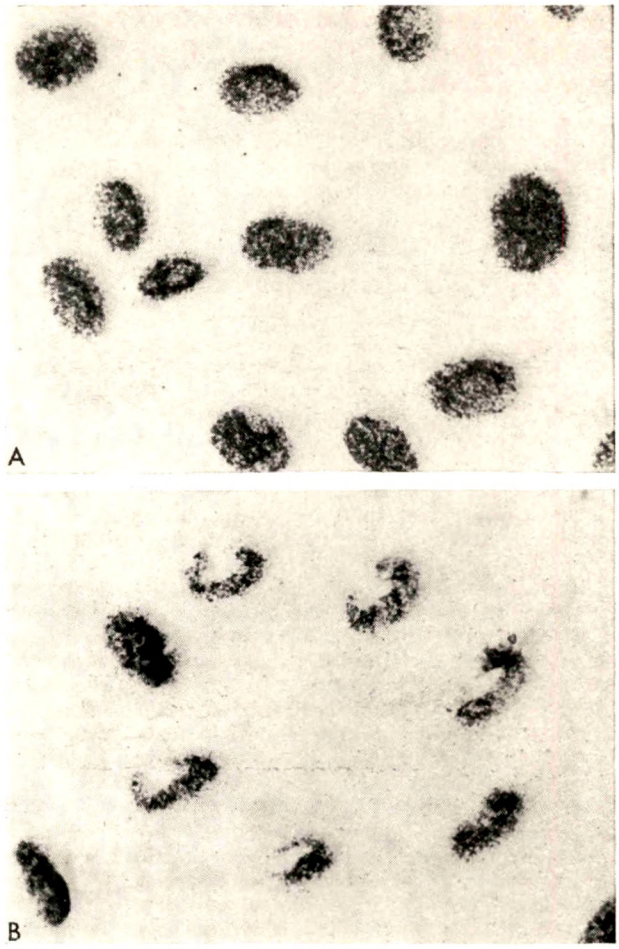


Fig. 4. Autoradiographic pictures (×288) of BS-C-1 (*A*) and the mutant *ts*-2 (*B*) grown at 39·5° C after exposure to ^{3}H-TD (30 μCi/ml.) for 48 h.

Cells were then washed twice with PBS, trypsinized and collected in PBS, centrifuged and washed twice in calcium and magnesium-free PBS buffer at 1,000 r.p.m. for 10 min. The centrifugate was resuspended in 2 ml. of Ca-Mg-free PBS (cell density $2·5 \times 10^8$/ml.) and layered over 10 ml. of 0·3 M sucrose and centrifuged at 1,000 r.p.m. for 20 min. The pellet containing whole cells was finally resuspended in 4·5 ml. of 0·3 M sucrose, disrupted by the Ultra-Turrax disintegrator[7] until 90 per cent cells were disrupted (observed under the microscope). 0·05 ml. of 10 mM magnesium acetate and 0·5 ml. of 100 mM KCl were added. Cellular fractions[7,8] of labelled nuclei, mitochondria, microsomes and cell sap were separated and the specific activity of the TCA insoluble protein in each fraction determined[9] using the method of Lowry *et al.*[10].

Fractions of cellular organelles were collected as follows. The homogenates were centrifuged for 30 min at 2,000 r.p.m. in the horizontal rotor to sediment the nuclei. The supernatant fluid was used for the isolation of mitochondria and microsomes. The nuclear pellet was further purified by washing twice in 0·3 M sucrose containing 1 mM $MgCl_2$ and 0·5 per cent (w/v) 'Triton X-100' and twice with 0·15 M NaCl. The supernatant fluid remaining after the sedimentation of the nuclei was centrifuged for 10 min at 10,000 r.p.m. in the Sorvall SS-34 rotor to sediment the mitochondria. The pellet was resuspended in 0·3 M sucrose and resedimented for 15 min at the same speed. The last two supernatant fluids remaining after sedimentation of the mitochondria were centrifuged for 1 h at 50,000 r.p.m. in the MSE-50 rotor (10×10 ml.) to sediment the microsomal fraction; the

supernatant contained the cell sap. The pellet was resuspended in 0·3 M sucrose and centrifuged for 10 min at 10,000 r.p.m. in the Sorvall to sediment the small amount of mitochondrial material carried over in the previous steps. The supernatant fluid was then diluted with an equal volume of 0·15 M NaCl and the mixture centrifuged at 50,000 r.p.m. for 1 h. All steps in the cellular fractionation were carried out at 0°–2° C.

Table 1. LEVELS OF INCORPORATION OF ^{3}H-TD IN DIFFERENT CELLULAR FRACTIONS OF BS-C-1 AND *ts*-2 CELL LINES AFTER 48 HOURS EXPOSURE TO ^{3}H-TD AT 39·5° C, CALCULATED IN RESPECT OF RADIOACTIVITY IN TOTAL COLD TCA PRECIPITABLE PROTEIN

	c.p.m./mg protein ($\times 10^{-3}$)	
	BS-C-1	*ts*-2
Nuclei	310·0	78·6
Mitochondria	10·4	4·8
Microsomes	5·1	50·2
Cell sap	3·6	4·2

The results (Table 1) showed a remarkably high radioactivity per mg of protein in the microsomal fraction of the cytoplasm in the mutant *ts*-2 when compared with the identical fraction of the parental cell line BS-C-1. The nuclei of *ts*-2, on the other hand, had relatively little radioactivity as compared with the BS-C-1 cell line. This is consistent with the autoradiographic data. The low level of incorporated ^{3}H-TD in the mitochondrial fraction of the mutant cell line *ts*-2 at 39·5° C was thought to be significant. The number of c.p.m. per mg of protein were reduced by nearly a factor of four in the nuclei of the mutant when compared with the parental cell line, and that of mitochondria had decreased by nearly a factor of two, which in the mutant was similar to the amount of radioactivity in the cell sap (mitochondria : cell sap = 4·8 : 4·2). It is difficult to say from these data that the low level of 3H-TD incorporation by the mitochondria of mutant cell line *ts*-2 at the pm$^-$ temperature (39·5° C) is a direct effect of the inactivity of the nucleus. Further experiments are in progress to determine the exact nature of thymidine metabolism in the cytoplasm of the mutant cell line *ts*-2. It might be mentioned in this connexion that there have been several recent reports[8,11,12] of DNA associated with the microsomal fraction of mammalian cells which closely resembles nuclear DNA but is more rapidly labelled with isotopic precursors.

We tentatively conclude that the mutant cell line *ts*-2 was probably defective in the nucleic acid metabolism in the nucleus or in the nucleolus at the pm$^-$ temperature. The mutant, however, was visibly normal in growth at the pm$^-$ temperature of 33° C (Fig. 3*A*). The parental cell line BS-C-1 had remained unaffected between these two temperatures. The metabolic end product of the incorporated 3H-TD in the cytoplasm of the mutant at the restrictive temperature was not studied during these experiments. Long exposures to ^{3}H-TD indicated that a small percentage of the [methyl-^{3}H] thymidine had been demethylated into uridine. This part of the work is now being carefully investigated.

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Differences in Rh Type between Age Groups of Leukaemia Patients

IN view of the variation of the incidence of leukaemia in relation to age, sex and chronicity[1], I have investigated the ABO and Rh blood groups in subsamples of these categories. Significantly I found higher Rh negative frequencies among patients between groups 0–4 years old and more than 50 years old compared with those between 5 and 50.

The medical record numbers of all patients with a diagnosis of leukaemia were obtained from the Columbia–Presbyterian Medical Center, New York City, for the period 1945 to 1958; the data were collected in 1959. All information was taken from the medical record of each patient. The diagnosis of leukaemia was based on laboratory reports on bone marrow, blood and lymph node biopsy specimens, X-ray findings (children) and autopsy reports. Usually more than one of these sources of information was available for a given patient. There were 581 Caucasian patients—458 Gentiles and 123 Jews. Only the analysis of the Gentile sample was undertaken because the very limited size of the Jewish sample precluded meaningful analysis.

Because it is known that leukaemia is most frequent before 5 and after 50 yr (ref. 2), the study population was divided into three age groups: 0–4, 5–50 and more than 50 yr.

Table 1. DISTRIBUTION OF ABO AND Rh BLOOD GROUPS IN ACUTE AND CHRONIC LEUKAEMIA PATIENTS AND AGE AT DIAGNOSIS

Age at diagnosis	Chronicity	Rh type	ABO blood groups O	A	B	AB	Total	Per cent Rh
0–4	Acute	Positive	17	26	7	3	53	77·94
		Negative	5	7	2	1	15	22·06
	Chronic	Positive	3	3	0	0	6	66·67
		Negative	2	1	0	0	3	33·33
5–50	Acute	Positive	46	41	12	3	102	88·70
		Negative	8	4	1	0	13	11·30
	Chronic	Positive	11	19	3	1	34	89·47
		Negative	2	1	1	0	4	10·53
51–90	Acute	Positive	17	15	7	1	40	74·07
		Negative	6	8	0	0	14	25·93
	Chronic	Positive	32	27	9	3	71	78·89
		Negative	4	11	4	0	19	21·11

Blood group determinations were available for 374 Gentile patients, and the proportions of each sex within a given age group were comparable ($P > 0·15$ in each case). There is no suggestion of differences in the distribution of the ABO blood groups between categories of age or chronicity (Table 1), although a much larger sample is clearly required to give meaningful results. The Rh frequencies are remarkably similar between acute and chronic leukaemia patients within each age group, but the distribution of these frequencies among the three age groups differs significantly, $\chi^2 = 8·71$, 2 d.f., $P < 0·15$ (Table 2).

Table 2. DISTRIBUTION OF Rh TYPE IN RELATION TO SEX AND AGE OF PATIENT

Age groups	Rh type	Male *n*	Male Per cent	Female *n*	Female Per cent	Male and female *n*	Male and female Per cent	Total *n*
0–4	Positive	30	73·17	29	80·56	59	76·62	77
	Negative	11	26·83	7	19·44	18	23·38	
5–50	Positive	81	93·10	55	83·33	136	88·89	153
	Negative	6	6·90	11	16·67	17	11·11	
51–90	Positive	71	79·78	40	72·73	111	77·08	144
	Negative	18	20·22	15	27·27	33	22·92	
Total	Positive	182	83·87	124	78·98	306	81·82	374
	Negative	35	16·13	33	21·02	68	18·18	

When the data for males and females are analysed separately, the heterogeneity in the Rh frequencies among the age groups in males is statistically significant, but although the same trend prevails in females, it is not significant. None of the comparisons between males and females differs significantly.

Independent comparisons of age groups 0–4 and 51–90 yr with 5–50 yr indicate a significant excess of Rh negative patients in each of the two former groups in males, and in the combined data of males and females, $P < 0.02$ or less. Thus the increased Rh negative frequency is observed in the two age groups associated with a high incidence of leukaemia.

No factors seem to favour the admission of leukaemia patients to the hospital from which my sample was obtained on the basis of Rh type and age. Since for most patients the diagnosis was established at this hospital, it is not likely that blood groups were determined in relation to their disease before admission. Decreased agglutinability of the D (Rh) antigen, or two populations of cells (Rh positive and Rh negative), have been reported in very small samples of patients with leukaemia[3,4]. This evidence, however, does not warrant the assumption that "complete" loss of agglutinability of the D antigen can explain the excess of Rh negative patients in the age groups observed in this study. Furthermore, there is evidence of immunological similarities between certain autoimmune diseases and some leukaemic states[5], and that the thymus is involved in idiopathic autoimmune diseases and in malignant proliferation, such as leukaemia[6]. Thus it may not be fortuitous that an excess of Rh negative[5] was found in patients with autoimmune diseases[7], and, in this report, among patients in the age groups associated with the highest susceptibility to leukaemia.

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Effects of Anti-lymphocyte Serum on Uptake of Uridine by Lymph Nodes during Carcinogenesis

ANTI-LYMPHOCYTE serum (ALS) has been shown to influence the course of experimental carcinogenesis, enhancing susceptibility to oncogenic viruses[1,2] and the carcinogenic action of dimethylbenz(α)anthracene (DMBA)(ref. 3). Subcutaneous administration of ALS augmented the production of tumours formed in hamster cheek pouches by topical applications of DMBA and details of tumour incidence have been reported[3]; 95 per cent of pouches contained tumours after 12 weeks, compared with 70 per cent in untreated animals.

In vitro studies have demonstrated that lymphoid blast cells incorporate more RNA precursor than other cells of the lymphatic population[4]. We wish to describe here some general aspects of regional lymphatic involvement during carcinogenesis in an immunologically privileged site, and an assay of lymphoid blast cells exhibiting RNA synthesis, possibly concerned in the elaboration of a cell mediated response to an antigen. The cheek pouches of twenty-eight conventionally bred and maintained golden Syrian hamsters (*Mesocricetus auratus*) were painted three times a week with 0·5 per cent DMBA in mineral oil (Sigma). Sixteen of these animals received ALS, which had been prepared in rabbits, had a cytotoxic titre greater than 256 as determined by the trypan blue exclusion test, and was administered according to a regimen described earlier[3]. Comparisons were made with ten untreated hamsters and seven animals whose cheek pouches were painted with butylated hydroxyanisole (BHA) for 15 weeks. The latter were a by-product of experiments to test the carcinogenic potential of hydroxyanisoles, chemicals known to cause chronic inflammation and structural changes in the hamster cheek pouch, comparable with the early effects of DMBA (ref. 5).

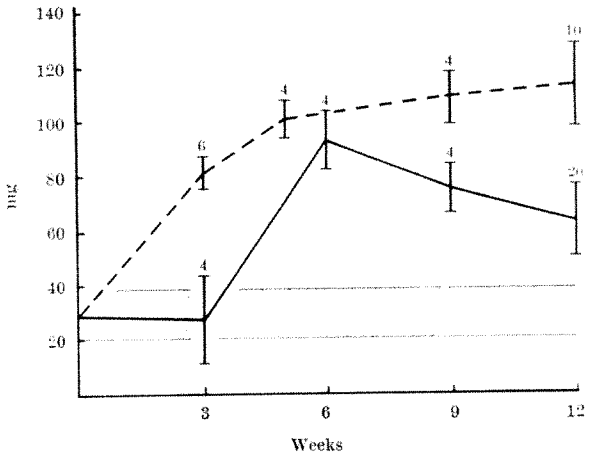


Fig. 1. Weights of submandibular lymph nodes; untreated hamsters (hatched area), treated with DMBA (- - -), treated with ALS/DMBA (———). Numbers of lymph nodes weighed are indicated.

When treatment commenced the animals were 4–6 weeks old and weighed on average 85 g. The BHA group was killed after 15 weeks; others were killed at intervals. Sub-mandibular lymph nodes were removed, stripped of extraneous tissue and weighed.

For autoradiography, two animals were taken at each of the following states: (i) without treatment; (ii) after 15 weeks of BHA; (iii) after 3 weeks of DMBA; (iv) after 3 weeks of ALS/DMBA; (v) after 12 weeks of DMBA; (vi) after 12 weeks of ALS/DMBA. Hamsters in groups (v) and (vi) carried one or more tumours in each pouch. Ninety minutes before being killed, each received intraperitoneally 10 μCi/g of 5-^{3}H-uridine (specific activity 5·0 Ci/mole; Radiochemical Centre, Amersham). Material for light microscopy was fixed in buffered formol saline and processed using Ilford K5 dipping emulsion. Tissue for electron microscopy (EM) was fixed in phosphate buffered 2 per cent osmium tetroxide and exposed with Ilford L4 emulsion. Most light autoradiographs were stained with haematoxylin and eosin but some were prepared with methyl green and pyronin. Cells were counted on six or more light autoradiographs of each node, examining twenty-four random areas of the node core at a magnification of × 800.

At the beginning of the experiment the lymph nodes were considered to be fully developed[6]. Their weights were recorded (Fig. 1) throughout the experiment and it was found that DMBA treatment caused increasing hypertrophy of the lymph nodes. ALS partially suppressed this enlargement.

In the light autoradiographs, the paracortical regions of all nodes examined contained large, pyroninophilic

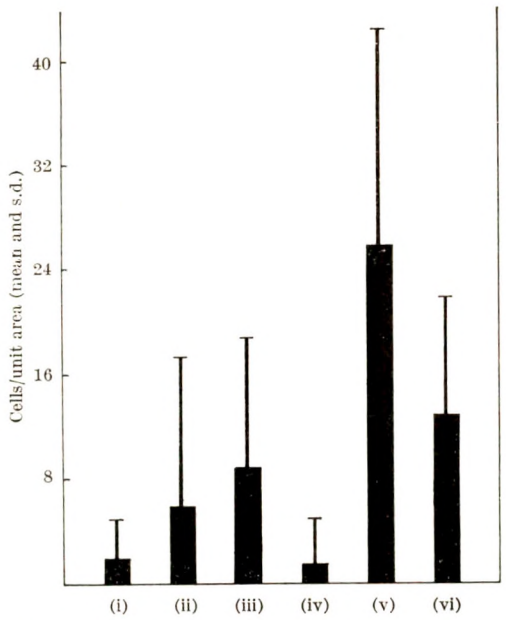


Fig. 2. Numbers of large, pyroninophilic, labelled cells counted/unit area in light autoradiographs. (i) Untreated; (ii) BHA 15 weeks; (iii) DMBA, 3 weeks; (iv) ALS/DMBA, 3 weeks; (v) DMBA, 12 weeks; (vi) ALS/DMBA, 12 weeks.

cells, some of which were labelled with uridine; their relative numbers are presented in Fig. 2. In electron microscope autoradiographs (Fig. 3) the labelled cells resembled lymphoblasts. They had a maximum diameter of 24 μm, their cytoplasm contained abundant polysomes and their nuclear chromatin was almost totally in the expanded form. They were surrounded by unlabelled small lymphocytes; medullary plasma cells with dilated endoplasmic cisternae, neutrophils, eosinophils and some blast-like cells were unlabelled.

The degree of lymph node hypertrophy was paralleled by the relative number of RNA synthesizing blast cells. The strongest reaction was evoked in the animals treated with DMBA which bore pouch tumours but whether this large number of active blast cells could be attributed to the cumulative effects of DMBA or to the presence o tumour antigens was not ascertained. Lymphoid hyper trophy and blast cell formation have been observed i mice receiving chronic ALS treatment[7] and, althoug similar hyperactivity was evident in the lymph nodes o the hamsters treated with ALS and DMBA, their respons was poor when compared with those on DMBA alone.

Lymphatic connexion between the hamster chee pouch and the submandibular lymph node can be demon strated by tracing with Evans blue (unpublished work c C. J. Smith), and therefore it is not surprising that a antigenic stimulus in the pouch, although the latter i immunologically privileged, may affect the regional node Treatment with ALS can influence the incidence an generation time of chemically induced cheek pouc carcinomas[3] and also partially suppresses regional lym phoid activity, which suggests that ALS interferes wit efferent and/or afferent cell traffic between the pouc and the regional node.

Impairment of the cell mediated immune response an blast cell activity by ALS, as evidenced by the relativ numbers of cells able to take up large quantities of uridine may be related to the enhancement of pouch tumours.

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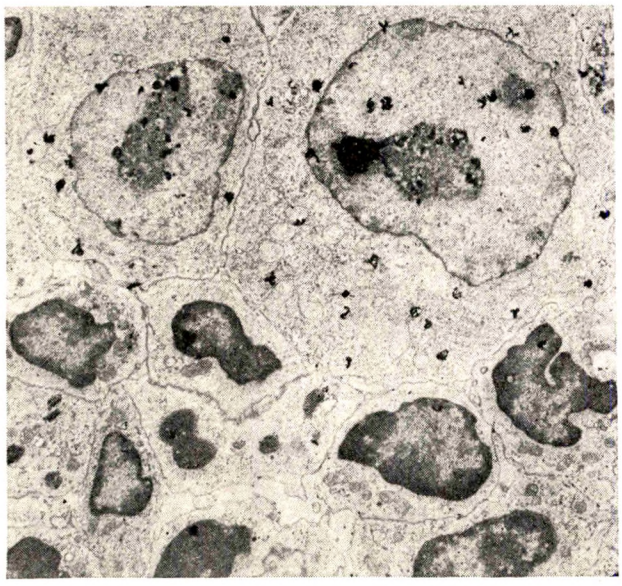
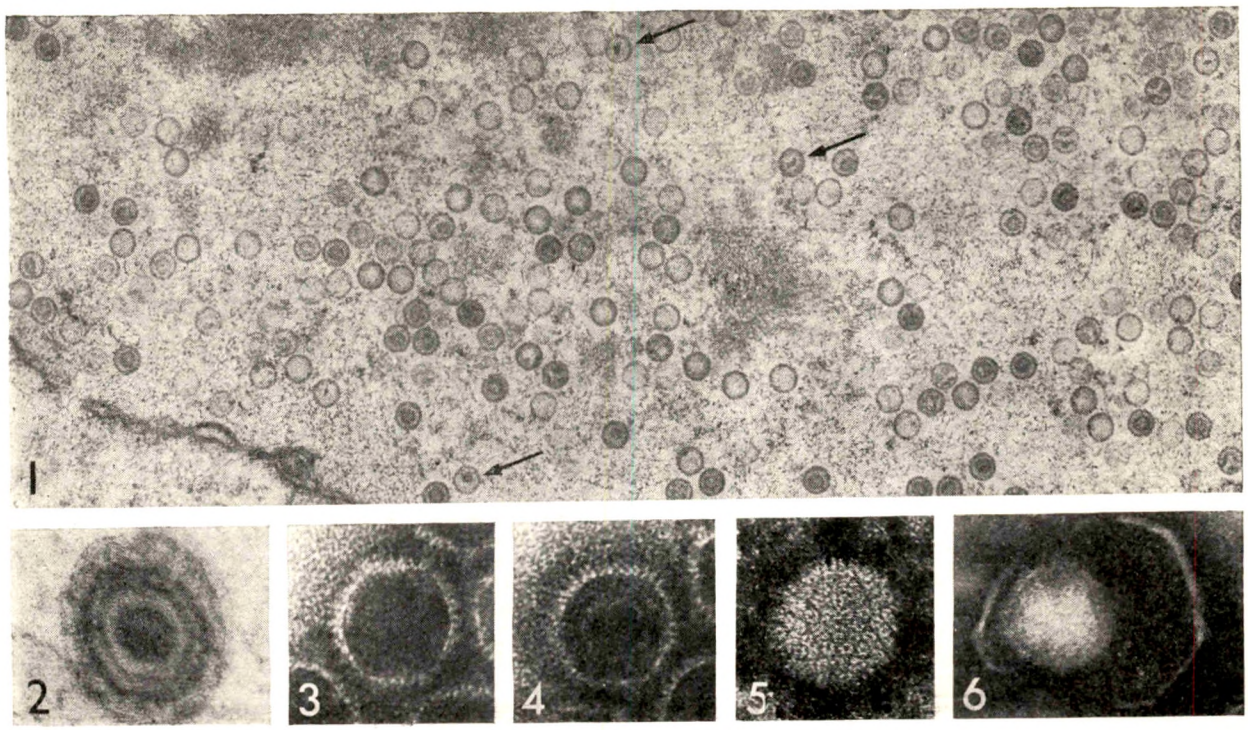


Fig. 3. Electron micrograph of large, blast-like cells labelled with 5-^{3}H-uridine. (×3,000.)

Morphological Confirmation of the Herpes Nature of a Carcinogenic Virus of Primates (*Herpes saimiri*)

Interest in the possible relation of herpes viruses t cancer has been aroused by the discovery of the herpes like EB virus in cultured Burkitt tumour cells[1,2]. Th association of EB virus with malignant change has subse quently been strengthened by sero-epidemiological studie on patients with Burkitt's lymphoma[3,4], by observation on the effects of the virus when it infects and stimulate human lymphoid cells *in vitro*[5–7], and by its incriminatio as the aetiological agent of the limited *in vivo* lymph proliferation of infectious mononucleosis[8–10].

The importance of these findings has been confirme by new developments showing herpes viruses to b carcinogenic in animals. A herpes virus long known t occur in cells of the Lucké frog kidney carcinoma[11] ha been shown to cause malignant kidney tumours whe injected into frog embryos[12], and this carcinogenic fro agent shares a common antigen with EB virus[13]. I addition, a virus has at long last been isolated fro Marek's chicken lymphoma[14,15], has been identified as th causative agent[16,17], and has likewise been shown to be typical herpes virus[18,19].



Figs. 1–6. Electron micrographs of material from owl monkey kidney cultures infected with *Herpes saimiri*. Figs. 1 and 2 show thin sections which have been stained with uranyl acetate; Figs. 3 to 6 show whole-mount preparations in PTA. Fig. 1, part of a nucleus containing profuse, hexagonal, immature virus particles, either empty or with dense central (arrows) or ring-shaped nucleoids. (×35,825.) Fig. 2, mature particle with its additional outer enveloping membrane lying within a cellular space bounded by a fine membrane. (×125,000.) Fig. 3, empty hexagonal immature particle which has been penetrated by PTA. The structure of the hollow tubular surface capsomeres is seen well in side view. (×150,000.) Fig. 4, hexagonal immature particle; a central ring-shaped nucleoid is revealed following penetration by PTA. (×150,000.) Fig. 5, undamaged, complete, hexagonal immature particle with hollow capsomeres in end on view arranged in triangular facets. (×150,000.) Fig. 6, mature particle with damaged outer enveloping membrane; PTA has entered to reveal the hexagonal immature component within. (×150,000.)

More recently, a new virus has been propagated in tissue culture from normal squirrel monkey kidney cells[20], and, although without pathogenic effects on its natural host, it regularly induces fatal lympho-reticular tumours on inoculation into owl monkeys and marmosets[21]. The agent is thought to be a herpes virus on the basis of its biological properties, and has been designated *Herpes saimiri*[20], and we now provide definitive morphological confirmation that this squirrel monkey virus is indeed a member of the herpes family.

Confluent owl monkey kidney cell cultures were infected with sufficient virus to give moderate widespread cytopathic change after incubation at 37° C for 12 days. The infected cells were then gently scraped from the glass into suspension in 1 ml. of culture fluid. Thereafter, the cells were either fixed in glutaraldehyde followed by osmium, dehydrated and embedded in epoxy resin for thin sectioning as already described[22], or made into whole-mount negative-contrast preparations in phosphotungstic acid (PTA) by the method of Parsons[23]. Observations were made with a Philips EM 300 electron microscope.

On examination all the infected cultures contained profuse virus particles of herpes-type morphology (Figs. 1 to 6) which were absent in uninoculated control cultures. In thin sections immature hexagonal particles, either empty or with central ring-shaped or dense nucleoids and of about 108 nm diameter, were seen in both nuclei (Fig. 1) and cytoplasm. Mature particles with an additional enveloping membrane measured 140 nm across and lay in the perinuclear space, in cytoplasmic spaces (Fig. 2), or close outside the cell surface.

Whole-mount PTA preparations confirmed the size of the particles (Figs. 3 to 6). Empty particles without nucleoids were penetrated by PTA (Fig. 3) as were some with nucleoids (Fig. 4); intact particles were also seen and often showed triangular surface facets (Fig. 5). All the immature particles were covered by hollow tubular capsomeres 12·5 nm long and with a centre-to-centre spacing of 12·5 nm (Figs. 3 to 5). Although the exact number of capsomeres along the edge of a facet could not be accurately determined, the findings suggest that the immature particle is an icosahedron covered by 162 capsomeres. Mature particles appeared structureless except where the outer enveloping membrane was damaged and allowed PTA to enter and reveal the immature component within (Fig. 6).

On morphological grounds, therefore, *Herpes saimiri* has been confirmed as a member of the herpes virus group. Taken in conjunction with the frog kidney tumour herpes virus[6] and the herpes virus of Marek's disease[9], a carcinogenic herpes virus of primates has important implications for the problem of EB virus and human cancer. Our findings reinforce the need to explore fully the role of herpes viruses in the whole field of neoplasia.

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Copper in Hepatocyte Lysosomes of the Toad, *Bufo marinus* L.

ALTHOUGH copper is essential for life, excessive concentrations are usually toxic and may lead to pathological changes in various tissues and eventually death. Acute disturbances, sometimes fatal, may occur in sheep and cattle grazing on pastures enriched with copper. Chronic copper toxicity in animals is unknown, although it occurs in human beings as Wilson's disease (hepatolenticular degeneration)[1]. In these patients greatly increased hepatic copper concentrations, approaching 2,000 μg/g dry tissue (normal: 20–45 μg/g)[2] cause degeneration of hepatocytes, necrosis and ultimately cirrhosis[2,3].

Beck, working in Australia, found copper concentrations as high as 1,640 μg/g dry tissue in the livers of the toad, *Bufo marinus* L.[4]. This was by far the highest hepatic copper concentration of eighty-three species studied. The toad's liver is undamaged by this level of copper, so we have compared the intracellular distribution of the metal in the toad with the distribution previously described in patients with Wilson's disease[3] and in newborn human infants whose livers also tolerate large amounts of copper[3,5].

Samples of liver from ten female toads from the Dominican Republic were fixed in neutral formalin and embedded in paraffin. Sections were examined by light microscopy after staining with: haematoxylin and eosin for histological detail; a modification[3] of Timm's silver sulphide procedure[6] for demonstration of heavy metals; rubeanic acid, for identifying copper[3,7]; and Perls's prussian blue technique, for staining iron[8]. Lipofuscin was identified by Schmorl's[8] and the 3,3′ diaminobenzidine (DAB) reactions[9]. To demonstrate lysosomes frozen sections of tissue fixed in formalin were incubated for acid phosphatase activity with β-glycerophosphate as substrate[10] and β-glucuronidase activity with naphthol-AS-Bl glucuronic acid as substrate[11]. Frozen sections were also stained with oil red O (ref. 10) to demonstrate fat. Portions of four livers were cut into small cubes, fixed in 3 per cent glutaraldehyde in 0·1 M cacodylate buffer, *p*H 7·4, osmicated, dehydrated and embedded in Epon. Thin sections were stained with uranium and lead for examination in a Siemens Elmiskop 1*a* electron microscope.

Grossly, these toad livers, like those of other amphibians, were generally black and under the light microscope there were large numbers of cells, probably macrophages[12], filled with melanin granules. Some livers were yellow, however, and in these specimens the hepatocytes contained numerous large lipid droplets, up to 10 μm in diameter. Cytoplasmic fat was seen in the black livers, but the droplets were about a tenth as large and far less numerous. No evidence of necrosis or fibrosis was seen in any of the ten livers.



Fig. 1. Copper demonstrated with rubeanic acid is localized in the pericanalicular pigment granules (arrows) of toad hepatocytes. (×1,800.)

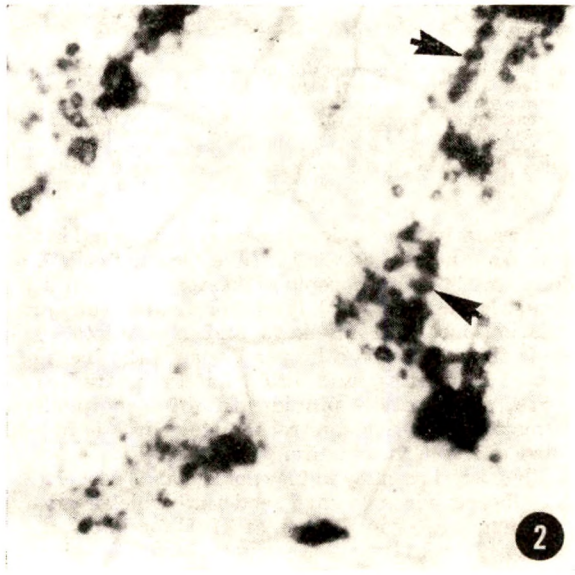


Fig. 2. Many pericanalicular granules (arrows) show the staining characteristic of lipofuscin in a section of toad liver stained with Schmorl's technique. (×2,000.)

In unstained sections numerous brown granules were seen arrayed along the bile canaliculi of hepatocytes and these contained heavy metals demonstrable with the silver sulphide procedure. Staining with both rubeanic acid (Fig. 1) and Perls's prussian blue procedure showed that the metal was copper and not iron. No copper was demonstrated elsewhere in the cell. The granules were Schmorl positive (Fig. 2), and showed heat stable peroxidase activity with DAB and acid phosphatase and β-glucuronidase activities, all characteristic of lysosomes that contain lipofuscin[5,9]. In the electron micrographs they were delimited by a single membrane and contained membranous, vesicular and granular components of varying electron density (Fig. 3).

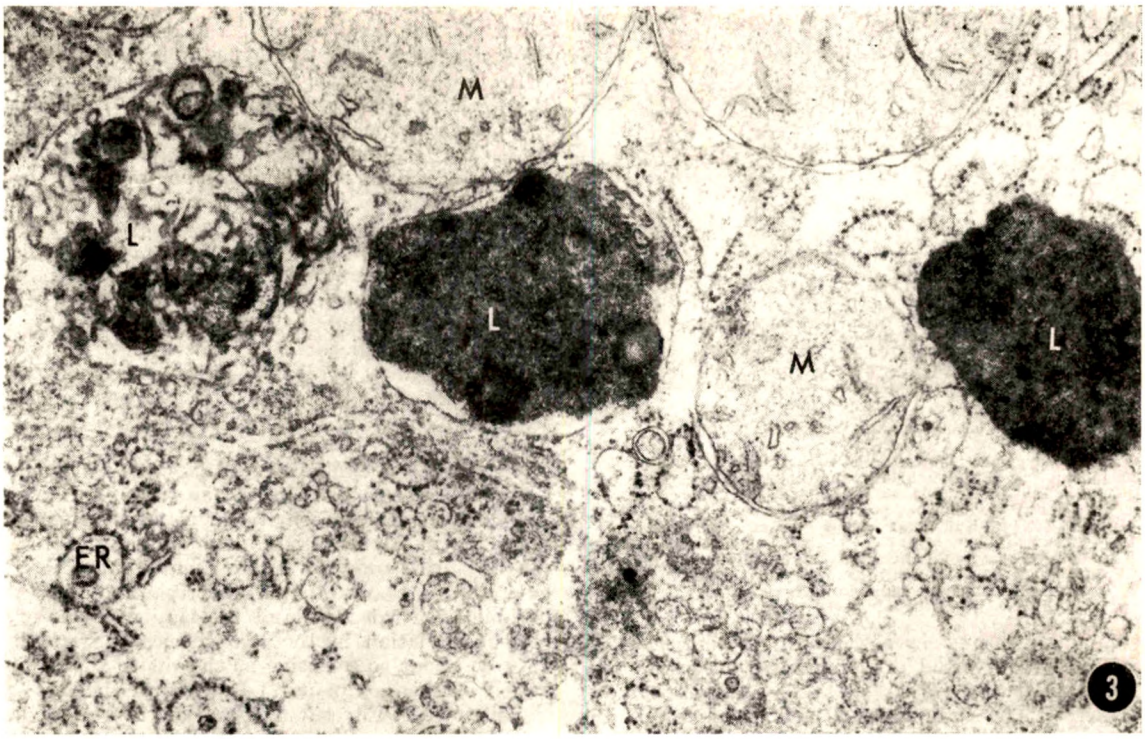


Fig. 3. Electron micrograph showing a portion of a toad hepatocyte with membrane bounded dense bodies or lysosomes (L) the contents of which are similar to those of human lipofuscin granules. Mitochondria (M) and endoplasmic reticulum (ER) are noted. (×20,000.)

Copper concentrations were determined quantitatively[13] on aliquots of liver from six of the toads. The black livers contained about three times as much copper as the fatty, yellow organs (Table 1).

Table 1. HEPATIC COPPER CONCENTRATIONS IN SIX TOADS

Specimen No.	Colour of liver	μg/g dry liver
1	Black	1,265
2	Black	1,248
3	Black	2,091
4	Yellow	367
5	Yellow	559
6	Yellow	459

The localization of these astonishingly high concentrations of copper to lysosomes is identical to our earlier findings in normal human neonates[3,5]. In both toad and baby no pathological effects are associated with these deposits. In contrast, very similar concentrations of copper are diffusely distributed in the cytoplasm of hepatocytes in patients with Wilson's disease and are associated with severe and often fatal pathological changes. Our present findings provide additional evidence of the view that sequestration of copper in lysosomes protects the cell from the toxic effects of the metal[3,14–16].

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Metabolism of Dimethylnitrosamine by Human Liver Slices *in Vitro*

THE occurrence of carcinogenic nitrosamines in various environmental conditions and the possible hazard to human subjects and animals exposed to them have been discussed in several reviews[1–4]. A wide range of species is susceptible to the carcinogenic action of the nitrosamines but there is no direct evidence for the susceptibility of man. In the absence of such evidence, which could be obtained only by epidemiological studies, an attempt has been made to get indirect evidence by studying the metabolism of dimethylnitrosamine by human liver slices *in vitro* because it seems probable that this compound requires metabolic conversion to form an active carcinogenic intermediate. Dimethylnitrosamine is demethylated in the body to products which are further oxidized to carbon dioxide. One of these products is an alkylating intermediate which methylates cellular com-

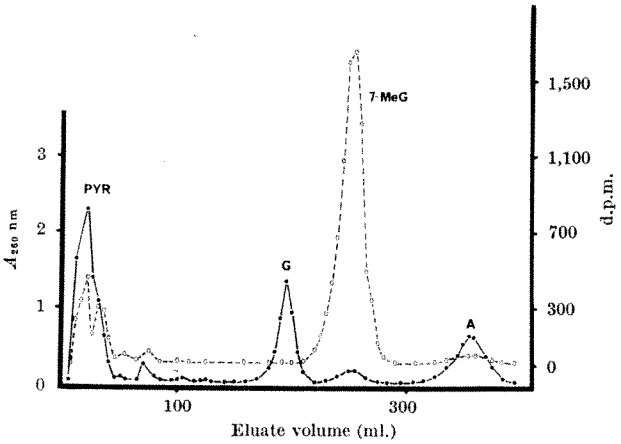


Fig. 1. Ion-exchange chromatography of nucleic acids of human liver slices incubated with [^{14}C]dimethylnitrosamine *in vitro*, conditions of incubation as in Table 1. Nucleic acids (DNA and RNA) were extracted from pooled slices by the Schneider procedure[12], followed by hydrolysis in 1 M HCl at 100° C for 1 h and ion-exchange chromatography on Dowex 50 (×12, H^+ form) with exponential 1–4 M HCl gradient elution, 5 ml. fraction volume. ●, A_{260}; ○, radioactivity; PYR, pyrimidine nucleotides; G, guanine; 7-MeG, 7-methylguanine; A, adenine. Carrier 7-methylguanine was added to the hydrolysate.

ponents including nucleic acids and proteins. DNA and RNA are methylated predominantly on guanine to yield 7-methylguanine, other sites being alkylated considerably less[5]. The significance of nucleic acid alkylation in cellular injury and carcinogenesis by nitrosamines has been discussed[1,6].

In the present experiments, incubation of human liver slices with [^{14}C]dimethylnitrosamine has resulted in the production of labelled carbon dioxide and in methylation of the slice nucleic acids, indicating metabolism of the carcinogen. The results have been compared with those obtained with rat tissue slices. The respiration rate of the rat liver slices (Table 1) was comparable with published data[7,8] and the human liver slices showed a lower rate. The ratio of the mean values of oxygen uptake by rat and human liver was 3·2 which is in good agreement with the report of Booth *et al.*[8].

The rate of oxygen consumption was relatively constant over the period of incubation (100 min) and not influenced by the presence of dimethylnitrosamine, as shown before[7].

Table 1. METABOLISM OF [^{14}C]DIMETHYLNITROSAMINE BY TISSUE SLICES *in vitro*

Tissue	Average weight/flask (mg)	O_2 uptake (μl. O_2/g wet wt/h)	Total radioactivity of $BaCO_3$ (d.p.m.)	$^{14}CO_2$ production (per cent ^{14}C added)
Rat liver	162	760	213,280	5·2
Human liver	162	240	126,100	3·0
Rat kidney	147	2,560	21,680	0·51

Rat tissues were obtained from female Wistar derived albino rats (about 200 g body weight) bred at the Courtauld Institute. Small pieces of human liver weighing approximately 1 g were placed in ice-cold incubation medium (Krebs–Ringer phosphate containing glucose) immediately after surgical removal. Slices were cut with a tissue slicer, weighed and transferred to Warburg flasks containing 2 ml. of ice-cold incubation medium. [^{14}C]Dimethylnitrosamine dissolved in medium (0·2 ml. containing 70 μg and 1·89 μCi) was added to each flask. Incubation was at 37° C in an atmosphere of O_2, with shaking, for 100 min, O_2 uptake being measured at 10 min intervals. Radioactive CO_2 was trapped by NaOH in the centre well and its radioactivity determined, after conversion to [^{14}C]$BaCO_3$, by scintillation counting as previously described[9].

Previous studies with rat liver slices *in vitro* showed that the extent of breakdown of dimethylnitrosamine was directly proportional to the amount of tissue as well as to the time of incubation[7]. In the present experiments (Table 1) the weights of the tissues were roughly the same and the time of incubation was constant, which allowed comparison of the results obtained with different tissue slices. The amount of dimethylnitrosamine (70 μg) was well above the level which could be metabolized by the amount of tissue present in each flask[7]. The rate of production of radioactive CO_2 with human liver slices was rather less than with rat liver slices but it was considerably greater than with rat kidney slices (Table 1). Similarly, the level of nucleic acid methylation (Fig. 1) in the human liver slices (0·13 per cent) was slightly lower than in the rat liver (0·19 per cent) but considerably greater than in the rat kidney slices (0·01 per cent). The degree of methylation of the nucleic acids, which is expressed as the percentage of the guanine converted to 7-methylguanine, was calculated as previously described[9].

The higher level of nucleic acid methylation in rat liver than in rat kidney observed *in vitro* is similar to previous findings *in vivo* when the percentages of methylated guanine in nucleic acids of liver and kidney were approximately 1 per cent and 0·1 per cent 5 h after injection of [^{14}C]dimethylnitrosamine (30 mg/kg body weight). In the rat a single dose of dimethylnitrosamine at this level produces a significant incidence of kidney tumours[10] and continuous feeding of this carcinogen induces liver tumours even with concentrations in the diet as low as 5 or 2 p.p.m.[11].

Although the significance of alkylation of nucleic acids in carcinogenesis by nitrosamines is not known, there is a correlation between the levels of methylation in an organ and the distribution of tumours in rats treated with dimethylnitrosamine[9]. These results show that human liver can metabolize dimethylnitrosamine at a rate comparable with rat liver and the similar levels of nucleic acid methylation in the two species suggest that man may be about as sensitive as the rat to the carcinogenic action of this compound. In view of the high carcinogenicity of the nitrosamines and of their presence in the environment, our findings emphasize the potential human hazard from exposure to this group of carcinogens.

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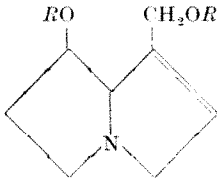
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Role of the Acid Moieties in the Toxic Actions of Pyrrolizidine Alkaloids on Liver and Lung

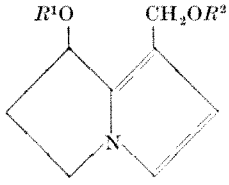
MANY pyrrolizidine alkaloids have toxic effects in the livers and sometimes the lungs of experimental animals[1], and there is evidence that these effects are caused by metabolites formed by enzymic dehydrogenation of the alkaloids in the liver[2–4]. These metabolites are highly reactive dihydropyrrolizine esters which when released

in the liver cell can react with nucleophilic tissue constituents. The heterocyclic moiety can become bound to the tissue while the acid moiety is liberated as the anion.

This implies that the acid moiety may play only a secondary part in the toxic reaction. Yet alkaloids with the same basic moiety, esterified with different acids, have widely different toxic effects[1]. Thus retrorsine, monocrotaline and the semisynthetic diisovaleryl retronecine[5,6], all esters of retronecine (I, $R = H$), have to be administered to rats in doses of about 35, 120 and 500 mg/kg body weight respectively to produce comparable liver damage, while diacetyl retronecine (I, $R = Ac$) has to be given in doses above 900 mg/kg together with an esterase inhibitor to produce liver necrosis (my unpublished results). Moreover, of these compounds, only monocrotaline consistently damages the lungs as well as the liver.



(I)



(II)

Characteristic toxic effects in the lungs and livers of rats have been produced by intravenous injections of chemically prepared dihydropyrrolizine derivatives[2] into the tail vein (5 mg/kg) or a mesenteric vessel (15 mg/kg) respectively[7]. Similar doses of retrorsine pyrrole and monocrotaline pyrrole gave identical effects in both organs, but retronecine pyrrole (II, $R^1 = R^2 = H$), which has weaker alkylating ability[3], was ineffective. To test the importance of the acid moiety, diacetyl retronecine pyrrole (II, $R^1 = R^2 = Ac$) was prepared by acetylating retronecine pyrrole. This compound, when injected intravenously into rats, produced lesions identical to those given by retrorsine and monocrotaline pyrroles at similar dose levels. Like the other dihydropyrrolizine esters it had high alkylating reactivity and was strongly bound to lung and liver tissues after injection. I have previously shown that a crude mixture containing monoacetyl pyrrole derivatives, such as II ($R^1 = Me$, $R^2 = Ac$), can cause the characteristic lung lesions[2].

Thus the qualitative and quantitative differences in toxic effects produced by different alkaloids having the same base moiety (retronecine) but different acid moieties, are not found when the corresponding pyrrole derivatives are applied directly to the appropriate organs. The differences between the alkaloids seem to be due to (1) the different amounts which are metabolized to pyrrole derivatives, and (2) different stabilities of the reactive metabolites in the liver cells. Both these factors are influenced by the acid moieties. The latter can affect distribution of the alkaloids, binding to metabolizing enzymes, and ease of hydrolysis (leading to detoxication)[1]. The alkaloids are apparently not metabolized by lung tissue (unpublished results of I. White and myself). For lung damage to occur, sufficient reactive metabolite must be transported from liver to lungs. The concentrations of pyrrole metabolites found in the lungs of rats 2 h after being dosed with monocrotaline or retrorsine were approximately 34 and 7·5 per cent respectively of the amounts found in the livers. Monocrotaline pyrrole is less reactive than retrorsine pyrrole, requiring twice as long as the latter to react completely with 4-*p*-nitrobenzylpyridine (ref. 3); this probably accounts for its better ability to survive while being transported from liver to lungs.

The nature of the acid moiety seems to be unimportant at the sites of the toxic reactions themselves, for acetate, which is physiological, can be as effective as the complex acids of the natural alkaloids when incorporated in the reactive metabolite. Thus it may be inferred that it is reactions of the dihydropyrrolizine moiety with tissue constituents which are responsible for the toxic effects.

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Stability of Cardiac Monoamine Oxidase Activity after Chemical Sympathectomy with 6-Hydroxydopamine

Monoamine oxidase (MAO) of sympathetically innervated organs has been classically considered to be involved in the regulation of intraneuronal catecholamine concentrations. This function of MAO implies that the enzyme is located within the presynaptic element of the adrenergic neuroeffector system. Recent studies, however, have indicated that MAO may be located extraneuronally[1,2].

To confirm these findings we performed a chemical sympathectomy, using 6-hydroxydopamine (6-OH-DA) because of its relative specificity in producing a total sympathectomy of most peripheral organs[3,4]. Data from experiments measuring catecholamine concentrations and tyrosine hydroxylase activity after the administration of 6-OH-DA support the evidence that a complete chemical sympathectomy has taken place[5].

Male Sprague–Dawley rats (5 weeks old, 100–125 g) were given four intravenous injections of 6-OH-DA. The first two injections (50 mg/kg of the salt in 0·01 N HCl bubbled with nitrogen) were given 8 h apart, as were the second two injections (100 mg/kg) 7 days later. Control animals received four intravenous injections of the acid bubbled with nitrogen on the same schedule. Animals were killed 1 day, 5 days or 14 days after the second series of injections and the hearts were removed, blotted dry and placed on ice. The cardiac MAO activity was determined using 5-hydroxytryptamine as a substrate according to the procedure of Weissbach *et al.*[6]. Cardiac noradrenaline concentrations were measured by the method of Chang[7], as modified by Maickel *et al.*[8].

The MAO activities of hearts from rats treated with 6-OH-DA did not vary significantly from controls 1 day after the second series of injections (Fig. 1). Enzyme levels determined 5 days and 14 days later were also well within the control range. Cardiac noradrenaline was not detectable 1 day after the last injection of 6-OH-DA (control, $0{\cdot}48 \pm 0{\cdot}09$ μg/g; 6-OH-DA pretreated, $< 0{\cdot}10$ μg/g). Groups of rats of different ages were also tested; the cardiac MAO activities are not reported because they also failed to show any changes in activity. The use of kynuramine as a substrate for measuring MAO activity did not reveal any changes in the enzyme level following 6-OH-DA (unpublished observation).

The dramatic drop in noradrenaline concentrations indicates that 6-OH-DA did produce a chemical sympathectomy of the heart, especially in view of the reports of the loss of tyrosine hydroxylase activity in rat heart after administration of 6-OH-DA[5]. If 6-OH-DA produces a chemical sympathectomy as stated by various workers, it may have been assumed that cardiac MAO activity would also decrease, especially if the enzyme is localized intraneuronally. The failure of 6-OH-DA to lower the MAO activity in rat heart is of special interest because it

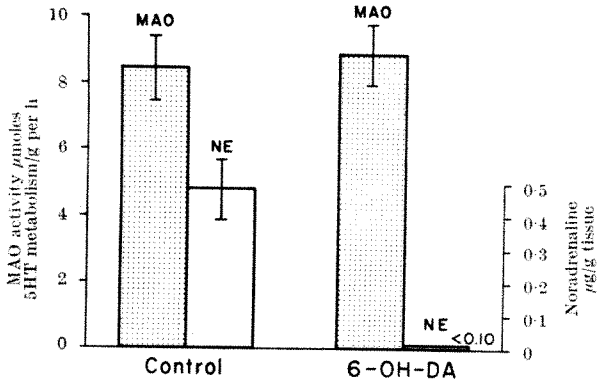


Fig. 1. Cardiac monoamine oxidase activities and noradrenaline levels in control rats and in rats 1 day after the administration of 6-OH-DA. Enzyme activity was determined on individual rat hearts of controls, or of 6-OH-DA pretreated animals (four or five per group). Noradrenaline levels were assayed on individual rat hearts (three per group) of both groups. Results are expressed as the mean ± the standard deviation of the mean. A t test for differences between groups give a P value of $< 0{\cdot}05$ for the noradrenaline levels.

immediately implies that most of the enzyme is of extraneuronal origin.

Several investigators have indicated an extraneuronal localization of MAO in certain sympathetically innervated organs, but its significance has not been pursued. Immunosympathectomy of the rat with the antiserum to nerve growth factor does not lower cardiac MAO[9]. Surgical denervation, as accomplished in autotransplantation of the heart of the dog, does not abolish cardiac MAO activity[1]. These investigations tend to support our contention that MAO of the heart is not totally intraneuronal. In fact, our results indicate that most of the MAO in the rat heart is extraneuronal. The results of our experiments are not specific only for 5-hydroxytryptamine because the use of kynuramine as a substrate for the MAO assay gives the same results.

The MAO activity of the rat heart is attributable to microsomal and mitochondrial components obtained by differential centrifugation[10], so it is possible that the cardiac MAO normally assayed is composed of different fractions, some contributed from neuronal tissues and others from non-neuronal tissues. Because the preponderance of MAO in the rat heart does not appear to be within the sympathetic nerves, it must be located in some other cellular component of the heart.

The finding that most of the cardiac MAO of the rat is not neuronal suggests a role for cardiac MAO other than that of regulating intraneuronal catecholamine levels. If catecholamines are transported across the sarcolemma of cardiac cells in their normal role as sympathetic neurohumours of the heart, then it would be possible to postulate an intracellular localization of MAO in cardiac muscle cells of the rat. The histochemical studies of Mueller and Pearse[11] and the differential centrifugation studies of deChamplain *et al.*[10] indicate that mitochondria and microsomes are the most likely subcellular locations of MAO in the rat heart.

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Effects of Morphine and its Antagonists on Release of Cerebral Cortical Acetylcholine

Several studies have shown that the narcotic analge morphine and morphine-like compounds reduce t release of acetylcholine (ACh) at peripheral cholinerg junctions[1–3], but there is relatively little evidence that th have a similar action on the central nervous syste Beleslin and Polak[4] have shown that an intraventricul infusion of morphine in the anaesthetized cat leads to modest decrease in the release of ACh into the cereb spinal fluid. The morphine antagonists nalorphi ('Nalline') and levallorphan ('Lorfan') have been sho to reduce ACh released from the ileum of the guinea-p but a comparable action on the release of ACh from t brain has not been demonstrated. There is evidence th the brain contains a system of cholinergic neurones wi terminals which project to the cerebral cortex[5,6] a release ACh at the cortical surface[7]. If morphine impair the release of ACh at the cortical level, and if this acti was antagonized by narcotic antagonists such as lev lorphan, nalorphine and naloxone, such a reversal morphine action by the antagonists could yield importa information about the neurohumoral changes associat with the hyperactive state which follows the acute wit drawal of morphine in animals and man[8]. We ha therefore investigated the action of morphine on the cor cal release of ACh and compared this action with th of morphine antagonists.

Twenty-two adult cats were anaesthetized with sodiu pentothal and the anaesthetic was continued until t end of surgical procedures, when the animals were mai tained under very light 'Dial' anaesthesia (Dial Co pound, Ciba Ltd, 0·4 ml./kg). ACh was collected bilat ally for 15 min periods from the exposed sensorimotor a left auditory cortices in 'Perspex' cylinders, filled wi artificial cerebrospinal fluid[7] which contained 5×10^{-5} g/n of neostigmine bromide, and rested lightly on the corte After several control samples had been collected, morphi was applied topically on one side only and sampli continued from both sides. In most experiments morphi was injected intravenously following a noticeable effect morphine, topically applied onto one sensorimotor corte when ACh was released from both cortical hemisphere Antagonists were administered intravenously. AC released in the cylinders was assayed biologically on hear from the bivalve mollusc, *Mercenaria mercenaria*. Aft assaying, the hearts were treated with an ACh antagonis benzoquinonium ('Mytolon'), and the samples we re-tested to ensure that responses to both standard a unknown solutions were abolished.

Fig. 1*A* (i, ii and iii) illustrates an experiment in whi topical applications of morphine (1 in 1,000 and 1 in 10 to the right sensorimotor cortex reduced the resti release of ACh not only from the ipsilateral side but al from the contralateral cortex. To rule out the possibili that morphine was reducing the output of ACh on t contralateral side by depressing neural activity in t transcallosal pathway, the resting output of ACh w monitored simultaneously from the contralateral audito

cortex, an area of the brain not connected transcallosally to the morphinized sensorimotor cortex. Fig. 1*A* (iii) shows that there was a parallel decrease in the release of ACh from the auditory cortex. This result suggests that the contralateral effect of topical morphine may have been caused by absorption of the drug into the systemic circulation. Concentrations of morphine lower than 1 in 1,000 did not significantly affect the release of ACh on either side.

The results obtained with drugs administered intravenously are summarized in Table 1. Morphine in doses of 0·1, 1·0 and 5·0 mg/kg consistently reduced the resting release of ACh both in animals which were respiring spontaneously and in those undergoing artificial ventilation. After a reduction of ACh release by morphine to very low levels, levallorphan (5 mg/kg) restored the output to control levels (Fig. 1*B*, i and ii). Subsequent injections of morphine had no effect on release. Small doses of levallorphan (0·1, 1·0 mg/kg) alone decreased the release of ACh as did morphine, but they did not antagonize the effects of morphine. Naloxone itself did not decrease ACh release when given in doses of 0·1 or 1·0 mg/kg, but it readily antagonized the reduction in ACh release induced by morphine or levallorphan. Nalorphine produced effects which were essentially similar to those produced by naloxone in that, in doses of 0·1–5·0 mg/kg, it antagonized the actions of morphine but did not itself reduce the release of ACh.

These findings suggest that morphine depresses the resting cortical release of ACh when applied topically or given systemically. Levallorphan, which is a partial agonist, shares this property with morphine when given in small doses, but antagonizes the depressant effects of morphine in higher doses. Naloxone, which has been claimed to be a true antagonist of morphine[10], does not affect the output of ACh by itself in doses up to 1 mg/kg but readily antagonizes the depressant action of morphine on the cortical release of ACh. These results have not established the exact site of action of morphine and related drugs on the ACh releasing system in the brain. Such a site of action may involve cholinergic nerve endings located in the cerebral cortex or it may involve deeper elements, located subcortically. Our observations suggest that more than one site of action in the brain may be involved.

The exact significance of this decreased release of cortical ACh in the analgesic response produced by these agents has yet to be ascertained. It seems, however, that the immediate increase in the release of cortical ACh after treatment with a narcotic antagonist is partly responsible for the acute withdrawal syndrome precipitated by the administration of narcotic antagonists to morphinized animals. In view of this it is interesting that ACh antagonists have been recommended to ameliorate the narcotic abstinence syndrome[11].

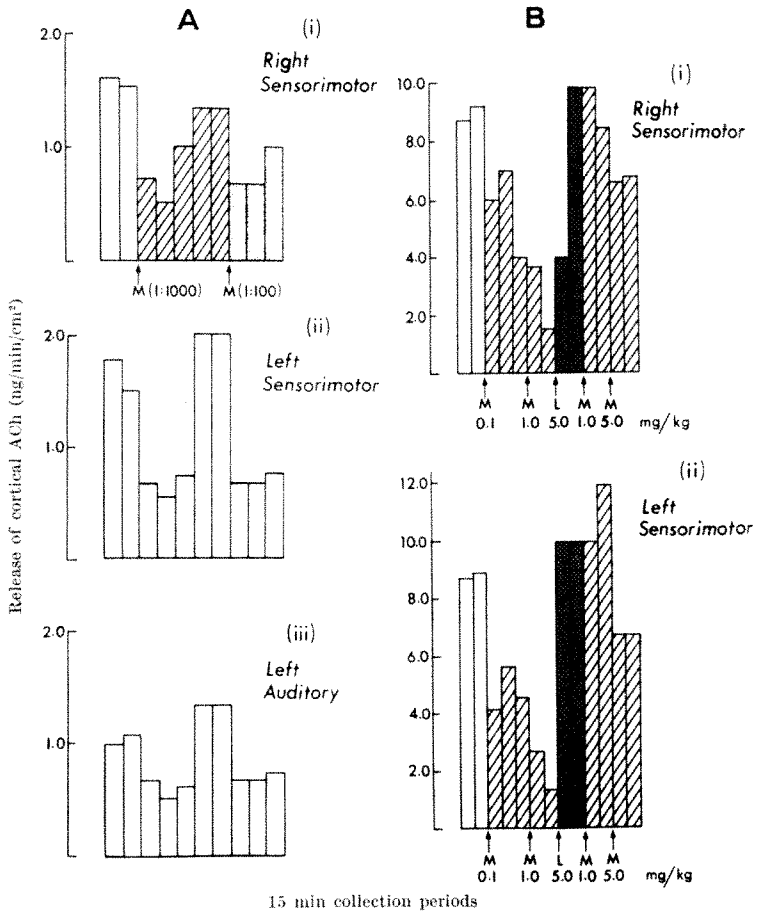


Fig. 1. *A*, Rates of release of ACh from different cortical areas of a cat anaesthetized by 'Dial' compound. Cups were placed bilaterally on the sensorimotor cortices and on the left auditory cortex. At the points indicated by the arrows the ACh collections were interrupted while morphine solutions (1 : 1,000 and 1 : 100) were placed in the cylinder over the right sensorimotor cortex for periods of 30 min. These solutions were replaced at frequent intervals during the periods of exposure. Collections of ACh from the three areas continued after a 5 min period during which the surface of the right sensorimotor cortex was washed repeatedly. *B*, Rates of release of ACh from (i) right sensorimotor cortex and (ii) left sensorimotor cortex after intravenous injections of morphine (M) and levallorphan (L). There was a 5 min break between collections following each drug injection. Actual doses (in mg/kg) of morphine and levallorphan are indicated on the figure.

Table 1. EFFECTS OF INTRAVENOUSLY ADMINISTERED DRUGS ON CORTICAL RELEASE OF ACh

Drug	Release of cortical ACh (ng/min/cm²) Control*	In first 15 min after administration	In second 15 min after administration
Morphine sulphate, 1·0 mg/kg; 12 hemispheres	3·41 ± 0·74	1·30 ± 0·25	0·76 ± 0·16
Levallorphan tartrate, 0·1 mg/kg; 6 hemispheres	2·94 ± 0·87	0·74 ± 0·27	0·91 ± 0·38
Naloxone hydrochloride, 1·0 mg/kg; 4 hemispheres	4·03 ± 0·29	4·75 ± 0·74	5·0 ± 0·57
Nalorphine hydrochloride, 1·0 mg/kg; 4 hemispheres	1·95 ± 0·09	1·95 ± 0·18	2·95 ± 0·61

* Control release represents average of release during two 15 min periods immediately preceding drug administration. Post-drug administration collections commenced 5 min after injection. Values ± standard error.

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Production of Poly-unsaturated Ruminant Body Fats

THERE seems to be a significant relationship between serum cholesterol concentrations and the incidence of atherosclerosis in man[1-4]. In the past, considerable attention has been focused on the possible relationships between the consumption of ruminant products (for example, lamb, beef, milk, butter, cheese and cream), high concentrations of serum cholesterol and coronary heart disease in man[5]. These relationships have been attributed largely to the relatively high proportions of saturated fatty acids that occur in ruminant fats, and the possible role of these fatty acids in increasing serum cholesterol concentrations[6,7]. Moreover, it is now firmly established that the substitution of poly-unsaturated fats for saturated fats in human diets will cause a reduction in the concentration of serum cholesterol[5-10]. For these reasons, ruminant fats are largely excluded from the diets of patients being treated for coronary heart disease[5,11]. The availability of poly-unsaturated ruminant products would therefore assist in the formulation of special diets for experimental work and for therapeutic use in patients with atherosclerosis.

It has not been possible, in the past, to increase the proportion of poly-unsaturated fats in ruminant animals by feeding poly-unsaturated oils or by intraruminal infusion of these oils[12,13]. This is because the oils are extensively hydrogenated by the microorganisms present in the rumen[14-16]. If the oils are administered into the abomasum or small intestine, to by-pass the rumen and escape hydrogenation, however, there are substantial increases in the proportion of poly-unsaturated fatty acids in ruminant adipose tissues[13,17].

A procedure has been described for protecting dietary supplements of poly-unsaturated fatty acids from ruminal hydrogenation; when these supplements were fed to lactating ruminants there were substantial increases in the proportion of poly-unsaturated fatty acids in the milk fat[18].

Here we show that feeding these special supplements of poly-unsaturated oils will cause an increase in the proportion of C_{18} poly-unsaturated fatty acids in ruminant depot fats.

Poly-unsaturated lipid-protein particles were prepared by spray-drying a homogenate consisting of equal parts by weight of safflower oil (75 per cent linoleic acid) and sodium caseinate[18]. This procedure stems from previous work on the production of butter powders[19]. The lipid-protein particles (approximate size 15–50 μm) were protected against ruminal hydrogenation by spraying with formalin (37 per cent formaldehyde)[18,20], at a rate of approximately 50 ml./kg of powder. To ensure uniform treatment with formalin, the particles were sieved through a fine screen into the top of a cylindrical tower (3·6 m high and 0·75 m in diameter). As the particles descended through the tower, they were treated with formalin vapour which was introduced into the system through four injection ports located approximately half-way up on the wall of the tower. The formalin treated particles were collected and stored in sealed polyethylene bags for at least 24 h.

Six lambs (8–10 weeks old) were fed daily a pelleted diet of chopped lucerne hay and oats (800 g, 1:1 w/w) and formaldehyde treated safflower oil-protein particles (200 g). Two control lambs of similar age were fed a diet of chopped lucerne hay and wheaten hay (1,000 g/day). All lambs consumed approximately 85–90 per cent of the ration offered. Half of the lambs on each diet were slaughtered after three weeks of feeding; the remainder were killed after 6 weeks. Samples of perinephric, mesenteric and subcutaneous fat were removed, frozen on solid carbon dioxide and transferred into chloroform and methanol (2:1 v/v). The tissues were homogenized, lipid extracts were prepared, and fatty acid compositions determined as previously described[21].

The results in Table 1 show that feeding formaldehyde treated safflower oil supplements caused a 3 to 5-fold increase in the proportion of C_{18} di-unsaturated fatty acid in the depot fats; the greatest increase was observed in the perinephric fat. The change that was induced by feeding the supplement for 6 weeks was not greatly different from that after 3 weeks. This suggests that in lambs of this age there is a fairly rapid exchange between the fatty acids of plasma triglycerides and depot fats. The fatty acid compositions of fats from the control lambs were similar to those reported previously[22,23]. Preliminary analyses on the fatty acid composition of muscle show that the proportion of linoleic acid in the triglycerides is also significantly increased and the values are similar to that obtained for subcutaneous fats. Further studies are required to examine the relation of poly-unsaturated fatty acid concentration in tissues to the dietary intake of protected lipid. Possibly higher levels than those reported here could be attained in a shorter period of time. Comparable experiments with beef animals (12 months old) have shown that the proportion of poly-unsaturated fatty acid is significantly increased after feeding protected lipids for 4 to 8 weeks.

The results show that it is now possible to produce poly-unsaturated ruminant fats by feeding diets containing formaldehyde treated safflower oil-protein particles. Using these procedures it is only necessary to feed the animals for a few weeks before slaughter to produce significantly elevated proportions of poly-unsaturated fatty acids. Although in these experiments only safflower oil was used, the principle would be applicable to other oils (sunflower, soybean, linseed).

The availability of poly-unsaturated ruminant meats (for example, beef, veal, mutton and lamb) and dairy products[18] should offer patients who are being treated for cardiovascular diseases a greater variety of edible

Table 1. FATTY ACID COMPOSITION OF FATS FROM LAMBS ON A DIET CONTAINING SUPPLEMENTS OF FORMALDEHYDE-TREATED SAFFLOWER OIL-PROTEIN PARTICLES

Fat	Period of feeding (weeks)	Treatment	Fatty acid* (weight per cent) 14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2†	18 : 3†
Perinephric	3	Control	6·0	20·0	2·9	24·8	29·9	4·3	1·9
		Experimental	4·4 (0·8)	15·7 (0·8)	2·1 (0·2)	22·0 (1·9)	33·1 (1·2)	15·6 (2·0)	1·5 (0·1)
	6	Control	3·5	19·5	2·9	32·9	26·5	4·4	1·8
		Experimental	3·3 (0·5)	15·7 (0·5)	2·2 (0·2)	22·3 (1·2)	34·6 (2·5)	18·6 (2·2)	1·2 (0·4)
Mesenteric	3	Control	8·6	24·9	3·2	21·2	29·6	1·9	2·4
		Experimental	7·1 (0·7)	20·8 (1·2)	2·7 (0·1)	19·3 (1·1)	32·1 (0·4)	11·3 (0·8)	1·2 (0·4)
	6	Control	5·5	23·3	3·6	26·0	28·2	3·0	1·8
		Experimental	4·9 (0·6)	18·7 (1·4)	2·2 (0·1)	17·8 (0·8)	33·7 (0·7)	15·7 (1·4)	2·1 (0·3)
Subcutaneous	3	Control	8·8	25·1	3·5	18·2	33·5	1·6	1·6
		Experimental	4·0 (1·6)	19·3 (0·8)	2·7 (0·1)	17·6 (0·6)	37·1 (2·3)	12·2 (0·4)	3·7 (1·3)
	6	Control	4·7	27·1	2·5	23·7	30·8	3·7	1·8
		Experimental	5·8 (0·8)	19·1 (0·9)	3·1 (0·3)	12·7 (0·4)	34·7 (2·8)	13·9 (1·6)	2·8 (1·8)

* Fatty acid nomenclature is that used by Ahrens *et al.*[24] and the values for the experimental lambs are the mean of three lambs with standard error in parentheses. Values for the minor components such as 12 : 0 and the C_{15} and C_{17} branched chain acids are not included in this table, but they account for the residual proportion of fatty acids in the depot fats.

† The safflower oil contained 75 per cent 18 : 2 but only 0·2 per cent 18 : 3.

products. It should also assist research workers in their studies on the pathogenesis of atherosclerosis.

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Prostaglandin E_1 and the Release of Growth Hormone *in Vitro*

THE presence of prostaglandins in the hypothalamus of the dog[1] raises the interesting possibility that they are important in regulating the release of pituitary hormones. This possibility is strengthened by the demonstration that prostaglandin E_1 (PGE_1) acts on the anterior pituitary *in vitro*, activating adenyl cyclase and increasing the concentration of adenosine 3′5′-cyclic monophosphate (3′5′-AMP)[2]. Theophylline also increases the concentration of 3′5′-AMP in the anterior pituitary, by inhibiting cyclic nucleotide phosphodiesterase[3], and has been shown to stimulate the release of growth hormone *in vitro*[4]. If the increase in 3′5′-AMP in the tissue mediates the stimulation of release by theophylline, prostaglandin E_1 should also stimulate release. The effect of PGE_1 on the release of growth hormone has therefore been determined using the incubation system and radioimmunoassay which I have described before[5].

Pituitary slices, obtained from heifers[4], were incubated at 37° C during transport to the laboratory in 100 ml. of bicarbonate buffered salt solution[6] which had been gassed with oxygen: carbon dioxide (95: 5) and which contained glucose, sodium hydroxybutyrate and sodium glutamate at 0·26 mM. The slices were then divided along the midline and pairs of half slices were incubated with shaking in 3 ml. of the same medium for 30 min. They were then distributed among 10 ml. flasks containing 3 ml. of the same medium and the appropriate concentration of PGE_1, and incubated at 37° C for 45 min. In a second series of experiments the incubation medium also contained 0·5 mM theophylline at this stage. At the end of the incubation the slices were removed, blotted and weighed, and the incubation medium was centrifuged at 3,000*g* for 5 min, and a 50 μl. sample was diluted for growth hormone assay[5].

Table 1. EFFECT OF PGE_1 ON RELEASE OF GROWTH HORMONE IN THE ABSENCE OF THEOPHYLLINE

Concentration of PGE_1 (M)	Rate of release of growth hormone (μg/mg wet weight/h)
0	1·63 ± 0·09
5×10^{-8}	1·46 ± 0·17
5×10^{-7}	1·70 ± 0·13
5×10^{-6}	2·12 ± 0·12*

Results are expressed as mean standard error of the mean for sixteen slices at each concentration of PGE_1, obtained from three pituitary glands.
* $P < 0·005$ compared with control without PGE_1.

Table 2. EFFECT OF PGE_1 ON RELEASE OF GROWTH HORMONE IN THE PRESENCE OF THEOPHYLLINE

Concentration of PGE_1 (M)	Rate of growth hormone release (μg/mg wet weight/h)
0	0·94 ± 0·08
5×10^{-8}	1·53 ± 0·12*
5×10^{-7}	1·70 ± 0·14*
5×10^{-6}	1·40 ± 0·08*

Theophylline (0·5 mM) was present in all samples. Results are expressed as mean standard error of the mean for sixteen slices at each concentration of PGE_1, obtained from four pituitary glands.
* $P < 0·005$ compared with control without PGE_1.

The effect of PGE_1 on the release of growth hormone is shown in the tables. In the absence of theophylline, PGE_1 increased the release of growth hormone by about 30 per cent ($P < 0·005$) in the largest concentration tested (5×10^{-6} M) but had no effect in lower concentrations (Table 1). In the presence of theophylline, the release of growth hormone was increased at a PGE_1 concentration of 5×10^{-8} M, and a maximum increase of about 80 per cent ($P < 0·005$) was obtained at 5×10^{-7} M (Table 2). The ability of theophylline to lower the effective dose and potentiate the action of PGE_1 is to be expected if 3′5′-AMP is involved in growth hormone release, for PGE_1 activates pituitary adenyl cyclase[2] and theophylline inhibits pituitary cyclic nucleotide phosphodiesterase[3]. PGE_1 has been shown to increase *in vivo* release of insulin[7] and ACTH[8], and there are conflicting reports of its effects on the release of catecholamines[9,10]. My finding that PGE_1 stimulates the release of growth hormone *in vitro* demonstrates that stimulation by PGE_1 is not specific to one cell type or to one hormone. Prostaglandins may function as modulators of the action of the well documented hypothalamic releasing factors at the pituitary receptor level, in the same way as they have been proposed as modulators of the action of transmitters in smooth muscle[11]. It is perhaps relevant that lipid extracts of the hypothalamus have been reported to cause weight gain in young rats[12].

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Effects of High Levels of Yeast Feeding on Uric Acid Metabolism of Young Men

THE possible use of single cells of yeast and bacteria as food is receiving much attention[1,2]. It has long been recognized[3–9] that feeding yeast can increase urinary uric acid excretion, but the levels of its use in processed foods have been so low that no problems have arisen. If yeast and other single cells are used, however, as sources of protein rather than of vitamins, the intakes involved are much higher.

Man and higher apes lack the enzyme uricase, which catalyses the oxidation of uric acid to the more soluble allantoin, so that in individuals with a genetic tendency to primary over-production of uric acid there may be precipitation of uric acid crystals in joints (gout), soft tissues (tophi) or the formation of stones in the urinary tract. The increase in uric acid secondary to a high intake of dietary purines and other exogenous factors may have a similar effect.

Four healthy male MIT students aged 18 to 27 years, weighing 65 to 90 kg, were fed a controlled, low-purine diet providing adequate calories, minerals, and vitamins together with 100 g of protein and about 11 mg purine nitrogen per day. The subjects received the basal diet for 16 days followed by consecutive 9-day periods with 45, 90, and 135 g of food yeast (*Torula utilis*, NF 12 Type 300, Lake States Division, St Regis Paper Co.) distributed equally among four meals. Total protein intake was maintained at 100 g per day. The weight of the subjects remained constant throughout the experiment.

The experimental diets contributed 2·9, 5·8 and 8·7 g of yeast nucleic acid (DNA and RNA) by analysis[10]. Uric acid was determined[11] on plasma taken at 8.00 h, 11 h after the start of the last meal. Creatinine excretion[12] was measured daily throughout the experiment.

The results in Table 1 show that 45 g of yeast containing 2·9 g of nucleic acid was sufficient to raise the serum uric acid levels of the subjects to approximately 7·0 mg/100 ml. Doubling this quantity increased the uric acid levels of all subjects to unacceptable levels, and tripling it brought about a further but much smaller increase.

The only comparable data are those of Waslien *et al.*[13], who fed healthy young men 0, 2, 4 and 8 g of yeast RNA for 5 consecutive days in a constant 75 g egg-protein diet distributed among four equal meals. The resulting average uric acid levels of 4·9, 6·0, 7·7 and 9·4 mg/100 ml. are similar to ours when allowance is made for differences in the exact level of nucleic acid fed. All five of their subjects fed 2 g of yeast RNA had serum uric acid level which remained within normal limits, while all four of our subjects fed 2·9 g of nucleic acid had levels which were at or slightly above 7·0 mg/100 ml.

Because this is a level which separates most patients with primary gout from most normal subjects[14–16], the combined results for blood uric acid levels suggest that 2 g of single-cell protein (SCP) nucleic acid per day is probably a safe limit to feed most normal subjects, but 3 g of SCP nucleic acid per day might place some normal subjects at risk.

The influence on urinary uric acid excretion of feeding the yeast is shown in Table 1. The lowest level of yeast nucleic acid fed, 2·9 g, increased uric acid excretion to more than 1,000 mg per day, and the additional increase was proportional when nucleic acid ingestion was doubled. A further increase in nucleic acid intake did not produce any consistent further change.

The urinary uric acid excretion values reported by Waslien *et al.*[13] were lower—373, 667, 939 and 1,393 mg per day for the intakes cited previously—but our data suggest that for most normal individuals a daily intake of approximately 3 g of SCP nucleic acid results in undesirably high levels of urinary uric acid because the majority of normal persons on ordinary diets excrete less than 600 mg of uric acid per day[17,18]. A dietary level of 2 g of SCP nucleic acid would seem to involve little risk of stone formation; slightly higher levels probably pose no hazards as long as urine volume and *p*H are not abnormally low. At no time was any gastrointestinal disturbance encountered even at the level of 135 g of yeast per day.

Table 1. EFFECT OF YEAST NUCLEIC ACID ON SERUM AND URINARY URIC ACID IN YOUNG MEN

	Serum uric acid (mg/100 ml.)				Urine uric acid (mg/24 h)			
Yeast fed (g/day)	0*	45	90	135	0*	45	90	135
Yeast nucleic acid intake (g/day)	—	2·9	5·8	8·7	—	2·9	5·8	8·7
Subject number								
1	4·5	7·5	9·2	10·1	533	1343	2007	2100
2	4·7	7·4	9·1	9·9	405	1200	1880	2400
3	4·5	6·8	8·6	8·5	504	1100	1800	1585
4	4·3	7·2	8·5	9·1	599	1125	1725	1400

* 100 g "purine-free" protein per day.
The values for serum and urine uric acid are the means of the values for the 5th, 6th and 7th days of each dietary period.

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New Process for Reducing the Nucleic Acid Content of Yeast

It has been shown that if single-cell protein is to be used as a primary protein source for human populations, the nucleic acid content should be reduced to a level which would allow a maximum intake in the range of 2 g of nucleic acid per day[1]. For a processing method to be acceptable, it must not only decrease the nucleic acid level to the required degree, but it must also be inexpensive and must not contaminate the product with undesirable chemicals.

Candida utilis was grown in continuous culture[2] with glucose as the carbon source at *p*H 4·0 and at a dilution rate of 0·19 h^{-1} ($\mu_{max} = 0{\cdot}4\ h^{-1}$). The initial nucleic acid content of the cells in these growth conditions was approximately 7 per cent. After collection, the cells were treated in a three-step heating process to produce cells with 1·0–1·5 per cent nucleic acid and approximately 50 per cent protein. The successive steps involved were: (1) heat shock of a few seconds duration at 54°–70° C; (2) incubation at 45°–50° C; and (3) incubation at 55°–60° C. All three steps seem to be necessary for optimum results although a compromise temperature between 50 and 60° C is possible for the last two steps.

The heat shock was performed by passing a suspension of *C. utilis* in spent medium through a 56 cm length of 1/32 inch inner diameter (1/16 inch outer diameter) stainless steel tubing immersed in a vigorously stirred water bath at the desired temperature. For the remaining steps a nominal residence time of 5·5–6·0 s and a temperature of 68° C were chosen. After heat shocking, the cells were collected at 0° C or were treated immediately at 45°–50° C. The method of collection did not affect the final results.

The data in Fig. 1 indicate an optimum temperature range of 62°–68° C for a heating time of 17 s and an optimal temperature of 68° C for treatments of shorter duration. Other experiments have shown that longer heating times at lower temperatures or shorter heating times at higher temperatures do not lead to an optimal process under any conditions of subsequent incubation. When the heat shock is omitted the remaining two steps result in only a 10 per cent reduction in nucleic acid compared with an 80–85 per cent reduction when heat shock was used. After heat shock alone the nucleic acid content of the cells is not significantly altered.

Recalculation of data presented by Bodley[3] for the rate of *E. coli* ribosome denaturation indicates a half life of 0·6 s at 68° C. Haight and Ordal[4] have shown a ribonuclease associated with staphylococcal ribosomes which is activated by heat and attacks only the RNA closely bound within the same ribosome. The heat shock in our process may serve either or both of these functions. The final two steps probably allow a complex series of enzymatic hydrolysis reactions to occur culminating in the leakage of these products from the cells into the surrounding medium.

One of the principal functions of the third step is to allow leakage of the accumulated hydrolysis products from the cells, but there is also a significant amount of additional hydrolysis of polynucleotides. If the second step is conducted at 45° C there is essentially no leakage of UV absorbing material into the medium in the absence of step 3. The optimal time and temperature of incubation of this third step were found to be 1 h at 55° C when the second step was conducted at 45° C. A slightly higher temperature of 60° C results in poorer nucleic acid removal, possibly because of the denaturation of the endogenous ribonucleases preventing further degradation of polynucleotides or alternatively of the formation of RNA aggregates resistant to enzyme action. The optimum range for the overall process is between *p*H 5·0 and 6·5.

In view of the close similarity between the optimum conditions of heat shock for breakdown of *C. utilis* RNA and the conditions required for irreversible thermal denaturation of *E. coli* ribosomes[3], we expect that our method can be applied to other types of microbial cells. We have begun to scale up the process of *C. utilis* in order to prepare enough product to conduct feeding studies. We are also trying to determine the exact nature of the reactions involved in each of the steps.

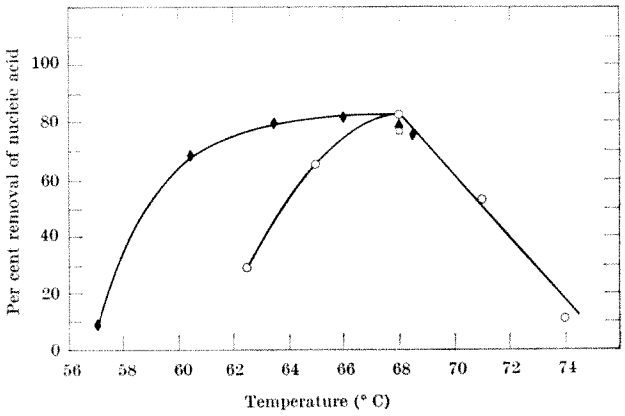


Fig. 1. Effect of heat shock temperature and time on nucleic acid removal. Step 1: variable; step 2: 45° C, 2 h; step 3: 55° C, 1 h. Residence time: ▲, 3 s; ○, 6 s; □, 12 s; ◆, 17 s.

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Absence of the Pigments of Photosystem II of Photosynthesis in Heterocysts of a Blue–Green Alga

All filamentous blue-green algae capable of fixing elementary nitrogen have heterocysts. Stewart *et al.*[1] have strong evidence that these differentiated cells are the sites of nitrogen fixation. They did not, however, show that photosystem II, responsible for the evolution of molecular oxygen (O_2), is not functional in heterocysts. Because high oxygen tension inhibits nitrogen fixation, heterocysts should not possess the pigments of photosystem II. Fay[2] produced evidence *in vitro* suggesting that heterocysts do not contain c-phycocyanin (c-PC), a principal constituent of photosystem II. Stewart *et al.*[1] found much less c-PC in heterocysts than normal cells and believed that photosystem II could be absent in heterocysts. Others[3,4], however, have demonstrated the evolution of O_2 from blue–green algae in the absence of c-PC. Thus the points to be resolved are whether heterocysts *in vivo* contain c-PC and the other pigments comprising photosystem II, and whether, in the absence of one or more of these, photosystem II is functional in heterocysts. A comparison of the *in vivo* pigment composition of normal cells and heterocysts has indicated that heterocysts lack a functional photosystem II.

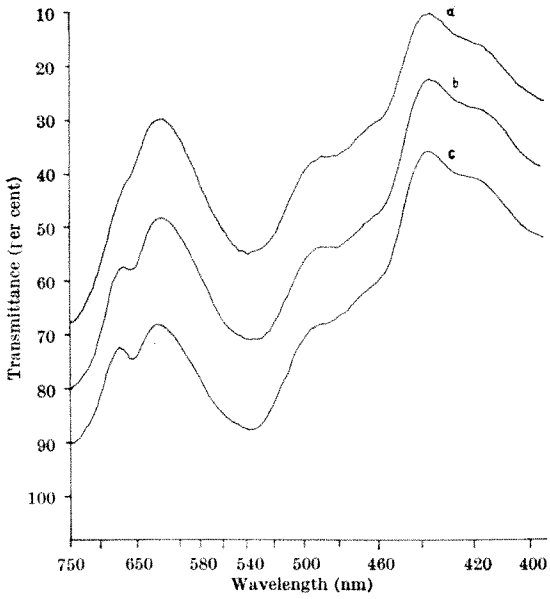


Fig. 1. Typical absorption spectra of single normal cells of *Anabaena* sp. L-31: (*a*) very young cells, (*b*) young cells and (*c*) old cells spectra. *a* and *b* are superposed by twenty and eight divisions respectively in percentage transmittance scale to facilitate comparison.

Anabaena sp. L-31 was grown in batch and continuous cultures in Watanabe's medium[5] without combined nitrogen. Cultures were aerated at a rate of 5 l./min. Light intensity was about 5,000 lux, and the algal suspension was 5 cm deep. The temperature was maintained at $29 \pm 1°$ C. Details of the continuous culture procedure have been reported before[6]. Samples were pipetted out aseptically and either centrifuged, washed and suspended in 50 per cent glycerol or directly mounted on quartz slides.

I used the microspectrophotometric technique, which is suitable[7] for the *in vivo* determination of the spectral characteristics of the pigments of single algal cells. Using a Universal Microspectrophotometer (UMSP I, Zeiss), all measurements were made on an area of diameter 3·2 μm in the spectral range 400 to 750 nm on single cells of *Anabaena*. Absorption spectra of nearly 180 normal cells and 80 heterocysts were measured.

Fig. 1*b* shows a typical absorption spectrum of normal cells, which comprised nearly 60 per cent of the population examined. The principal chlorophyll *a* peak is near 666 nm, while the c-PC peak is near 620 nm. C-phycoerythrin (c-PE) is not distinct, except for a slight change of slope of absorption near 580 nm. The absorption maxima due to carotenoids are near 490 and 460 nm. The spectrum in Fig. 1*a* is characteristic of very young cells constituting nearly 18 per cent of the total population and is distinctly different. The chlorophyll *a* (Chl *a*) and c-PC peaks are not well separated. Allophycocyanin (allo-PC) absorbing near 650 nm has been shown to be present in blue–green algae[8,9]. In young cells there seems to be more allo-PC. Absorption near 650 nm is enhanced and in the resultant overall envelope distinct peak characteristics are obliterated. Older cells, constituting nearly 22 per cent of the population, contain less allo-PC, so that there is a progressively deeper trough near 650 nm (Fig. 1*c*).

With the decrease of allo-PC in old cells there is a less drastic decrease in the peaks of Chl *a* and c-PC. Moreover, the Chl *a* peak is then no longer near 666 nm but is close to 680 nm, which indicates that it is the short wave form of Chl *a*, generally referred to as chlorophyll C*a* 670, which is decreasing in quantity.

The typical absorption spectrum of fully differentiated heterocysts characterized by thick walls, distinct polar bodies and homogenous protoplasm is shown in Fig. 2*c*. The chlorophyll peak is much reduced and near 680 nm, demonstrating that the long-wave form of Chl *a* (chlorophyll *a* 680) is present while the short-wave form, chlorophyll *a* 670, is absent. The peak of c-PC is absent, or, when present, appears as a very small hump. Peaks due to allo-PC and c-PE are also absent. The carotenoid peaks near 490 and 460 nm are, however, present and there is no significant change in their heights.

Spectrum 2*c* is typical of heterocysts of *Anabaena* grown in continuous culture and in samples examined during the exponential phase of batch cultures when light-saturated growth is prevalent. But in samples collected from batch cultures during the late linear phase of growth when growth is limited by light, heterocysts with distinctly different spectral features are observed (Fig. 2*b*). Under the microscope, these appear more opaque than those visible before. c-PC is present, but in much smaller quantities than normal. c-PE is also present. Low light intensities seem to favour the synthesis of biliproteins in heterocysts, as in normal cells of other blue–green and red algae[10,11]. Allo-PC seems to be absent, causing a deep fissure in the absorption curve near 650 nm. The chlorophyll peak is much reduced and is near 680 nm, which suggests that chlorophyll *a* 670 is not present.

These results indicate that heterocysts have two distinct types of spectral characteristics depending on culture conditions. But all heterocysts, irrespective of type, seem to lack the short-wave form of Chl *a* and allo-PC (Table 1). The range in the wavelength of absorption maxima of Chl *a* peak in the normal cells is between 662 and 672 nm, with a mean at 666 nm. In heterocysts the range falls within 678 to 685 nm, with the average value at 681 nm.

Table 1. WAVELENGTH OF MAXIMUM ABSORPTION OF CHLOROPHYLL *a* IN NORMAL CELLS AND HETEROCYSTS OF *Anabaena* sp. L-31

Normal cell		Heterocyst	
Range in λ max. (nm)	Mean λ max.* (nm)	Range in λ max. (nm)	Mean λ max.† (nm)
662–672	666	678–685	681

* Average value obtained from 179 measurements.
† Average value obtained from 76 measurements.

Photosystem II of blue–green algae responsible for the evolution of molecular O_2 is chiefly composed of c-PC and Chl *a* 670. The accessory pigments allo-PC and c-PE are also thought to be involved. It is generally believed

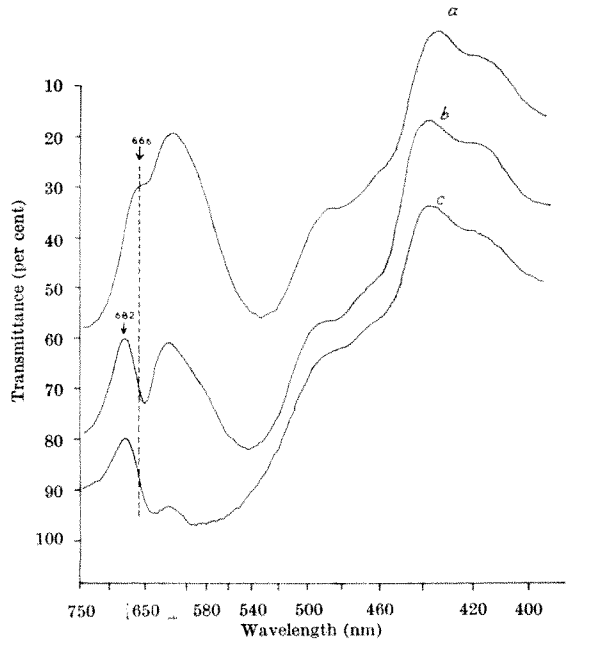


Fig. 2. Absorption spectral characteristics of single normal cells and single heterocysts of *Anabaena* sp. L-31. Typical spectra of (*a*) a normal cell, heterocysts in (*b*) light limited and (*c*) light saturated growth respectively. Spectra *a* and *b* are superposed by thirty-three and seven divisions respectively in percentage transmittance scale to facilitate comparison.

that the energy absorbed reaches the reaction centre by sequential resonance transfer between pigments with overlapping absorption bands, down the gradient from the region of higher energy (shorter wavelengths, accessory pigments) to regions of lower energy (longer wavelengths, chlorophyll *a*)[12]. In blue–green algae the sequence would be

c-PE→c-PC→allo-PC→Chl *a* 670→reaction centre

Even when c-PC is present, as in the second type of heterocysts, the absence of allo-PC and chlorophyll *a* 670 would seriously hamper energy transfer. Photosystem II, responsible for the evolution of molecular O_2, thus may not be functional in heterocysts.

The presence of chlorophyll *a* 680 and carotenoids indicates that photosystem I exists in heterocysts. Goedheer[13] showed that in blue–green algae energy transfer from β-carotene to chlorophyll occurs exclusively in photosystem I. This suggests that in heterocysts there is a functional photosystem I and that ATP generated by cyclic photophosphorylation can promote the reduction of nitrogen.

The evolution of O_2 observed by others[3,4] in the absence of c-PC in normal cells of blue–green algae is compatible with our results. Even if c-PC is absent, molecular O_2 would still be evolved under white light in the presence of chlorophyll *a* 670. The findings that both chlorophyll *a* 670 and allo-PC are invariably absent in heterocysts and that a functional photosystem I is present suggest that these differentiated cells would be ideal sites for nitrogen fixation in blue–green algae.

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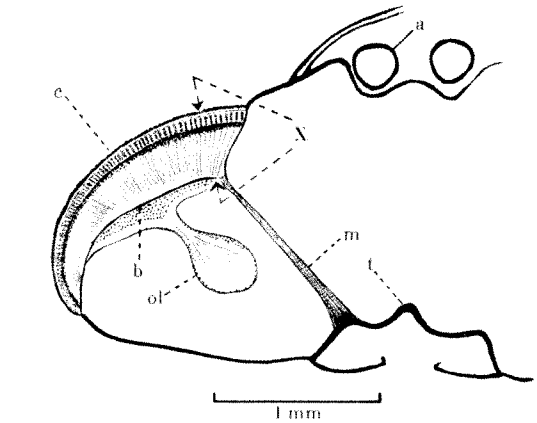
Internal Muscle in the Eye of an Insect

THE muscles associated with the vertebrate eye are well known, comprising those responsible for eye movements and the muscles within the eye controlling the iris and lens. The compound eyes of insects form part of the head capsule, and movements of the eye as a whole seem to demand that the whole head be moved as in the peering movements of young locusts[1]. There is only one little-known reference to a muscle within the compound eye itself, in the blowfly *Calliphora*[2]. Lowne called it a "ciliary muscle", but only noted its outer attachment. Fig. 1*a* shows that the muscle is attached externally to the medial border of the membrane at the base of the receptor layer, and internally to the tentorium (the internal supporting structure of the insect head). It is supplied by a fine branch of the antennal nerve. We have found comparable muscles in other Diptera, for example, syrphids, tipulids and culicids.

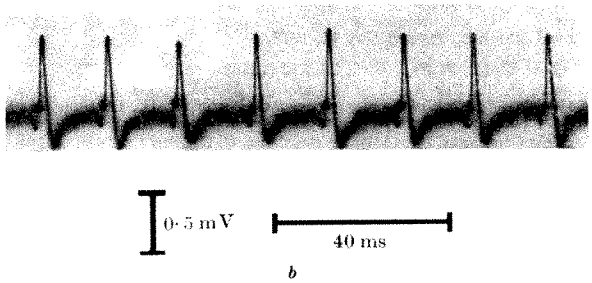
Extracellular recordings using a 30 μm tungsten electrode in contact with the muscle have been made in several species of syrphids, but mostly in *Calliphora vomitoria* L. The responses of the muscle in the intact head consist of a repetitive discharge of potentials of up to 1 mV amplitude and 25–100 Hz frequency (Fig. 1*b*). These potentials, to judge from their complex wave form, change in frequency with temperature and position of recording, and are the same as those described in *Calliphora erythrocephala* by Leutscher-Hazelhoff and Kuiper as "clock spikes"[3]. They described them as coming from the optic ganglion, but as the muscle is near the optic lobe (Fig. 1*a*) this location of the response could be due to electronic spread. They did not find any response to light.

We have found a marked response to light. Fig. 2*a* refers to flies which were adapted to white tungsten light of 6,500 lux and suddenly darkened, when there was a sharp increase in the rate of discharge of the muscle (shown by the shorter interspike interval). Conversely, as Fig. 2*b* shows, illuminating a dark adapted eye results in a slower rate of discharge (that is, a longer interspike interval).

Changes in the rate of response of the eye muscle would be expected to change its length and alter the position of structures to which it is attached. The tentorium would be expected to remain relatively static and the base of the eye would be expected to move. Two pieces of evidence suggest that this happens. First, light adapted insects were fixed rapidly while under light of 6,500 lux by injecting Bouin's fluid into the head; and dark adapted insects were fixed in the same way under red light to which



a



b

Fig. 1. *a*, Horizontal section of the compound eye of *Calliphora vomitoria* L., female. a, Angenna; b, basement membrane; c, cornea of the compound eye; m, muscle; ol, optic lobe; t, tentorium; X region used for measurement of depth of eye. *b*, Extracellular recording from surface of muscle (m).

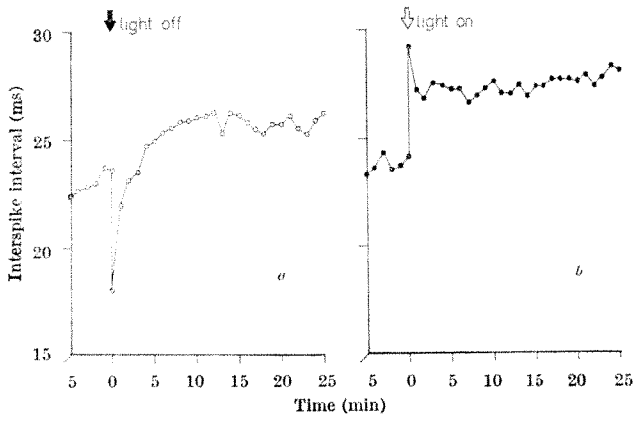


Fig. 2. Interspike interval plotted against time for female *Calliphora vomitoria* L. (means of ten animals). *a*, Light adapted in response to light "off". *b*, Dark adapted in response to light "on".

Calliphora is less sensitive. Frozen sections were compared in two groups each of thirty eyes from female flies by measuring the depth of region X in Fig. 1*a*. This was deeper in the light adapted state—$459 \pm 3{\cdot}9$ against $445 \pm 3{\cdot}5$ μm (s.d. of mean) in darkness, $P = 0{\cdot}01$–$0{\cdot}002$. Second, the eye was illuminated from within the head as described by Gemperlein and Jarvilehto[4], and the illuminated rhabdomeres were observed by a microscope through the outer surfaces of the eye. Changes in illumination of the eye in these conditions produced readily visible movements of the rhabdomeres.

Changes in the rate of discharge of the muscle can also be caused by moving large striped patterns across the visual field, and (which is rather unexpected) by air currents on the head.

Considering the function of the muscle, we thought earlier that it might cause the proximal migration of the rhabdomeres on adaptation to light first reported in Hemiptera[5] and we have found a similar muscle in Notonecta. Among Diptera, rhabdomere movements have been found in culicids[6], tipulids[7] and chironomids[8]. They also occur in Coleoptera[9]. In *Calliphora*, however, there are no movements of the rhabdomeres relative to the crystalline cone of the eye when the insect undergoes light adaptation. The occurrence of a response in the muscle to moving striped patterns (which in themselves would not cause a change in total illumination on the eye) suggests that the muscle causes scanning movements of the compound eye by displacing the photoreceptors. Movements of the photoreceptors relative to the lens system occur in the eye of salticid spiders[10] and in the copepod *Copilia*[11]; while the importance of small rapid movements of the eyes is seen in the human eye[12] and in the eye-stalks of crabs[13].

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Analysis of Running and the Prediction of Ultimate Performance

Several people have attempted to analyse world running records in the hope of explaining why man runs slower as the distance run increases. Meade[1,2] and Lietzke[3–5] have pointed out that the ratio of records for two distances has remained more or less constant since reliable athletic data have been kept. By showing that the rate of running during world record performances varies smoothly with distance (when plotted in logarithmic form), Francis[6] pointed out inconsistencies in the records and predicted which records should be broken and by how much. A physiological approach by Henry[7] yielded a polynomial expression relating mean running speed and total running time. The coefficients of the polynomial were related to the consumption and production of chemicals involved in muscle contraction, for example, glycogen and lactic acid.

Henry's work highlights the defect in all previous approaches in that each attempted to explain the process of fatigue (slowing down with distance) of one man by an analysis of world records set by several men. The hypothesis presented here applies to the fatigue of a single man and yet may explain many of the factors noted earlier, namely, smooth variation of record running rates with distance and the constant ratio of record times for two distances. In particular the success of this analysis allows a realistic estimate of the ultimate running performance.

We assume intuitively that the rate of running decays naturally (exponentially) with distance to be run. The expression

$$V^{-1} = f \ln d + c \qquad (1)$$

where V^{-1} is reciprocal of running rate (min/mile is a convenient unit); d is distance; c is a constant, and f is a constant called "fatigue coefficient", applies to many athletes engaged in aerobic running (distances above 1/4 mile). For example, in Fig. 1 the dotted lines and points demonstrate that equation (1) applies to three well known athletes, J. Ryun (United States, world record holder at 1/2 and 1 mile), R. Clarke (Australia, world record holder at 3 and 6 miles) and R. Hill (Great Britain, world record holder at 10 miles and second fastest marathon runner) and shows how f can vary considerably. Ryun has a high fatigue coefficient of 1·30 which is about double that of Clarke (0·62) while Hill (0·51) has one of the lowest fatigue coefficients we have found. The full line in Fig. 1 shows the smooth current world record curve and the data for Ryun and Clarke show that this arises

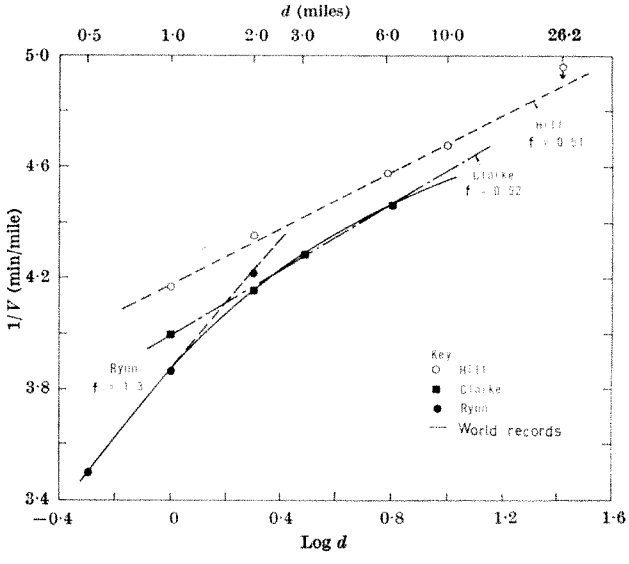


Fig. 1. Equation (1) plotted for three athletes and the current world records. Common logarithms are used.

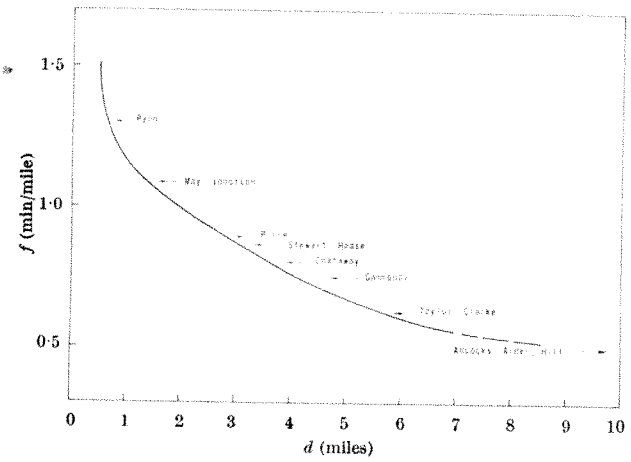


Fig. 2. Variation of f with distance for the current world records. Horizontal arrows indicate the coefficients established for a selection of prominent athletes.

from the overlapping of their individual straight lines; this curve explains the "constant ratio observation". Also, although there is an abrupt break in the world record curve above 6 miles, the linear relationship is retained by an individual up to the marathon distance (for example, Hill). This implies that the present 10 miles track record is slower than it should be.

Tangents to the world record curve at distance d will give the fatigue coefficient of the athlete likely to hold that particular record and thus f will vary smoothly with d. This is shown by Fig. 2 and so are the values of fatigue coefficient determined for several prominent athletes, past and present. Thus Fig. 2 shows effectively the event at which the athlete can perform best—the distance at which the slope of his "straight line plot" most closely approaches the tangent to the world record curve. The athlete has in most cases found by experience at which event he is best, but the significance of this idea to the non-world class or junior athlete is that it provides a means of determining which is his best event and also tells him, for example, whether his 9 min 2 mile run today is as good as his 14 min 3 mile run a month ago.

Equation (1) seems to hold for all runners but breaks down when the exercise is anaerobic. (At distances below about 1/4 mile $f \simeq 0$, that is, there is little loss in speed with distance.) This means that the performance of any aerobic exercise such as swimming or cycling should also obey the hypothesis, and this is found to be so. As the athlete improves, c is reduced and the performance line approaches the anaerobic region. But the data available for mature athletes indicate that f is constant and therefore an innate characteristic of the body.

In adult runners maximum running speed is almost invariant and the fatigue coefficient is probably a constant, and so we have a means of estimating the best possible performances of which an athlete is capable; these are the performances which lie on a line that passes through the runner's maximum running rate, for example, his 100 yards rate. Further, we can estimate the ultimate running times by taking the lowest observed fatigue coefficient, $f = 0{\cdot}5$, and a 100 yards time of 9·0 s (0·1 s below the present world record) and using equation (1) calculate the rates and times at other distances from 1/2 mile up to the marathon. The predicted limit of human performance is shown in Table 1 and compared with the present world records. If the present linear improvement in running records continues the ultimate performances should be reached in about 100 yr.

Table 1. ULTIMATE RUNNING PERFORMANCES

Distance (miles)	$f = 0{\cdot}5$ Present world record	100 yards in 9·0 s Ultimate performance
0·5	1 min 44·9 s	1 min 27 s
1·0	3 min 51·1 s	3 min 00 s
3·0	12 min 50·4 s	9 min 43 s
6·0	26 min 47·0 s	20 min 24 s
10·0	46 min 44·0 s	35 min 00 s
Marathon	2 h 8 min 56 s	1 h 37 min 30 s

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Quantitative Assessment of the Flight of *Archaeopteryx*

The coracoids, humeri and sternum of *Archaeopteryx* indicate that it was a poor flier[1]. Recent work on pigeon flight[2] suggests that an analysis of the strength of the wing bones should allow a quantitative evaluation of the flight potential of *Archaeopteryx*. While Pennycuik's experimental techniques could not be used, a theoretical approach seemed feasible.

The bones which are subjected to the highest stresses in flight are the humeri because these have to bear the total lift force. I decided to calculate the maximum bending moment which the humerus in *Archaeopteryx* could tolerate and from this to determine the ultimate load factor of the wings.

The shaft of the humerus is almost circular in cross-section and its narrow distal end approaches a cylindrical form. This bone was not pneumatic[1] and it was therefore assumed that the distal end was solid. The equation relating the maximum bending moment M to the tensile strength of the bone T and its radius r is as follows[3]

$$M = \frac{T\pi r^3}{4}$$

The radius at the narrowest portion (both London and Berlin specimens) is 0·15 cm and T is taken as $1{\cdot}3 \times 10^9$ dynes/cm^2, this being the highest recorded value[4,5].

The maximum bending moment of 3,500 g cm which the humerus could sustain can be related to flight performance if the weight and the position of the centre of lift are known. I agree with Jerison's estimate[6] of 500 g for the weight. Locating the centre of lift and relating this to the orientation of the humerus requires an accurate reconstruction of the wing profile. That shown in Fig. 1

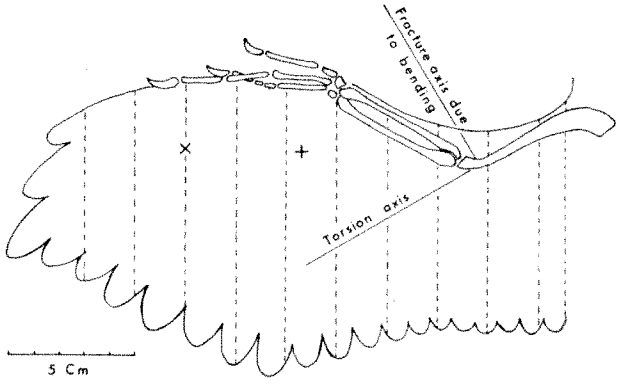


Fig. 1. Wing of *Archaeopteryx lithographica* spread out in the manner adopted during flight. Dashed lines divide the wing up into the eleven strips which were used in calculating the positions of the centre of lift while gliding (+) and hovering (×).

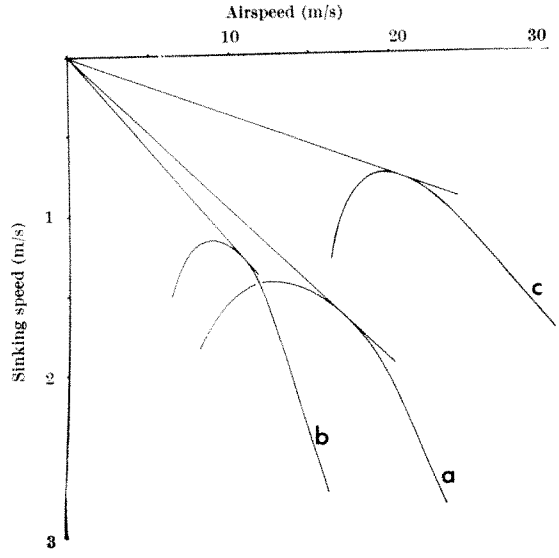


Fig. 2. The gliding polar illustrating the relationship between sinking speed and airspeed for (*a*) *A. lithographica*, (*b*) the fulmar petrel and (*c*) a typical man-made glider (*b* and *c* after Pennycuik[10]). The tangent to each curve shows the best gliding angle.

is based on the known feather pattern[1]. The centres of lift for gliding and hovering were located using Pennycuik's method[2] of dividing the wing into strips and calculating the lift generated by each strip. After locating the centres, the humerus was re-oriented slightly so that the ratio of the distances to the fracture axes was the same as that for the pigeon. In this configuration the humerus would have offered maximum strength to bending and torsion[2].

Considering first gliding flight, the distance from the centre of lift to the suggested fracture axis caused by bending is 5·9 cm, giving a maximum permitted lift on each wing of 590 g. We can ignore the lift which could be generated by the large tail, because, in ordinary flight, it would have acted so far back as to seriously upset the animal's balance. If the body also provided negligible lift, then the wing had an ultimate load factor of 2·4. This seems remarkably low and compares very unfavourably with the figure of 8·8 for the pigeon[2]. This weakness must have limited the animal's manoeuvrability, particularly in its circular paths. Assuming that during its tightest turns the wings were subjected to a load factor of 2·0 (that is, 0·4 reserve), then the maximum angle of bank was 60° (ref. 7). The minimum radii of these circular paths can be computed if the velocity is known. This is calculated from the same equation as that used by Bramwell and Whitfield in their analysis of the flying speed of *Pteranodon*[8], that is,

$$v = \sqrt{\frac{2L}{C_L \rho A}}$$

Here the lift L had a maximum value of $9{\cdot}8 \times 10^5$ dynes, the area of the wings A was 373 cm^2, the lift coefficient C_L is taken as 1·0 (that is, a cambered plate)[9], and the density of the atmosphere ρ is assumed to have been the same as at present. The maximum velocity is found to have been 20·9 m/s.

In pursuing a horizontal circular path the horizontal component of lift balanced the centrifugal force from which it is found that the minimum radius was 27·7 m, that is more than eight times that of the pigeon[2]. In pulling out of a dive the wings had to provide sufficient force to balance both centrifugal force and the bird's weight. At the lowest point the lift required to maintain a circular path (radius p) is as follows

$$L = \frac{mv^2}{p} + mg$$

The minimum radius of 44·5 m again compares unfavou ably with the 4·4 m calculated for the pigeon.

The performance of *Archaeopteryx* in gliding flight c be estimated theoretically[7]. The equation relating sinki speed to airspeed at low gliding angles (ø) is

$$r \sin \varphi = \rho \frac{AC_D r^3}{2\ mg} + \frac{2k\ mg}{\pi \rho S A r}$$

where C_D is the drag coefficient at the angle of inciden at which there is no lift, S is the aspect ratio and k is constant. C_D is taken as 0·04, this being the value for cambered plate at Reynolds numbers within the ran of *Archaeopteryx*[9]. According to Alexander[3] k cannot less than 1·0 and this value may be taken for most practic purposes.

The computed curve (Fig. 2*a*) shows the minimu sinking speed to have been 1·5 m/s and the best glidi angle 6°. Compared with fulmars and a typical man-ma glider (Fig. 2*b* and *c*)[10], it can be seen that *Archaeopter* was inferior to both. In view of the high sinking spe it is surprising that its pelvis was not better adapted withstand the impact of landing. De Beer pointed ou that the skeleton in modern birds is increased by t uncinate processes on the ribs, the extensive sacru and the elaborate articulations between the vertebra *Archaeopteryx* had none of these safeguards, from whi he concluded that it could not have landed from a gre height nor at great speed. No matter what method landing was adopted it would have been more viole than that of the fulmar and probably of most mode birds for, as will be shown, flapping its wings would on have produced a fraction of the lift needed for hoverin

Flapping flight imposed greater stresses on the win than gliding because the centre of lift moved further o from the body, the limiting case being reached if hovered. This centre during hovering was 9·8 cm fro the fracture axis caused by bending (see Fig. 1); thus t humerus would have fractured at a wing loading 360 g. In spite of this low figure the wings were stro enough to bear the animal's weight, but the possibili of hovering must be excluded on other evidence. T wings were aerodynamically poor because the long cla on the leading edge would have disrupted the air flo Taking a maximum lift coefficient of 1·5 it is calculat that in order to hover, *Archaeopteryx* would have had flap its wings at 20 beats/s, that is, more than twice t rate of a pigeon[2]. To achieve this the pectoral musc would have had to be even more powerful than t pigeon's, but it is clear from the anatomical eviden that they were not.

In every aspect of flight it seems that *Archaeopter* was inferior to modern birds. Calculations on the strengt of the humeri of some pterosaurs and other fossil bi have led to similar conclusions. These will be discuss elsewhere[11].

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Book Reviews

PLEASURES OF RESEARCH

First and Last Experiments in Muscle Mechanics

By A. V. Hill. Pp. xv+141. (Cambridge University: London, May 1970.) 55*s*.

It was a pleasure to read this book by Professor Hill, not only because it provides an excellent up to date review of the subject of the mechanics of muscle, but also, and perhaps chiefly, because the pleasure which the author took in writing it shines through so clearly in every chapter. In his years of retirement he looks back over everything he has done on this special aspect of muscle physiology during his long career. He traces the development of the various theories and notes where he went wrong and why. He shows where the work of others caused some revision in his thinking and he very generously gives credit, sometimes perhaps too much credit, where he feels it is due to others. He takes the reader through what he calls "the bad old days of the visco-elastic theory" and shows why the theory needed modification. Actually it was not a bad theory and served very well for many years, but the author did not hesitate to discard it in the light of new evidence as it appeared. For one who at one time worked in Professor Hill's laboratory and who participated to a small extent many years ago in some of the work described, the book is fascinating reading and a fine example of the power of simple but meticulous experiments coupled with sagacious and sophisticated interpretation.

The first experiments by Gasser and Hill in 1923, with nothing more than a simple lever and a smoked drum, were as original and bold in concept as any in the book and they differed from the "last" experiments chiefly in the type of recording equipment available at the time. The book ends with a speech made by its author at the International Congress of Physiological Sciences held in Tokyo in 1965 when he was given the keys of the city by his former student, the governor of Tokyo, Ryotaro Azuma. He certainly enjoyed that occasion, but mentions that it interrupted his "last" experiments. Even after that gala occasion he was eager to get back to the laboratory to put the finishing touches to his work on the mechanics of muscle.

One does not get the impression from this book, however, that the subject is now closed. Rather the book indicates many places where more work is needed. One chapter, for example, ends with the plea: "Will someone please make the experiment in this way ?". At another point he asks for better time records of delayed isometric contractions in single fibres without an external series elastic component (s.e.c.): "Then we could answer the question at the head of this chapter", that is, "How much of the s.e.c. can be accounted for within the sarcomere ?".

The book makes particularly clear that a muscle is a population of many fibres differing greatly in their velocities of shortening and in their response times. Indeed, the book might have been entitled a reappraisal of muscle mechanics in terms of the statistical distribution of fast and slow fibres, for this explains many of the anomalous records obtained. Similarly, the differing population of sarcomeres within a single fibre is considered, because the stronger units stretch the weaker ones, thus modifying the rate of development of tension externally.

Another complication, clearly recognized by Hill, is the fact that the excited state does not develop instantaneously at the moment of stimulation, nor at the same speed in all fibres. To avoid this, many experiments had to be carried out with "delayed isometric contractions" when the muscle is stimulated so that it would shorten a bit before reaching the length desired for recording. With this procedure the excited state is fully developed when the important part of the record begins, thus eliminating one complicating factor.

One interesting new fact in the book is the observation that "a preliminary high tension makes the series elastic component (s.e.c.) less extensible, but the greater stiffness rapidly passes off under low tension". With regard to this odd finding Hill remarks with characteristic humour, "There are more things in heaven and earth, Horatio—and even in frog muscles". True, but it takes a keen mind or an A. V. Hill to discover them. Many such "things" are described in this book. They are all in a sense trivial, but they add up to provide a penetrating view of muscle mechanics which is by no means as simple as it sounds.

Professor Hill's many contributions to this subject will always be remembered as "classics". It is only to be regretted that he is no longer active in the laboratory where he inspired so many younger physiologists by his approach to research, which was fundamentally so serious but outwardly rather gay and humorous. He thoroughly enjoyed his work and so did his students. This book accurately reflects the stimulating atmosphere of his laboratory.

Wallace O. Fenn

INTRODUCTION TO LIFE

The Life Process

By J. A. V. Butler. Pp. 256+12 plates. (Allen and Unwin: London, August 1970.) 60*s* boards; 35*s* paper.

This book is in the same style as its two predecessors by the same author, *Inside the Living Cell* and *The Life of the Cell*. It is intended as a relatively elementary book for students beginning university studies and is probably set at about the right level to achieve this aim. Like its predecessors it covers a wide range from the chemistry of proteins and DNA, on the one hand, to the function of the brain and ageing on the other. Professor Butler has a knack for couching complicated facts in simple language and he manages to present a large amount of information in digestible form. But, perhaps inevitably, the book is patchy. Some parts are extremely well done, especially the chapters on molecular biology, that is, those on DNA and replication, the genetic code, viruses, the genetics of bacteria and viruses, and regulatory mechanisms of bacteria. These are quite detailed and yet easy to read. On the other hand, the chapter on the hormones provides little more than a list of known hormones. Those in sense organs, cell membranes, nerves and brain also receive superficial treatment. Because textbooks abound

on each of these topics, it is not easy to see what useful information could be crammed into chapters of five to ten pages in an elementary textbook. Perhaps it might have been better to limit its scope. Apart from this shortcoming the book provides a readable and up to date introductory text.

An interesting feature of Butler's style is that he is meticulous in mentioning the names of the scientists associated with each discovery. The book is well illustrated with clear line drawings and plates of moderate quality. It has a well-selected bibliography for each chapter.

JOHN PAUL

SCAVENGING CELLS

The Macrophage

By Nancy N. Pearsall and Russell Weiser. Pp. x+204. (Lea and Febiger: Philadelphia, 1970. Distributed in Great Britain by Henry Kimpton, London.) 76*s*.

IT is impossible in a short review to do justice, critically and otherwise, to this little book in which the authors have compressed an enormous amount of information, in fact, more than it can hold, and under chapter headings which frequently indicate overlap.

Although the book is called *The Macrophage*, great emphasis is laid on the diversity of macrophage populations and the great inter- and intra-species differences that exist among these populations morphologically, biochemically and functionally. Strangely, the fact that cultured fowl macrophages proliferate actively in homologous serum is not mentioned; yet the special macrophage growth factors necessary for the proliferation of macrophages ("mammalian" should have been added) receive due consideration.

In the first half of the book, the structure of macrophage (poorly illustrated by only a few electron micrographs), phylogenetic and ontogenetic aspects, sources and lifespan, cellular interrelationships (incidentally, the claim that lymphocytes transform into macrophages only in the presence of polymorphs still awaits confirmation), metabolic activities, phagocytosis and functions (sic!) of macrophages are reviewed and discussed. The chapter on metabolism contains a useful, comprehensive table on enzymes found in macrophages.

The second half of the book is concerned with the role of macrophages in immunological situations and is addressed more to the expert in this field than to the uninitiated reader. It deals with contributions of macrophages to the antibody response, with antibodies cytophilic for macrophages, with delayed sensitivity and antitissue and antimicrobial cellular immunity with special discussions of tuberculosis, leprosy, the *Listeria* and the *Salmonella* systems. There is no doubt that macrophages act as scavengers, trapping, processing and storing antigenic material, but whether they are specific effector cells remains still an open question; and it is perhaps significant that one of the authors' concluding sentences reads: "Overall, lymphocytes and macrophages cooperate in an effective cellular immune response".

The great merit of the book lies in the accumulation of numerous data on the subject which have appeared in recent years in widely scattered journals. Of the 699 references quoted, only 160 were published before 1960; this makes the work invaluable as a reference book. One can say, it is up to date as far as this is possible these days. Some of the papers quoted are reviewed in detail; others are summarized in such a condensed form that this does not give sufficient information or sometimes even a misleading or an inaccurate one; and any reader of this monograph would be well advised to go back to the original reference, because this is, after all, one of the aims of this book.

F. JACOBY

BEFORE THE ANTIBIOTICS ERA

A History of Medical Bacteriology and Immunology

By W. D. Foster. Pp. xi+232. (Heinemann (Medica London, July 1970.) 40*s*.

THERE is a wide variety of general books on the history medicine but, on the whole, the history of its special are is less well served. Thus this book is only the third histo of bacteriology in English to appear and the need for has been evident for some time because neither of i predecessors, Bulloch's *History of Bacteriology* (1938) a Ford's *Bacteriology* (1939), is entirely adequate. Bulloc for example, dealt at great length with the earlier perioc finishing at 1900, and did not cover the medical aspects the subject fully.

Dr Foster is ideally suited for the task of providing competitor for Bulloch and Ford because he has alreac published excellent accounts of the history of clinic pathology and of parasitology. He begins his book with brief introduction to the germ theory of disease which w established in the 1870s, and then discusses in detail tl contributions of Pasteur and Koch to its foundatio There is a good chapter on the discovery of the mo important human pathogenic bacteria, a vital aspect of tl subject but hardly mentioned by Bulloch. As his tit indicates, Foster includes the history of immunology, fir its scientific basis and then its practical application medicine; in this regard he might have mentioned *History of Immunization* by H. J. Parrish (1965). The la two chapters comprise an excellent account of the princip developments in bacteriology during the early twentiet century, and in the chemotherapy of bacterial diseas Medical students should be encouraged to peruse thes carefully in order to counter the commonly held view tha specific therapy commenced in the 1930s and 1940s wit the introduction of sulpha drugs and antibiotics. Or useful secondary source Foster omits to cite here is *Tl Impact of the Antibiotics on Medicine and Society* (edite by I. Goldston, New York, 1958).

A criticism of this book could be levelled at the intr ductory chapter dealing with events before the ger theory. From it important men such as Fracastoro, wl gave the first "modern" interpretation of contagio Semmelweis and Oliver Wendell Holmes of puerperal fev fame, and others of merit have been excluded. Admitted Bulloch perhaps discussed early concepts in too gre detail, but Foster would seem to have gone to the oth extreme. It can be argued, however, that, given a limite amount of space, more should be allocated to the mo modern rather than to the earlier period. This may be le acceptable to the historian but it certainly is an advanta to students and practitioners who prefer to know of tl immediate rather than the distant past.

Foster is to be congratulated on his achievement, for l has produced a history of bacteriology which is orientate adequately to medicine and which provides for the fir time an account of the most recent history of the subjec up to 1938, a convenient date because it marks the eve the antibiotics era, as well as the appearance of tl previous histories by Bulloch and Ford. Moreover, he giv evidence throughout of appreciating the meaning an methodology of history. His book can be warmly recon mended and parts of it should be required reading f medical students.

EDWIN CLARKE

TIPS FOR MICE BREEDERS

Methods in Experimental Embryology of the Mouse

By Keen A. Rafferty, jun. Pp. xi+94. (Johns Hopkin Baltimore and London, August 1970.) 81*s*.

WALTER HEAPE, the father of experimental embryology i mammals, wrote: "On the 27th April, 1890, two ova wer

obtained from an Angora doe rabbit which had been fertilized by an Angora buck 32 hours previously.... These ova were immediately transferred into the upper end of the Fallopian tube of a Belgian hare doe rabbit which had been fertilized three hours before by a buck of the same breed as herself.... In due course this Belgian hare doe gave birth to six young—four of these resembled herself and her mate, while two of them were undoubtedly Angoras". Although he tried on many occasions to repeat his earlier feat, it was not till 1897 that Heape achieved two further successful transfers. In the intervening years, he developed the less exacting technique of artificial insemination.

In the early 1950s, when Donald Michie and I began to transfer eggs from one mouse to another, nobody in Britain had used the technique; one worker on the Continent of Europe and two or three in the United States had recently revived the art. Like Heape, we had beginners' luck, but many months of frustration followed. We wrote to L. M. Boot in Amsterdam, the nearest practitioner, who replied with three close-typed pages of excellent advice, and our fortunes improved.

Had Rafferty's book been available at that time, our appeal to Dr Boot might not have been necessary. During the past 15 years, mammalian eggs (most often mouse eggs) have not only been transferred in their tens of thousands, but have been fused together and split apart, cultured *in vitro* and even fertilized *in vitro*, grown in ectopic sites, used for micro-injection experiments and subjected to biochemical analysis and electron microscopy. (Rafferty comments: "One hardly knows where it will all end".). All these techniques and more, together with the equipment which they demand, are described in lucid detail in this book.

Of course the book is not perfect. Rafferty is more successful in his description of techniques than in his accounts of the literature, which are often confused. For instance, it is not true that Billington, Graham and McLaren found that ova "cultured to the blastocyst stage and then transplanted to kidney or testis give much better embryonic development than do blastocysts which developed *in vivo*". The table of normal development, in appendix E, does not emphasize sufficiently that rate of development varies widely from strain to strain. The timing that it gives for blastocyst stages contradicts both my experience and his own Table 1.

Some of the procedures that he describes sound clumsy, and most experienced workers will probably continue to use the methods to which they are accustomed. A day spent in a laboratory where techniques are in constant use is worth many pages of text, however clear. Nonetheless, this book needed to be written and will be widely used.

ANNE MCLAREN

SURVEYS OF HUMAN GENETICS

Modern Trends in Human Genetics

Vol. 1. Edited by Alan E. H. Emery. (Modern Trends Series.) Pp. ix + 379. (Butterworth: London, March 1970.) 95*s*.

AMONG the multi-author publications which plague the scientific literature this is one of those rare exceptions which show unity and little repetition. The editor deserves congratulations for what must have been a tough assignment.

With the exception of the first chapter, the thirteen chapters, all by different authors or pairs of authors, cover most of the areas in which human genetics has made substantial advances recently. Surprisingly omitted are the analysis of autosomal linkage and population genetics. The first chapter, by Boyer, is somewhat out of context. It is, nonetheless, a valuable and stimulating discussion of the various levels at which, in higher organisms, one might expect that regulation of protein synthesis could operate. Hopkinson then summarizes the techniques and the results of the study of enzyme variation—especially electrophoretic variation—in man. The survey carried out in Harris's laboratory at University College, London, reveals the enormous degree of individual enzyme diversity in human populations. Out of eighteen randomly selected enzymes, seven show genetic polymorphism.

Two chapters of great topical interest survey exhaustively two facets of the genetics of the immunological system. One, by Schimke and Kirkpatrick, deals with the genetics of gamma globulin synthesis; the other, by Grove and Bach, with that of leucocyte antigens. How wide can be the range of refinement in genetic analysis is clear in the former: at one extreme, variation is analysed down to amino-acid sequences, for example, in the immunoglobulins; at the other extreme, variation is recognized only at the gross level of organ abnormality, for example, in thymus aplasia. The progress on leucocyte typing—an important tool in transplantation—is well summarized in the second of these two chapters.

Davidson covers the relatively new field of somatic cell cultures as tools in human genetics. The human genetic markers recognizable at the cell culture level so far identified are not far from forty. In addition the number of cellular enzymes known to differ interspecifically, for example, between mouse and man, is increasing rapidly. By using interspecific cell hybridization it has now become possible to identify which human chromosome determines a particular enzyme by finding which chromosome must be present in an interspecific hybrid for the human enzyme to be synthesized. This technique, first successfully applied by Weiss and Green in 1967, has recently led to most interesting developments for the identification of linkages and even of the linear order of human genes.

A chapter by Edwards and Fowler deals with female meiosis and the genetics of human pre-implantation, and their abnormalities, from non-disjunction and abnormal fertilization to errors in cleavage and chimaeras. Meiosis in males with normal or abnormal chromosome complements is treated by Kjessler. Sanger and Race's summary of the present state of mapping of the human *X*-chromosome makes most interesting reading. They conclude by stressing the extraordinary conservatism of the *X*-chromosomes in mammals—no less than five genes, *X*-borne in man, are also so in species ranging from rodents to ungulates.

Four chapters cover some more strictly clinical aspects of human genetics. Emery considers the antenatal diagnosis of genetic disease. In addition to gross chromosomal abnormalities, there are already more than ten conditions which can be diagnosed by culture of amniotic fluid cells or by analysis of the amniotic fluid. This is, of course, of importance in high risk families where early diagnosis can permit prevention (for example, in the case of pyridoxine congenital epilepsy) or termination. Finn gives a very stimulating account of the reasoning which led to the use of anti-Rh immunoglobulin for the prevention of Rh haemolytic disease. The wider implications for the prevention of genetically determined diseases are ably discussed. Wynne-Davies copes with a most difficult field, that of congenital malformations which represent only extreme deviations in a continuous distribution curve. Here the genetical component can only be inferred by comparing the incidence of the malformation among relatives of various degrees. Carter discusses the prospects in genetic counselling and its three purposes: to advise parents at risk on the chance of further affected children; to alert the medical profession on early diagnosis and possibly early treatment and to reduce the incidence of affected children in the population.

Finally, Smith deals with the ascertainment of those at risk in the prevention and treatment of genetic disease. The reduction of infant mortality in Britain has had the dramatic effect of bringing to the forefront the fraction connected with genetic conditions. These now account for

20 per cent of all infant mortality; four times more than at the beginning of the century. Similarly, in adult mortality the genetic component is now relatively greater than in the past. These two facts increase the importance of diagnosis, prevention and, in a small number of cases which will gradually increase, treatment. Carter surveys the various aspects of this increased importance of genetic disease and the formidable ethical problems it raises.

On the whole this very readable book is of interest both to the specialist and to the layman. G. PONTECORVO

BIOLOGY UNDER PRESSURE

High Pressure Effects on Cellular Processes

Edited by A. M. Zimmermann. (Cell Biology: A Series of Monographs.) Pp. xvi + 324. (Academic: New York and London, April 1970). 182*s*; $19.50.

THIS book has thirteen contributors, including the most distinguished recent investigators in the field, several of whom might now be regarded as reaching the peaks of highly productive careers. Each of the eleven chapters is a separate essay, with an extensive bibliography, but together they form a coherent whole, with author and subject indexes. The book is good value; it is packed with information, is well illustrated and is interesting to biochemists, physiologists, microbiologists, protozoologists, embryologists and marine scientists.

F. H. Johnson and H. Eyring, drawing on the theories of Arrhenius and of absolute reaction rates, show how the pressure-temperature relations of bacterial luminescence and muscle tension are probably connected with the volume changes involved in enzyme activity and inactivation. J. V. Landau, with full experimental details, similarly explains the effects of pressure on biosynthesis of protein and nucleic acids, and discusses the possibility that protein synthesis requires the maintenance of a gel matrix, which breaks down under pressure. R. Y. Morita and R. R. Becker, citing dilatometric and other relevant studies on enzymes and aqueous systems, point out the difficulties which arise because the effects of pressure are so various and impossible to control experimentally. T. H. Murakami surveys studies in Japan, covering pressure effects on mitosis, nerve and muscle action, cell permeability, oxygen consumption, enzyme inactivation and protein denaturation. C. E. Zobell broadly reviews effects on the morphology and metabolism of microorganisms.

No bacteria have been found capable of growth at pressures much greater than those in the deep ocean trenches. There are, however, many phyla of marine animals represented at the greatest depths, as H. Flugel and C. Schlieper show, and they report further that the tissues of different shallow-water species are pre-adapted to varying degrees for functioning at abyssal pressures. Some effects of pressure on freshwater heliozoans, suctorians and ciliates are described by J. A. Kitching, who discusses possible mechanisms of response and adaptation. In another chapter on Protozoa, Selma Zimmerman and A. M. Zimmermann show how earlier studies on the division and movement of cells have been extended to ultrastructural and molecular levels. A. M. Zimmermann contributes another shorter chapter, with T. H. Murakami, on the curious cathodal galvanotaxis of *Tetrahymena*, which weakens and eventually reverses if the pressure is increased sufficiently. He goes on to review previous and current research on biosynthesis in the eggs of sea urchins. Finally, D. A. Marsland, to whom the volume is dedicated (on or around his seventieth birthday), adds a particularly well organized chapter about the mechanisms involved in cell division. Knowledge of that has advanced considerably as a result of pressure experiments, but for each question tentatively answered, several new ones have arisen.

E. W. KNIGHT-JONES

DIVERSE MICROBES

The Actinomycetales

The Jena International Symposium on Taxonom September 1968. Edited by H. Prauser. Pp. 439. (Gusta Fischer Verlag: Jena, 1970.) 184*s*.

THIS book consists of papers presented at a symposiu held in September 1968. Although the actinomycet include microbes of economic importance and attract tl attention of a great many workers, a meeting on the sca of this one had not been held for some time. The need f such a publication was therefore considerable.

Treatment of actinomycetes has always reflected tl fact that they have affinities with both bacteria and fun so that there has been a considerable diversityof taxonom theory and method applied to the group, which is w illustrated by this collection of papers. Papers by specialis working on most of the best known genera are include with an understandable emphasis on the most studi *Streptomyces* genus, although it is somewhat surprisi that there is only one paper dealing specifically with t genus *Nocardia*. There is also a section on metho applied in taxonomy which covers techniques such infrared spectrophotometry, cell wall analysis, serolo and numerical taxonomy. The book is concluded by fo papers dealing with taxonomic problems in the Actin mycetales as a whole.

All papers are in English and in spite of occasion inaccuracies the choice of a single language has obvio benefits. Minor misprints are quite frequent and referenc are given in full in most but not all papers. The standa of production of plates and figures is satisfactory. It m confuse some readers to see the name *Actinomyces* used two different senses and it would have been useful to ha an indication in all cases when it was used to descri *Streptomyces* species. There is an index of taxa nam but no author or subject index.

To the specialist and non-specialist alike, this is valuable reference book. The comprehensive range current views and techniques presented provides an acc rate impression of the present state of the taxonomy this group and should serve as a stimulus for future wo Thus frequent discussions on the "importance" of par cular taxonomic characters and the variety of suggest classification schemes illustrate the need for more objecti assessments of character value and the purpose of clas fication schemes. Also, the many new methods providi additional characters may increase confusion if they are r integrated with existing characters in such schemes.

The editor and symposium organizer, Dr H. Prauser, to be congratulated for providing a much needed review this topic. S. T. WILLIAMS

WAR ON WANT

Evaluation of Novel Protein Products

Edited by A. E. Bender, R. Kihlberg, B. Löfqvist a L. Munck. (Proceedings of the International Biologi Programme (IBP) and Wenner-Gren Center Symposi held in Stockholm, September 1968.) (Wenner-G Center International Symposium Series, Vol. 14.) viii + 390. (Pergamon: Oxford and New York, June 197 140*s*; $18.50.

THIS book covers proceedings held two years ago Stockholm. Two years later, the Third Internatio Congress of Food Science and Technology on "Science Survival" in Washington DC again devoted its attent chiefly to "protein problems". A fair comparison therefore be drawn and progress can be measured.

It speaks well for the Stockholm meeting if I state tl no strikingly new approach or discovery was revealed Washington, and anybody reading this well edited b

may, as a listener in Washington, be entitled to say I have read it "all" before, and I mean "all", because this book deals with nutrition and malnutrition seemingly *in toto*.

J. Vogt takes cognizance of the nutrition problems as an economist and discusses the different attitudes expressed in the past two hundred years by men like Quesnay, Malthus and Marx. Few principles expressed are still pertinent today, others have been totally drowned by men with better appreciation of the problem.

J. C. Waterlow describes the protein requirements of men, and is then reminded by S. A. Goldblith how food habits and taboos have often prevented man from using available nutrients. Millions of persons will rather starve than eat food they are not accustomed to and this is a stark reminder to the food scientist trying to make a contribution to overcome world hunger. "You must first reach them, before you can teach them," Goldblith says, and we can only ignore this wise admonition at great peril to our effort.

Looking at the strategy in the evaluation of novel proteins, C. G. King begins by stating that malnutrition reflects a failure of man to adjust to his environment, and corrective measures must embrace not only an understanding of the science of nutrition, but also the essentiality of family planning. This underlines the stark naked truth that no effort, however far reaching, can resolve the world food problem without a measurable successful effort in limiting world population increase.

The second session of the book deals with world protein sources. Nobody seems better qualified than A. M. Altschul to begin this session with a review on oilseed proteins followed by G. Borgstrom's excellent address on fish proteins in all their various forms. New high protein cereal varieties receive a short mention, followed by an evaluation of leaf protein, and the session concludes with a very comprehensive address by L. Enebo on single-cell protein. These newcomers to the field of edible proteins will in time play an important part, and will receive increasing attention.

Nutritional evaluation of proteins by chemical and biological methods follows in session three, giving a much needed up to date picture of a field that has made great strides in the past few years. This valuable reflexion on the science of nutrition is followed gy a look at toxic, hygiene and functional properties of proteins giving the food scientist guidelines, hints and an understanding of this important aspect of food evaluation at a time when legislation in this field is more and more restrictive and embracing. No symposium on food could be complete without stressing the importance of processing and storage on protein quality. In the sixth session a number of well known authors deal with this area of food science competently and comprehensively.

The authors rightly conclude the book with a panel discussion on science and the world food problem, and here scientists who have become household names—while stressing a divergence of views—do not fail to impress on the reader the seriousness of the situation and the need to declare "a war on want" the fight is of global science against global hunger. Who could afford not to read this book ?

A. SPICER

VIRUSES AND PLANTS

Plant Virology

By R. E. F. Matthews. Pp. xv + 778. (Academic: London and New York, May 1970.) 275*s*.

THE rapid expansion of research on plant viruses and virus diseases in the past decade has outrun the few general textbooks available in English. Professor Matthews aimed his book at graduate students, teachers and research workers in virology and allied fields. They will welcome it, within the limitations set by its price, for its many attributes.

The book attempts, and succeeds in large measure, to cover the whole field of plant virology, beginning with assay methods and ending with speculation on the origin and evolution of viruses. One may cavil at the relative length of the sections—60 of its 700 pages devoted to replication and movement seems excessive—but in general the balance seems about right, for it reflects both topical emphasis in research and the fields in which the author has been most active himself.

The style, though often terse, is certainly clear. The author emphasizes his intention: "immunology has been plagued by a complicated and sometimes obscure terminology. Here we will attempt to use a minimum of such words". In defining terms in another context, he carries brevity without lucidity to the extreme: "The term protein shell means protein shell".

I noted some instances of pointless repetition and no doubt missed others. For example, the loss of transmissibility of potato yellow dwarf virus is quoted on pp. 547 and 549. The behaviour of cacao swollen shoot virus in transmission by its mealy bug vector is twice described in the same words on consecutive pages. Extra "polishing" could have shortened the text, although perhaps with little reduction in the price of the book. I noticed few misprints and these did not affect the sense. More serious is the occasional error of fact, such as quoting sudden death of cloves as an example of virus transmission through natural root grafts; the pathogen is a fungus.

The book is lavishly and clearly illustrated. In spite of the author's claim that he went to "some pains in selecting graphs and photographs to illustrate and supplement the text", a little more labour might have produced better examples than some he chose; others, including a full-page portrait of aphids possibly feeding and doing nothing else, would not be missed.

But, on the whole, this is an excellent book which fulfils its purpose and amply justifies the immense work carried out in its compilation.

A. F. POSNETTE

FUNDAMENTALS OF DESALINATION

Reverse Osmosis

By S. Sourirajan. Pp. xix + 580. (Logos, in association with Elek Books: London, August 1970.) 160*s*.

THIS book describes a new process which is developing rapidly. It is concerned primarily with the application of semipermeable membranes to separating water from solutions. This development occurred first because of its potential as a new and economically attractive method of desalination for the supply of potable water. Now its wider scope for separating the components of other aqueous solutions and possibly non-aqueous ones has been realized. The author was directly involved in the discovery of the method of manufacture of special forms of cellulose acetate membrane which formed a basis of this development, and is well fitted to write about the subject of reverse osmosis. He has produced a comprehensive publication dealing with important fundamental aspects and including much useful experimental data.

An introductory chapter deals with historical aspects and outlines the scope of the process. Subsequent chapters deal with the preparation and properties of suitable semipermeable membranes, transport through the membranes, fluid phase mass transfer and process design, the use as a general concentration technique, treating multicomponent mixtures and organic solutions and details of existing engineering designs. These chapters illustrate the much wider scope for the use of reverse osmosis and include appreciably more material than previous publications. Of particular interest is the treatment of stagewise operations to which this process lends itself on a large scale for concentrations and multicomponent separations. The precise mechanism of selective permeation through the

reverse osmosis membranes is not yet clear. The author's preferential sorption theory cannot be considered separately from the repulsion model for electrolyte solutions by Glueckauf and the diffusion models of others. The mass transfer theory correlates small-scale data but has yet to withstand the test of scale-up to large plant because only pilot scale equipment has so far been constructed. Nevertheless, the book is full of well presented informative detail and has brought together extensive information which, up until now, has been scattered through the literature. It can be recommended not only to scientists and engineers for research purposes but also to process and equipment designers who are concerned with the uses of the method for desalination, in the food and chemical industries, and for effluent treatment and other applications.

J. W. CARTER

ORGANIC REACTIONS

The Chemistry of Cyclic Enaminonitriles and *O*-Aminonitriles

By Edward C. Taylor and Alexander McKillop. Pp. xii + 415. (Advances in Organic Chemistry, Vol. 7.) (Wiley (Interscience): New York and London, July 1970.) 280*s*.

THIS book is the seventh volume of an excellent series which one of the authors edits. Whereas the series is chiefly composed of comparatively brief reviews of subjects of wide interest, this volume is devoted to a single subject, and that an esoteric one. Nevertheless, the authors' active interest in the subject over seventeen years has helped them to make a very thorough job of reviewing a field, which is of that kind for which the usual source of information, the indexes of *Chemical Abstracts*, is of little help. This is just the kind of review to present in book form and makes this one excellent value. In design it resembles a superior chapter from *Organic Reactions*, having extensive tables of the known syntheses of enaminonitriles in the first chapter and of *o*-aminonitriles in the second; the word *ortho* in the latter covers all 1-amino-2-cyano aromatic systems and is not restricted to the benzene ring. The first chapter is thus an extensive review of the Thorpe–Ziegler reaction: a persuasive case is made that, although this reaction is well known, it is undeservedly neglected by practising organic chemists. The second, longer, chapter is of more interest to a specialist in heterocyclic chemistry, but it clearly makes a good case for the usefulness of these compounds, and of the reactions used to prepare them, both in heterocyclic synthesis and in the transformations of heterocyclic compounds.

The price is a reflexion of the fact that only libraries will buy copies. They can do so in the full assurance that the book they get is concise, readable and extensive.

IAN FLEMING

PHYSICS OF SEMICONDUCTORS

Lectures on the Electrical Properties of Materials

By L. Solymar and D. Walsh. Pp. xiv + 350. (Clarendon: Oxford; Oxford University: London, August 1970.) 80*s* boards; 40*s* paper.

THE chief purpose of this book would seem to be the development of the physical arguments necessary for an understanding of semiconductor devices. This it does extremely well in nine chapters, starting with the concepts of the electron as a particle and as a wave, introducing Schrödinger's equation and using it to describe the hydrogen atom, bonding, free electron theory and zone theory before discussing semiconductors and devices. Four additional chapters, covering dielectric materials, magnetic materials, masers and lasers, and superconductivity, appear rather as afterthoughts. The treatment is clear, concise and logical, and that of the Ginsberg–Landau phenomenological theory of superconductivity is one of the best I have ever encountered. Non-physicists will have no difficulty in following the arguments as they are developed.

Much material has been omitted which, from the title, one might reasonably expect to be present. There is no discussion of the effect of the structure of real materials on electrical resistivity, dielectric properties, or the irreversible behaviour of magnetic and superconducting materials. To one whose understanding of electronics is extremely limited, the device chapter is the most interesting. Problems of switching speed are, however, neglected: a comparison between the speeds of semiconductor, thin film magnetic (completely ignored), and superconducting switches might have prevented the erroneous statement that the latter are the faster. The implication that lasers are a solution without a problem will please at least one of my colleagues.

The style is chatty and condescending; the many sneers directed at physicists ("honest engineers" obviously implies "devious physicists") do nothing to heal the unfortunate breach between the two professions. The treatment is uneven; an acquaintance with advanced mathematical techniques, such as Fourier transforms, is assumed while many simple physical concepts are laboured or dismissed as being too difficult.

As described in the preface, the book is very obviously an almost verbatim account of a lecture course given by the authors to second-year undergraduate engineers at Oxford and herein lies the source of the weaknesses criticized earlier. Careful editing could have produced an extremely valuable and self-sufficient textbook. As it stands, the book is unlikely to be used as a course text (most lecturers use an informal style similar to that of this book, and require the backing of a more authoritatively written text) but can be highly recommended for additional reading. The authors at least are to be commended for submitting their manuscript to a British publisher, thus ensuring that the price is still within the reach of some pockets.

DAVID DEW-HUGHES

FINDING EIGENVALUES

Latent Roots and Latent Vectors

By S. J. Hammarling. Pp. x + 172. (Hilger: London 1970.) 100*s*.

As the publishers say on the dust-cover of this book, latent roots, characteristic values, eigenvalues—call them what you will—are of fundamental interest in almost any field of study to which mathematics has been applied. For the mathematician there is Dr Wilkinson's authoritative treatise to refer to, but that work lacks the simple numerical examples which the engineer, physicist or economist needs before he can understand the many and various methods of finding eigenvalues and their associated eigenvectors and appreciate to which type of problem each is most applicable.

In this book, after two chapters in which basic theorems are proved and some applications presented, a separate chapter is devoted to each of the better known methods: Danilevsky, Givens, Householder, Krylov and Lanczos. Iterative methods and, in particular, the Q–R algorithm are described. Each method is illustrated by simple but not trivial examples and there are a number of well chosen exercises to consolidate understanding. A final chapter summarizes some other methods and indicates the reasons why they are not so popular.

This book gives an introduction to the problem of finding eigenvalues pitched at just the right level for the non-mathematician, but I am afraid that its price may prevent it being read as widely as it deserves.

A. H. CRAVEN

Correspondence

Apartheid for Whom ?

SIR,—Your review (*Nature*, **227**, 5; 1970) of my two papers in the *South African Journal of Science* (**65**, 329; 1969, and **66**, 12; 1970) gives a false impression both as to the extent of duplication of research in South Africa and its cause. The question asked in the survey was, "Have you ever had the experience of discovering after you had completed a piece of research that someone else had already discovered and published substantially the same facts ?". Although 17·5 per cent of the responses were affirmative, one is not justified in concluding that "nearly one South African scientist in five is duplicating research being carried out in other laboratories", or that this is a "feature of the isolation of South Africa". It is interesting to note that my figure for South African research workers is remarkably close to those obtained in similar surveys by Martyn[1] and Flowers[2] in the UK, Törnudd[3] in Scandinavia and Glass and Norwood[4] in the USA. Likewise the reasons for the failure of scientists to learn of other work in their fields in time to avoid duplication of research are much the same in those countries and are related rather to the literature searching habits of the scientists themselves than the political systems under which they work, as seems to be implied in your choice of headline.

Owing to a delay in the publication of my paper by the *S.A. Journal of Science*, the figures for the remuneration of research scientists are grossly out of date. I am happy to be able to report that currently the financial rewards of scientific research in South Africa are some 15 to 20 per cent better than those listed for various groups in my paper.

Yours faithfully,
D. RYLE MASSON

CSIR Natal Regional Laboratories,
Post Office Box 1, Congella,
Durban, South Africa.

[1] Martyn, J., *New Scientist*, **21**, 338 (1964).
[2] Advisory Council on Scientific Policy, *J. Doc.*, **21**, 83 (1965).
[3] Törnudd, E., *Proc. Intern. Conf. Scientific Information*, Washington DC, 19 (National Academy of Sciences/National Research Council, 1959).
[4] Glass, B., and Norwood, S. H., *Proc. Intern. Conf. Scientific Information*, Washington DC, 195 (National Academy of Sciences/National Research Council, 1959).

Phytopathology in Brazil

SIR,—I read with great interest the short report on the occurrence of coffee rust in Brazil (*Nature*, **226**, 997; 1970). I was also interested in the fact that many of the author's conclusions were based on observations made by Professor F. L. Wellman on the occasion of his recent visit to the rust-infected area.

I have known Professor Wellman for many years and also met him during his recent visit to Brazil, so I am sure he would be the first to agree with me about the need to add more details to the report and to give credit to a larger number of phytopathologists. He did not fail to give generous credit to others in another recent report[1].

The occurrence of the disease was observed by Brazilian phytopathologists, who also made a correct diagnosis of the causal agent. This diagnosis was later confirmed by other Brazilian colleagues. Among the Brazilian phytopathologists concerned with the problem I should mention Mr Arnaldo Gomes Medeiros (Centro de Pesquisas do Cacáo, Itabuna), Dr A. A. Bitancourt, and Miss Victoria Rossetti (Instituto Biológico, São Paulo) and Professor Charles F. Robbs (Universidade Federal Rural, Rio de Janeiro).

There is a relatively large number of phytopathologists working in Brazil, and they joined a few years ago to form the Brazilian Society of Phytopathology (Sociedade Brasileira de Fitopatologia.) In this connexion it should be mentioned that the first foreign phytopathologist approached by the Brazilian authorities to take part in the planning of control measures was Professor A. Branquinho D'Oliveira (Centro de Pesquisas sôbre ferrugem do Café, Oeiras, Lisbôa, Portugal). Some phytopathologists from other countries, such as Dr E. Schieber from Guatemala, were also given the opportunity to study the problem *in loco*.

Yours faithfully,
KARL M. SILBERSCHMIDT

Instituto Biológico,
Caixa Postal 7119,
São Paulo, Brazil.

[1] Wellman, F. L., *Phytopathol. News*, **4** (6) (1970).

The Definition of Aggression

SIR,—In his article in *Nature* (**227**, 1006; 1970) reviewing trends in neuroscience, F. O. Schmitt considers the role which neurophysiological investigations may play in the understanding and control of social behaviour, and of aggression in particular. I do not wish to challenge the methodology nor the interpretation of studies on the implantation of electrodes, etc, nor do I wish here to question the ethics of such research on human subjects. What is of concern, however, is the apparent failure to define the behaviour which is being modified.

Schmitt states, for example, that "violent and aggressive behaviour is an all too prevalent manifestation of social imbalance in many parts of the world", and he later refers to "aggressive and *other aberrant* behaviour" (italics added). It is debatable to what extent any scientist can evaluate or initiate research in the context of the bias inherent in the view that aggressive behaviour is an aberrant form of behaviour and that aggression is too prevalent in the world.

Aggression is a term which can be used to cover a wide variety of forms of behaviour and to be of any value in a scientific context it must be defined in as neutral and precise a manner as possible. Any investigation of social behaviour has to identify the structure of the behaviour and the structure of the situation in which that behaviour is displayed. It may be that a definition of aggression will include reference to injury or harm to another person, but an analysis of other components in the sequence of behaviour is necessary and account must also be taken of the situation in which the behaviour is manifested. Schmitt's view that aggression is a "manifestation of social imbalance" has prejudged and loosely defined the determinants of the behaviour and has stated an hypothesis in the context of a set of values which prevents rather than facilitates the understanding of the behaviour and its causes. In social science research it seems important to make explicit those value premises which may bias the form of research and the interpretation and utilization of the results of research.

Yours faithfully,
JOHN M. INNES

Department of Psychology,
University of Birmingham.

Theories of Electromagnetism

SIR,—McCaig gives a quite unwarranted impression of confusion in electromagnetism (*Nature*, **227**, 935; 1970). It is not the case that the Kennelly and the Sommerfeld formulations lead to self-contradictory results. McCaig has misinterpreted the basic claims of the two theories. The field of a physical magnet depends on its shape, so the statements that the H-field of a magnetic dipole is inversely proportional to μ_r in the one system, and inde-

pendent of μ_r in the other, are to be understood in reference to a particular shape. The early treatments of magnetism were based on the formulae for isolated poles, so when the same formulae are applied to magnets the shape assumed is that for which the magnet approximates to an ideal dipole, namely bar-shaped. The formulae must then only be used within their range of applicability. (For a magnet which is infinitesimally thin, the field at all points at a finite distance is indistinguishable from the field of a pair of isolated poles.) Kennelly is in this respect an MKS version of the traditional theory of electromagnetism, and his views presuppose the bar shape; Sommerfeld is explicit on the question of shape[1]. Thus it is incorrect to apply the relations uncritically to an ellipsoidal shape.

Both the traditional and the Sommerfeld systems are self-consistent, in magnetic media as well as vacuum. The two theories, however, make different statements about observable results; therefore they are not both consistent with the facts. The difference between them is of fact, not of arbitrary convention. The difference is not shown simply in the position of μ_r, for this difference can be accommodated by a different interpretation of magnetic moment. The difference in factual content comes when one asserts what magnetic quantities are constant. I gave the references previously[2].

An experiment has been performed[3], which decided in favour of the traditional view, and against the view now associated with the name of Sommerfeld; but it was not satisfactory and should be repeated. But in the meanwhile one can readily show that the factual falsity of Sommerfeld follows from the basic equations of magnetostatics, namely, div $\mathbf{B}=0$, curl $\mathbf{H}=0$, $\mathbf{B}=(\mathbf{H}+\mathbf{M})/\mu_0$, if one supposes that an ideal "hard" magnet is one in which magnetization $\mathbf{M}$ is unchanging. I propose publishing a fuller discussion on another occasion.

The clear understanding of electromagnetism is difficult; it is therefore important to eliminate mistakes as quickly as possible.

Yours faithfully,

H. V. Stopes-Roe

Department of Extra-mural Studies,
University of Birmingham.

[1] Sommerfeld, A., *Lectures on Theoretical Physics:* III, *Electrodynamics*, 41, 87 (Academic Press, New York, 1952).
[2] Stopes-Roe, H. V., *Nature*, **224**, 579 (1969).
[3] Sargant, E. B., *Phil. Mag.*, **14**, 395 (1882).

Disputed Pronoun

Sir,—It is simple to make the disputed sentence make sense by re-arranging the clauses in their syntactical order of importance—a sequence used by every competent journalist in this country. Thus: "John was surprised to learn (that) he had won the race." Mis-related participles are unacceptable because of their lack of precision.

I must confess surprise that the medical and scientific communities, who put so much stress on publication, are unaware of this basic rule, which is, I admit, related in practice to the degree of concentration available for sentence construction.

What does genuinely worry me is that people who can not set down their thoughts clearly may not be capable of thus assembling them. I trust, to preserve our belief in the omniscience of the communities concerned, I am forthwith proved wrong.

Yours faithfully,

Alistair Campsie

Highfield East,
Bridge of Allan,
Scotland.

Obituary

Professor W. O. Kermack

William Ogilvy Kermack, who died on July 20, 1970, made distinguished contributions to chemistry, biochemistry and statistics. He was born in Kirriemuir, Angus, on April 26, 1898, attended Webster's Seminary, Kirriemuir, and graduated at the University of Aberdeen in 1918. The following year he joined Professor W. H. Perkin, jun., at the Dyson Perrins Laboratory, Oxford, and succeeded in synthesizing heterocyclic compounds related to the alkaloid harmaline. In 1921 he became head of the chemical research laboratory, Royal College of Physicians, Edinburgh, but a laboratory accident in June 1924 rendered him totally blind. Such a catastrophe, which would have ended most research careers, presented a challenge to him to seek a way of resuming his scientific life. This he was able to achieve by the careful design of experiments which were undertaken by others under his direction. He kept abreast of the literature by having selected articles read to him, sometimes by colleagues, but especially by his wife without whose help he could not have continued. By 1930, in addition to his work in synthetic organic chemistry, he had studied the mechanism of flocculation of colloidal solutions and investigated certain aspects of glyconeogenesis.

After 1930, his organic chemical interests led him to look for new antimalarials. Many new quinoline and acridine derivatives were synthesized and, later, some substituted pyridoacridines and phenanthrolines, a few of which had great chemotherapeutic potency. The decade 1930–1940 was also the period of his greatest activity in the sphere of medical statistics, where he made important contributions to the mathematical theory of epidemics. At the same time, he began to write review articles on recent advances in biochemistry. Over a period of twenty years, few aspects escaped his notice. In 1938, he collaborated with Dr Philip Eggleton in writing *The Stuff We're Made Of*. This layman's guide to the basic sciences on which biochemistry is founded is still an entertaining book.

In 1949 he was appointed to the Macleod Smith chair of biological chemistry in the University of Aberdeen and characteristically accepted the task of creating a new department. His teaching commitments, University administrative duties, committee work in connexion with local research institutes, and plans for a School of Biochemistry left little time for research, yet he managed to study several biochemical problems principally concerned with amino-acids and enzymology. By the time of his retirement in 1968, he had established an active and effective department of high reputation.

During his career, many honours came his way, including Fellowship of the Royal Society of Edinburgh (1924), honorary LlD of St Andrews University (1937) and Fellowship of the Royal Society (1944). His scientific knowledge was broadly based and the clarity of his thinking, the logic of his arguments and his understanding of first principles were widely acknowledged. His advice was always generously given to colleagues who brought their problems to him. He enjoyed discussion within a small group, and had a good memory for scientific anecdotes and a sense of fun. His achievements would have been remarkable for anyone, but for a man blinded so early in life they could well be unique.

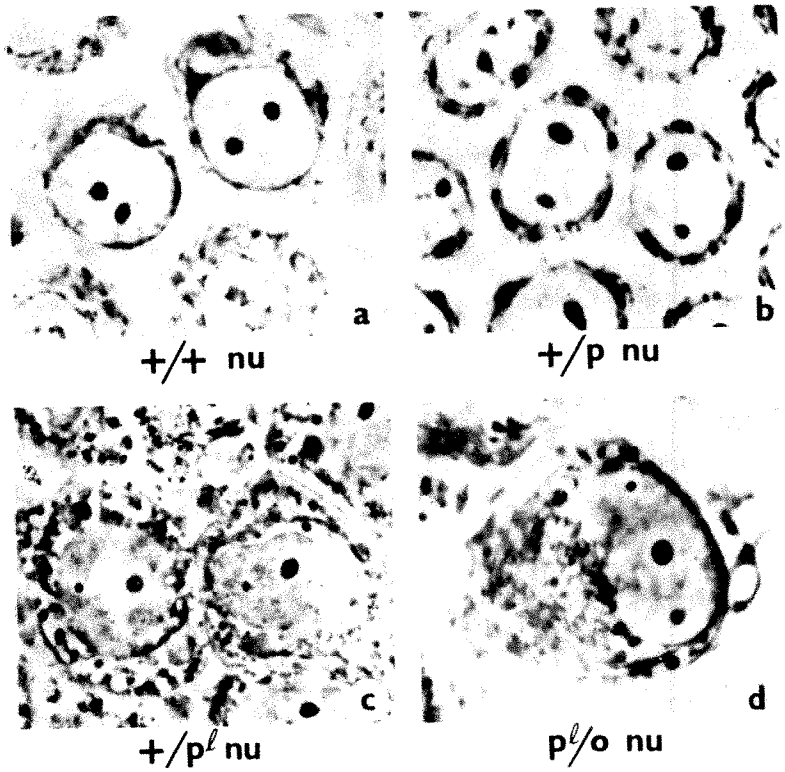


Fig. 1. Phase contrast preparations of epithelial cells from tadpoles having (*a*) two equal nucleoli (*+/+ nu*); (*b*) two slightly unequal nucleoli (*+/p nu*); (*c*) two very unequal nucleoli (*+/p^l nu*); and (*d*) one nucleolus and two extra nucleolus-like bodies (*p^l/0 nu*). *a* and *b* are from tentacles of stage 56 tadpoles, while *c* and *d* are from the tail tips of stage 42 tadpoles.

ERRATUM. Fig. 1 was unfortunately omitted from the paper "Mutations affecting the Size of the Nucleolus in *Xenopus laevis*" by Leo Miller and J. B. Gurdon (*Nature*, **227**, 1108; 1970). Under the subheading "Development of *p nu* Mutants", the third and fourth sentences should read: "The *p/0 nu* embryos, with a single large nucleolus in each cell, develop and metamorphose normally. In contrast, *p^l/0 nu* embryos, which contain extra nucleolus-like bodies in addition to a large nucleolus in some of their cells, die as tadpoles before starting to feed".

Announcements

University News

Mr P. E. Doak has been appointed to the Hawker Siddeley chair of theoretical acoustics in the **University of Southampton.**

Dr A. R. Lansdown has been appointed director of the Tribology Centre, **University College of Swansea,** in succession to Professor F. T. Barwell.

Appointments

Dr K. E. Scheer, University of Heidelberg, has been appointed director of the Division of Life Sciences in the Department of Research and Isotopes, **International Atomic Energy Agency.**

Dr Gordon K. Moe, director of research at the Masonic Medical Research Laboratory, Utica, New York, has been appointed to the National Advisory Heart and Lung Council of the **US National Heart and Lung Institute.**

Professor A. J. Cole, University of St Andrews, has been elected chairman of the **Inter-University Committee on Computing.** The committee reports to the Vice-chancellors and Principals Committee on matters of policy concerning the teaching of computer science and the development of computer services.

Mr H. J. Habakkuk, University of Oxford, **Professor J. B. Jepson,** Middlesex Hospital Medical School, and **Mr D. T. Richnell,** University of London, have been appointed to the **Advisory Committee for Scientific and Technical Information,** under the chairmanship of **Dr L. Rotherham.**

Miscellaneous

The Presidium of the Academy of the Sciences of the USSR has awarded the **I. I. Mechnikov gold medal for 1970** to **Boris L'vovich Astaurov** for his work in experimental genetics.

The European headquarters of the **Stanford Research Institute** has been established in London, and **Dr Torben Meisling** has been appointed managing director of SRI–Europe.

The first congress of the **European Association for Cancer Research** was held in Brussels last month. Membership of the association is open to individuals who are, or have been, engaged in either clinical or experimental cancer research. Further information can be obtained from the secretary, Dr G. J. V. Swaen, Wilhelmina Gasthuis, Amsterdam, Holland.

Price of Nature

From this week, the price of *Nature*, which has been unchanged since January 1963, will be increased from 4*s* to 5*s* for those who buy the journal through newsagents or who buy single copies. The subscription price of the journal will remain unchanged. The present increase, which will apply to some ten per cent of the weekly production of *Nature*, is made necessary by the increased cost of printing.

British Diary

Monday, October 12

Cytology of the Living Vocal Cords (5.30 p.m.) Dr Renato Segre, University of London, at the Institute of Laryngology and Otology, 330–6 Grays Inn Road, London WC1.

Jacking of Railway Bridge Abutments (5.30 p.m. informal discussion) Institution of Civil Engineers, at Great George Street, London SW1.

Tuesday, October 13

Driers (7 p.m.) Mr J. Turner, Oil and Colour Chemists' Association, at the Pendragon Hotel, Clarence Parade, Southsea, Hants.

Industrial Reciprocating and Rotary Compressors: Design and Operational Problems (three-day conference) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Medical Education in the United States, 1870–1970 (5 p.m.) Dr John Field, University of London, at St Mary's Hospital Medical School, Wright Fleming Institute Lecture Theatre, Norfolk Place, London W2.

Radiological Safety Aspects of Nuclear Power Stations (10 a.m.) Society for Radiological Protection, at London.

Some New Applications of Atomic-absorption Spectroscopy in the Metal Industry (7.15 p.m.) Mr P. Davy, Mr W. W. Fletcher and Mr E. Waugh, Society for Analytical Chemistry, Atomic Spectroscopy Group, jointly with the Modern Methods of Analysis Group of the Sheffield Metallurgical and Engineering Association, at BISRA Group Laboratories, Hoyle Street, Sheffield.

The Idea of a University (1.20 p.m.) Professor Richard Wollheim, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Traffic Engineering (7 p.m.) Mr T. D. Hobbs, Society of Environmental Engineers, at the University of Birmingham.

Wednesday, October 14

Aircraft and the Bird Hazard (6 p.m.) Mr G. W. Underwood, Society of Environmental Engineers, in the Mechanical Engineering Department, Imperial College, Exhibition Road, London SW7.

Cellular Growth, The Role of Hormones and Nutrition (5.30 p.m.) Professor Donald Cheek, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1.

Rheology and Processibility of Printing Inks (7 p.m.) Mr C. O. Rosted, Oil and Colour Chemists' Association, at the Borough Polytechnic, Borough Road, London SE1.

Some Aspects of Paint Formulation (4.30 p.m.) Mr T. W. Wilkinson, Oil and Colour Chemists' Association, at the Manchester Literary and Philosophical Society, 36 George Street, Manchester 1. (Student Lecture.)

Whither Fats in Animal Feeds (6.15 p.m.) Dr L. G. Chubb, Society of Chemical Industry, Food Group—Nutrition Panel, at 14 Belgrave Square, London SW1.

Thursday, October 15

Food from the Means—is the Future Really Promising? (1.20 p.m.) Dr I. Morris, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Problems of Sludge Treatment and Disposal (5.30 p.m. informal discussion) Institution of Civil Engineers, at Great George Street, London SW1.

Some Aspects of Coil Coating (6 p.m.) Mr J. Hortensius, Oil and Colour Chemists' Association, at the St Enoch Hotel, Glasgow.

Some Botanists of South and South East Asia (5 p.m.) Dr J. C. Marr, Linnean Society of London, jointly with the Royal Asiatic Society, at Burlington House, Piccadilly, London W1.

The NPL Mekometer and Its Application to Mine Surveying and Rock Mechanics, Mr C. M. St John and Dr T. L. Thomas; **The Hathern Anhydrite Series, Lower Corboniferous, Leicestershire, England,** Dr P. G. Llewellyn and Dr R. Stabbins; **The Hamersley Iron Pellet Plant,** Mr J. R. Hammond, (5 p.m.) Institution of Mining and Metallurgy, at the Geological Society, Burlington House, Piccadilly, London W1.

The Storage and Stability of Radiochemicals (6.30 p.m.) Dr Catch, Society for Analytical Chemistry, Radiochemical Methods Group, at the Leicester Lounge, Glasshouse Street, London W1.

What are Patents Anyway (5.50 p.m.) Mr Kingsley M. Wray, Society of Chemical Industry, Road and Building Materials Group, at 14 Belgrave Square, London SW1.

Friday, October 16

Engineering Aspects of Rock Weathering with Examples from the Dartmoor Area (5 p.m.) Mr P. G. Fookes, Mr W. R. Dearman and Mr J. F. Franklin, Geological Society, Engineering Group, at Burlington House, Piccadilly, London W1.

Polymer Developments—The Seventies and Beyond (6.30 p.m.) Mr G. L. Holbrow, Oil and Colour Chemists' Association, at the Chamber of Commerce House, 75 Harborne Road, Birmingham 15.

Saturday, October 17

Water and Plant Life (10 a.m. conference) Joint Biology Committee, in the William Beveridge Hall, University of London, Senate House, Malet Street, London WC1.

Monday, October 19

Development of Immersed Tunnelling in Eurpoe (5.30 p.m.) Mr Alex Christiani, Institution of Civil Engineers, at Great George Street, London SW1.

Karl Marx and the Withering Away of Social Science (5.30 p.m.) Mr G. Cohen, British Society for the Philosophy of Science, in the Joint Staff Common Room, University College London, Gower Street, London WC1.

Physiological and Clinical Results from Cardiac Pacing (4 p.m.) Dr Edgar Sowton, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

The Work of the ALRC (2.15 p.m.) Dr P. T. Haskell and Mr R. D. MacCuaig, Society of Chemical Industry, Pesticides Group, at the Anti-Locust Research Centre, College House, Wrights Lane, London W8.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Institute of Brewing National Collection of Yeast Cultures. Catalogue of Cultures maintained at the Brewing Industry Research Foundation. Pp. 73. (Nutfield, Surrey: Brewing Industry Research Foundation, 1970.) [19

Building Research Station. Current Paper 18/70: Stress-probe Experiments on Saturated Normally Consolidated Clay. By P. I. Lewis and J. B. Burland. (Reprinted from *Geotechnique*, 1970, Vol. 20 (1) pp. 38–56.) (Garston, Watford, Building Research Station, 1970.) *gratis.* [19

Fabian Tract 402: The New Politics: a Socialist Reconnaissance. By Anthony Wedgwood Benn. Pp. 28. 5*s* (25*p*). Fabian Research Series, No. 290: The UN—an Economic Institution. By Hamish Richards. Pp. 20. 4*s* (20*p*). (London: Fabian Society, 1970.) [39

Countryside Commission. National Park Guide No. 8: Exmoor. Edited by John Coleman-Cooke. Pp. xi+110+33 plates. (London: HMSO, 1970.) 8*s* 6*d* (40*p*). [49

The Natural Environment Research Council. Occasional Publications, No. 1: Humane Killing of Seals. By W. Nigel Bonner. Pp. 8. Publications, Series C, No. 1: Seal Deaths in Cornwall, Autumn 1969. Pp. 20. Publications Series C, No. 2: A Report on the Research Applications of the Submersible "Pisces II". Pp. 20. (London: Natural Environment Research Council, 1970.) [49

Imperial Chemical Industries Limited. Information Handbook. Pp. 42. (London: ICI Ltd., 1970.) [49

Other Countries

Organization for Economic Co-operation and Development: European Nuclear Energy Agency. Water Cooled Reactor Safety: An Assessment prepared for the Committee on Reactor Safety Technology. Pp. 179. 20 francs; 30*s* 6*d*; $4.50; 13.60 DM. Basic Approach for Safety Analysis and Control of Products Containing Radionuclides and Available to the General Public: a Guide prepared by an ENEA Expert Group. Pp. 31. 7 francs; 11*s*; $1.50; 4.90 DM. (Paris: OECD, European Nuclear Energy Agency; London: HMSO, 1970.) [29

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Entomology, 1968/1969. Pp. 69. (Canberra, ACT.: CSIRO, 1970.) [39

The Weizmann Institute of Science. Scientific Activities 1969. Pp. xvi+350. (Rehovot, Israel: The Weizmann Institute of Science, 1970.) [39

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NATURE Volume 228
OCTOBER 17, 1970

We Are All Luddites Now

TACTICALLY, the new British Government's proposals for trade union legislation have been a minor masterstroke. For one thing, they have acutely embarrassed the Labour opposition by stealing many of the proposals for legislation which the old Labour government first urged on the House of Commons but then withdrew in the face of opposition from the trade unions. Second, they are at first sight a judicious mixture of restraint on employers and employees, even though everybody must know that the balance of advantage will depend entirely on how the proposed system for regulating disputes functions in real life. Third, the new proposals have been introduced at a time when Britain seems likely to be plagued by a rash of industrial disputes in industries as different as coal mining, refuse collection and motor car assembly. Evidently the government will only win friends for itself by seeming to grasp the nettle of industrial disputes at this stage in the economic history of Britain, and this is undoubtedly just. Some regulation of this kind is no doubt necessary. Even if employers and employees have conflicts of interest, there is no reason why these should not be resolved within the compass of a legislative framework. But this is only half the battle and the more immediate half. The question which neither the government nor the opposition has tackled is that of providing a pattern for labour relations decades from now, when there is a danger that many of the jobs on which people's livelihoods at present depend may have become fossilized survivals from the past.

One sign of the way the wind is blowing can be seen easily enough in the pattern of the industries in which there have recently been labour troubles. Garbage collectors, and the army of low paid workers who work for the local authorities in Britain, are unfashionably employed. Nobody loves them, they are badly paid and they are denied even the glamour of technology. Nobody should be surprised that people trying to keep families alive on basic wages of less than $2,000 a year should now be up in arms. Unhappily, however, there is very little chance that their just demands on society could be satisfied without their jobs being priced out of the market. Local councils are prepared to pay garbage collectors less than a seemly wage for demeaning work, but would quickly install machines instead if the going rate were several times as much. And the same is true even in technically advanced industries such as motor car assembly. Here, too, the chief difficulty is that the jobs that are being carried out resentfully are jobs in the twilight that exists between work for human hands and work for machines. The higher the rates for which the motor assembly unions settle the more rapid will be the conversion to machines. What those now grappling with trade union legislation should appreciate is that the time will come when unfashionable jobs are entirely out of fashion. What will labour relations be like then?

What are the goals at which to aim? And if technology will change the pattern of employment in the years ahead, does it also suggest how industrialized societies should adapt to change? In the flurry of excitement about what is sometimes called technological forecasting, there has been too little attention to side-effects like these. In basic industries such as coal mining, however—one of those in which labour troubles seem unavoidable—it should be possible to make quite precise calculations of the effects of technical development.

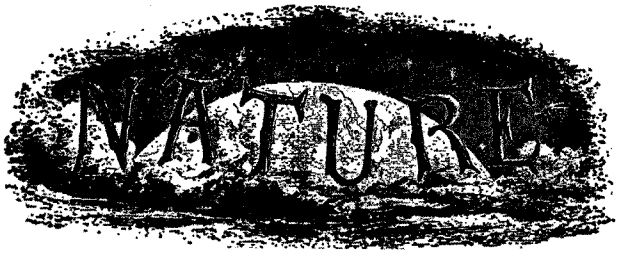
Ten years ago, 500,000 miners were able to produce close on 200 million tons of coal a year, but it was also clear that wages accounted for such a large proportion of the total cost of coal production that coal would price itself out of the market if there were not a rapid improvement of the productivity of miners—another way of saying that mechanization had become essential for the survival of the industry in competition with petroleum. In the event, there has been a dramatic improvement of the efficiency with which coal is extracted from British mines, but this has not been dramatic enough entirely to cancel out the rising cost of labour, which is quite properly linked with the prosperity of the country as a whole. Moreover, it now seems that the improvements of efficiency which marked the sixties are coming to an end (although this is a point on which the National Coal Board should have more to say). The result is that coal is moving rapidly into a situation in which miners feel hard done by, in which coal is, in any case, uncompetitive and in which even the most optimistic supporters of the industry can only look forward to a further decline of output. In circumstances like these, nobody should be surprised that the miners are up in arms. The best strategy for the National Coal Board to follow would be to abandon the concept that coal can still be an important source of power in Britain (which would also imply an abandonment of the tax penalties to which oil-burning power stations are subjected) and a determination to sell coal for chemical purposes (as in iron ore reduction) at a price high enough for miners to be paid a decent wage. No doubt there is also a moral in this for the declining industries.

The rash of strikes among the badly paid employees of the local authorities is, for example, reminiscent of the way in which trouble over coal was avoided in the

early sixties. If the local authorities wish, as they should wish, to contain the cost of municipal services, they can only hope to do so by mechanizing demeaning jobs such as the collection of garbage—still one of the most quaint mediaeval survivals in modern British cities. If it is not obvious how the job is to be done, then the local authorities will simply have to spend money on the necessary research.

But will the unions stomach changes of this kind? Perhaps the most serious defect of the government's discussion on paper is that it does nothing to provide the unions with an incentive to act in the best interests of the industries in which they are involved. For the years ahead, this should be the central problem. Out and out syndicalism is not quite inappropriate, but the objectives have something to be said for them.

100 Years Ago



The New Postal Act

AT page 474 Mr. Reeks complains as to the working of the new Postal Act. It seems intended to obliterate the old parcel post. He says, "Herbarium specimens are not excluded." Perhaps so; but they are not included. The provisions of the new Act are limited to books, written and printed matter, genuine trade samples and patterns, so far as regards the two ounces for a halfpenny. All parcels other than books, &c., as described above, go at the letter rate of one penny for each half-ounce.

The postal card is the thin end of the wedge that will hereafter open to us a regular letter rate of a quarter of an ounce for a halfpenny. For instance: we may now send ordinary business communications up to two ounces, thus embodying the matter of twenty postal cards, for the halfpenny, if folded in a paper wrapper. An ordinary business communication of half-ounce weight goes for the halfpenny, if folded as a letter but left unsealed.

I ask, If Government will now take an open letter for a halfpenny, why not take it closed at the same rate? Common sense will hereafter equalise this disparity. The parcel post, however, is at present discontinued. P. N. Row

October 14

Science and the Government

THE reason that the Government has refused to aid "the expedition to observe the approaching eclipse" is, that it is perfectly assured that "men of science and culture" are nothing but a set of lying impostors, and would swindle the public out of thousands of pounds to take an observation which might be done for 10*l.* and much less. The nation is fast beginning to perceive that astronomy is a monstrous cheat—and the Transit of Venus has no more to do with the distance of the sun than it has with the number of fingers on my hand.

JOHN HAMPDEN

[We congratulate Mr. Lowe on his ally, merely remarking that the same Government which has refused the Eclipse Expedition has granted 20,000*l.* for observations of the Transit of Venus. Perhaps Mr. Hampden can explain the cause of this inconsistency on the part of the Government.—ED.]

From Nature, **2**, 494, *October* 20, 1870.

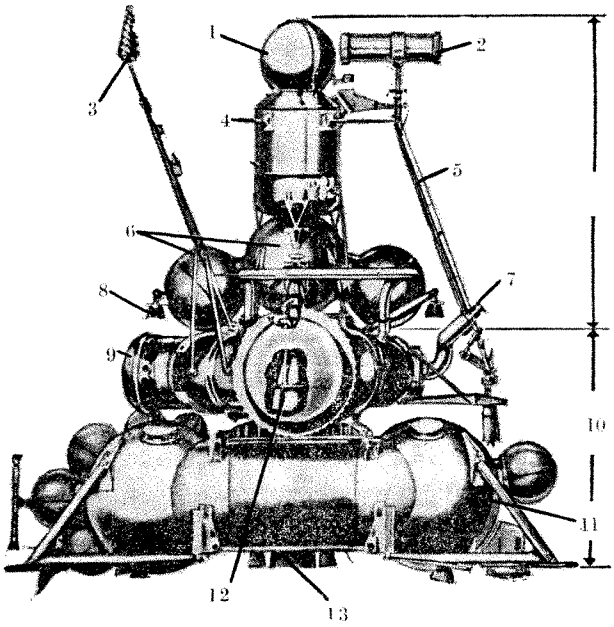
OLD WORLD

SPACE

Moves towards Cooperation

from a Correspondent

As the annual COSPAR symposium (in spring) gets duller and more specialized, the autumn congress of the International Astronautical Federation (of national rocket societies) becomes more lively. The most productive aspect of last week's meeting at Konstanz, Germany, was the discussion on the standardization of manned spacecraft docking, so that the craft of one nation could come to the rescue of that of another. For the foreseeable future only the United States and the Soviet Union are likely to have manned spacecraft in orbit, and so this amounts to a joint enterprise between the chief space rivals with far reaching implications.



The overall structure of Luna 16 (*Pravda*, No. 227, 1970). 1, Return craft; 2, drilling mechanism; 3, antenna; 4, instrument compartment of the rocket; 5, rod for drilling mechanism; 6, fuel tanks of the rocket; 7, telephotometer; 8, guidance nozzle; 9, instrument compartment of descent stage; 10, descent stage; 11, fuel tank; 12, rocket motor for journey from Moon to Earth; 13, descent stage motor.

The move has been endorsed by a letter from the Soviet Academy of Sciences to the American chairman of the Space Rescue Studies Committee. No Russian delegate attended the previous two meetings of the committee, but this year the leading Soviet space medicine expert Dr O. G. Gasenko took part, accompanied by one of the three Soviet cosmonauts present at the congress, Boris Yegorov. The academy letter undertook that there would be a picked Soviet delegation of four at the next full committee meeting, which will be held at the 1971 congress in Brussels. There is also widespread expectation that bilateral exchanges will be pursued during the visit of four Soviet cosmonauts to the Houston Manned Spacecraft Center in a fortnight's time.

Delegates to the congress also heard detailed accounts

of the rigors of the Apollo 13 mission, when three American astronauts survived by using one compartment of their vehicle as an emergency lifeboat, and of the eighteen day spaceflight by two Soviet cosmonauts in Soyuz 9 last May. Reporting on the Soyuz 9 flight, the ship's captain, Andrian Nikolayev, commented that month-long flights already seemed feasible, but anything longer called for additional and more effective conditioning procedures and new medical treatments. He reported that the post-flight effects lasted much longer than anything encountered on shorter flights. During the first few days both cosmonauts found walking a considerable strain, they were red in the face and they stumbled frequently. It was almost impossible to climb stairs and hard to stand upright. There had been considerable wasting of their lower limbs—as much as 4 cm round the hips and 2 cm in the measurement of the shins. This is attributed to the loss of muscle tissue in the legs, used to supporting the body's full weight at 1 G. Loss of total body weight was about 5 kg for the novice cosmonaut, Sevastianov, and rather less for Nikolayev, but both took about three weeks to recover the loss. This was in contrast to earlier post-flight experience, when lost weight was regained within a day or two. In spite of the weight loss both men felt two to three times heavier than usual for the first few days back on Earth.

In a report on the successful flight of Luna 16 (see illustration), which took place two weeks before the Congress, the deputy director of the Moscow Institute of Space Research, Mr Y. Hodarev, and Mr J. Surkov of the Geochemistry Institute of the Academy of Sciences said little more than the account released last week through the Novosti press agency. Privately, however, the experts revealed that Luna 16 had recovered 200 grams of lunar material, all contained in a single core 35 cm long and 4 cm in diameter. The Soviet members of the congress stressed the role of man in space with as much vigour as the role of automated instruments. There was no sign that NASA intends to revise or review its plans for the remaining four Apollo flights in the light of the new proof of what instruments alone can do.

SEWAGE

River at Risk

THE emphasis during the second week of the strike by British sewage workers shifted suddenly from the exaggerated concern over the danger to human health (*Nature*, **228**, 102; 1970) to justifiable anxiety about the threat to river life. Only two years after the lower reaches of the Thames had become so clean that more than forty species of fish were recorded there, a conservation specialist described the situation as "very serious, if not yet desperate".

Limited stretches of the Thames only have been affected so far, but it has been estimated that 10–20,000 fish may have been destroyed. It will take from three to five years for the rivers to recover completely from the damage caused by the sewage which has poured into them during the past two weeks. Lord Nugent, chairman of the Thames Conservancy Board, said last Sunday that if the strike was settled in a week, normal conditions could be substantially restored by early November. A spokesman for the board said later that as long as the sewage works remained in operation the situation would remain in hand; the staffing problem was getting worse each day, however, and if breakdown or closure of any of the sewage works resulted in further pollution of the river, the threat to river life would be grave.

Toxic substances apart, the presence of sewage in a river makes heavy demands on the amount of oxygen available to the inhabitants of the river. A heavy deposit of sewage on the river-bed requires a great deal of oxygen over a very long period for complete oxidation. Many fish are extremely sensitive to the amount of oxygen in the water in which they live, and cannot survive if the concentration falls below a particular value. Furthermore, the upper reaches of the Thames have, in recent years, become very clear with increasing sewage control, and in consequence the growth of algae and rooted water plants has been much encouraged. Such growth would be sadly cut back in the cloudy and turbid conditions which inevitably follow gross river pollution. Fish and plants will not be the only organisms affected. Invertebrates, such as water fleas and insect larvae, would perish, with disastrous consequences for the short and long term ecological balance of the river.

If the worst comes to the worst it will take a conservatively estimated ten years for the Thames to return to its former healthy state. It is unlikely, however, that the river could again become devoid of life, for fish, insects and plants will quickly return when pollution decreases to a tolerable level. But it would be a pity if the time and money spent during the past ten years in cleaning the Thames were to go to waste for the sake of an extra few thousand pounds on the municipal wage bill.

OCEANOGRAPHY

Mountains under the Mediterranean

from a Correspondent

FRESH information about the geological history of the Mediterranean Basin has been gleaned during the most recent stage of the National Science Foundation's Deep Sea Drilling Project (DSDP). On its voyage through the Mediterranean between August 13 and October 6—leg 13 of the DSDP—the Glomar Challenger collected twenty-seven corings from fourteen sites. Part of NSF's broader National Ocean Sediment Coring Programme, leg 13 was planned by an advisory panel of JOIDES, the Joint Oceanographic Institutions for Deep Earth Sampling.

According to Dr Kenneth J. Hsu, speaking in Paris on October 9, Glomar Challenger's scientific team recovered "long cores of sediments and rocks astonishingly similar to those found in many parts of the Alpine chains of Europe and North Africa". Using these cores it will be possible, for the first time, to compare the nature, age, thickness and sequential relationships of the material of the ocean with those of the surrounding land.

Signs were detected that in at least two places sedimentary formations have been displaced from their original sites of deposition beneath the floor of the Mediterranean. In one case, in the Hellenic Trough, 120 million year old Lower Cretaceous limestones were found immediately above young, soft Pliocene oozes not more than a few million years old. In another place,

north-west of the mouth of the Nile, a huge thickness of abyssal sediments (1–2 million years old), originating in the Nile River, was found upheaved on the submarine Mediterranean Ridge. "These circumstances represent the embryonic stage in a development process," Dr Hsu noted, "which when continued for millions of years will lead to the formation of mountains from the oceanic crust." It seems that Africa and Europe are pressing towards each other and squeezing the Mediterranean sea smaller and smaller, causing parts of the oceanic crust to rise and form mountains.

Glomar Challenger's scientists also found in the Atlantic Ocean samples of the igneous rocks called ophiolites, including gabbros and serpentinites formed at great depths beneath the sea's bed. A large thrust fault seems to have borne these high enough to come within reach of the ship's drill. Just above the ophiolites were Early Cretaceous and Late Jurassic pelagic oozes, the oldest yet to be recovered in the eastern North Atlantic and "strikingly similar" to some recovered 3,000 miles away during leg 11. Discovery of this old sediment, west of Portugal, confirms that the Iberian and African masses broke away from Europe, during one of the first phases of the broadening of the Atlantic, more than 130 million years ago.

Cores from the Mediterranean's three deep basins also yielded dolomites, gypsum anhydrite, diatomites and organic sapropels. According to Dr Hsu, the discovery of an abrupt transition, 5,000,000 years before the present, between evaporites and normal open marine pelagic oozes "marked an abrupt drowning of associated basins through the opening or connexion with the Atlantic Ocean". In the intervening several million years, life has "flourished with very high organic productivities", whereas the eastern Mediterranean's deeper sections were stagnant for several millenia at a time when the enormous ice sheets of the Pleistocene spread and receded. During the same period of about 2 million years "dozens of major volcanic explosions" seem to have occurred in these Mediterranean regions.

CONSERVATIVE PARTY

Mr Davies's Debut

THE Conservative Government's plans for matters technological have at last been spelt out in broad outline by Mr John Davies and others at the Conservative Party conference at Blackpool. In his first and possibly almost last principal speech as head of the Ministry of Technology in its present form, the word denationalization was rather obviously absent and Mr Davies spoke only of private capital "playing its part" in the development and management of already nationalized industries. This is a concept rather different from that feared by Mr Anthony Wedgwood Benn in his sombre warnings to the Labour Party conference the week before. Mr Davies's implication was that there should be no wholesale denationalization but that extra capital should be injected, possibly by the sale of nationalized industry bonds. Could this be symptomatic of a general easing of Conservative policy or is it merely a sop to the more progressive elements?

As far as state aid for private industry is concerned, the cry was very much in favour of help for industries which are temporarily weak, but against any further assistance for those which clearly had no chance of ever regaining strength. This seems to be an economically sane philosophy on the surface, but carries with it the risk of considerable unemployment. The probable fate of the Industrial Reorganization Corporation, which was partially responsible for the merger of British Leyland and BMC, will not please as many people as might be expected. The removal of such an interventionist body ought to be popular, but there is support both among Conservative MPs and throughout industry for the continuation of the corporation in a reduced capacity. Its rapid and complete demise, however, seems inevitable. Casualties among the little neddies—ancillaries of the National Economic Development Council responsible for particular industries—will probably be lighter, and decisions are likely to be taken for reasons that are definitely practical rather than doctrinal. Future plans for the regions were distinctly lacking at the conference although the Conservative Government claims to attach a fair amount of importance to them.

If other proposals for ministry mergers go ahead as planned, it would seem that Mr Peter Walker will eventually be overseer of environment and planning, because his Ministry of Housing and Local Government is likely to expand by swallowing up large parts of the Ministries of Transport and of Public Building and Works. Mr Walker showed good intent by announcing that more green belt would be designated during the lifetime of the present Parliament. Rumours are also flying that the Ministry of Aviation is to be revived under the general eye of Mr Davies.

There is time before the official announcements are made to Parliament for plans to be modified or shelved, but the overall picture must now be considered to be fairly accurate although the details are probably awaited with as much interest as the broad outline.

CERN ACCELERATOR

Two Months to Decide

THE conflicting arguments for and against the construction of a 300 GeV accelerator at CERN are likely to take a firmer shape in the next few weeks, now that the CERN council has officially asked the governments of the member countries to consider the fresh plans for the accelerator (see *Nature*, **226**, 301; 1970). At its meeting on October 8 the council approved the text of the plan prepared by Dr J. B. Adams, the director of the project, and asked the member governments to report back on the documents in time for the council to take a final decision on the project at its next meeting in December.

Three of the four pamphlets which the governments will be considering are concerned with the broad timetable and expenditure involved during the eight year construction programme. The fourth pamphlet is a report on the proposed site for the accelerator, which straddles the French and Swiss frontier near Geneva. One of the attractions of the revised scheme, produced earlier this year, is that the accelerator would latch on to the beam from the 28 GeV accelerator already operating at CERN. Personnel at the 300 GeV machine could also make use of the established support facilities at CERN, although one of the points raised at the recent meeting was whether the increase in the staff for the new accelerator—about a thousand new people are anticipated—could be linked to the building of a European University in the neighbourhood.

The result of borings and investigations of availability suggest that it will be possible to acquire an area large enough to house an accelerator of 2·2 km diameter. This is more than adequate for the revised plan, which envisaged a ring of 1·8 km diameter, but would preclude the option of reverting to the old 1964 design of accelerator if that were ever seriously reconsidered. Discussions are also in progress to arrange for cooling water to be transported from Lake Geneva to the CERN site and for the necessary facilities to draw the vast amount of electric power for the accelerator from the French and Swiss grids.

At the recent meeting the Italian and German delegations expressed concern that the cost of the accelerator should not exceed 1,150 million Swiss francs (£110 million), the amount foreseen in the Adams proposals. Of this about Sfr 250 million is expected to come from economies in the existing installations at CERN. The Italians also stressed the importance of Britain's participation in the new accelerator, which will cost about a third less than the 1964 proposals which the previous British Government found too expensive.

COMPUTERS

Market looks Ahead

IT has been said that the fourth generation of computers may be a long time coming, because the emphasis in the computer world has shifted from the design of bigger and better computers to the efficient use of those currently available or in the pipeline. To a certain extent this has been borne out by Computer 70, an international exhibition held last week at Olympia, London.

Acclaimed as the biggest computer show so far outside the United States, and certainly the most comprehensive in Europe, Computer 70 displayed a vast range of computer peripheral equipment, including countless alphanumeric display terminals and not a few complete computer mailing systems. British manufacturers do not on the whole seem to match up very well to the mainly American competition, and it is widely believed that there are more manufacturers of computer equipment in the northern suburbs of Los Angeles than in the whole of the United Kingdom.

Computers as such were not much in evidence at the exhibition, and the fact that the machines themselves are now taken very much for granted is probably the key to understanding the direction in which computing is heading. Traditional names in the peripheral market, such as IBM and ICL, have now been joined by hundreds more. For several firms, such as Scientific Data Systems Israel Ltd, Computer 70 is their first exhibition appearance. Together these manufacturers seem to cater for the organizational and decorative schemes of any imaginable office. A piece of computer equipment can certainly no longer be called laboratory apparatus, for it has become as basic as an office desk or a typewriter. These may be the first indications that the computer is coming out of the woods as far as society is concerned and is at last becoming almost respectable, in spite of the suspicions of a continuing hard core of opinion.

The general impression created by Computer 70 was underlined by Mr Alex d'Agapeyeff, when he delivered his presidential address to the British Computer Society at its annual dinner last week. Giving his views on the definition of computing and its role in society, he was at pains to make the point that this is no longer a branch of mathematics or physics as it was at one time, but of communications if, indeed, it is possible to classify it at all. He summed up his philosophy neatly by suggesting that computing should now be understood principally as the technology of computer use, with those who follow the profession doing so in a way very similar to the practice of lawyers or engineers.

LABOUR RELATIONS

Upholding Chemists' Rights

THE Royal Institute of Chemistry, one of the largest organizations of its kind, has shouldered the responsibility for negotiating directly with employers the wages and conditions of its 25,000 members. A statement of these plans, issued this week by the institute, comes at a time when graduate scientists are worried that they are being left behind by the progress made by militant trade unions.

The institute has made this change in policy because it is unconvinced of the suitability of trade union policy and tactics for the specific purpose of looking after the interests of professional chemists, particularly those in private industry. The institute claims to have the necessary background of information and experience which the unions lack, and to have better facilities for acquiring it and keeping it up to date. It believes that it should quickly develop the necessary competence to handle negotiations where a most careful appraisal, requiring a thorough understanding of the responsibilities involved, is essential. The institute is also convinced that its high standing in the eyes of the employers will be a valuable asset.

Among the steps that the institute intends to take will be to develop the already considerable records of prevailing salary levels, so that these can be used to provide evidence in actions taken by the institute. In order that individual members may be able to obtain advice locally, regional advisory panels will be established. These panels will recommend action by the institute if they think it is necessary. Arrangements for mediation and arbitration under the supervision of the institute will be made so that disputes between members and their employers can be resolved without expensive litigation.

In no circumstances will the institute call on its members to strike, for it believes that employer and employee should work together to reach agreement rather than act as two opposing factions. That is not to say that the institute considers membership of a trade union to be incompatible with membership of the institute. It is neither hostile to trade unions nor opposed to the principle of collective bargaining. Indeed, collaboration with trade unions and other associations may sometimes be desirable, although the institute says it would prefer to make its own representations on behalf of members. The statement also makes it clear that, in spite of the scheme for amalgamation between the institute and the Chemical Society, the institute will continue to act as a separate and wholly autonomous organization.

The example set by the Royal Institute of Chemistry may well be followed by the other science institutes;

and their greater influence will not merely be of benefit to members. The swing away from science in schools may be checked, and greater incentive provided for students embarking on long industrial training courses.

MEDICINE

Council for Postgraduate Training

THE concern with which both the government and the medical profession regard the training of medical students is reflected in the announcement last week by the Department of Health and Social Security of the establishment of a Council for Postgraduate Education in England and Wales. The new council, whose formation was recommended in the Todd report (Royal Commission on Medical Education, HMSO, 1968), will be chaired by Professor H. N. Robson, vice-chancellor of the University of Sheffield.

The Todd report, after describing postgraduate medical education in this country as "chaotic", laid the blame firmly on a lack of coordination between the universities, the medical profession and the National Health Service. Professor Robson sees the future of the new council as "a channel of communication between the various regional organizations, an advisory body whose decisions will be flexible, but whose efforts will be directed towards ending the present state of free for all in medical training which wastes time and causes needless frustration".

Organizations to be represented on the council include the British Medical Association, the NHS, the Committee of Vice-Chancellors and the various royal colleges of the medical profession. A recently qualified doctor who described his own postgraduate education as "rather disorganized" felt that the new council could do much to improve the situation both for trainee hospital doctors and intending general practitioners.

MEDICAL RESEARCH

New Building for Nuffield Group

THE Nuffield Institute for Medical Research has formally taken over its new laboratories on the University Medical Site, Headington, Oxford. The new building, which was officially opened on October 6 by Sir Geoffrey Gibbs, chairman of the Nuffield Foundation, cost almost £400,000. The building was financed jointly by the Nuffield Foundation and Lord Nuffield's Medical Benefaction to the university, and further funds have been made available from the University Grants Committee to buy furniture and research equipment.

The Nuffield Institute for Medical Research was established in 1935 and was originally housed in the old Radcliffe Observatory in Woodstock Road. The new building, which provides 25,000 square feet of laboratory and office accommodation, represents the first stage in the development scheme; the building is closely linked to the newborn nurseries and the Nuffield Department of Obstetrics in the new maternity hospital alongside, due to be opened next year. The second stage of the scheme should be completed in 1975 with the opening of a new teaching hospital and associated medical school buildings.

From its earliest days the institute has been predominantly concerned with the problems of the developing foetus; these activities will be expanded and developed under the present director, Dr C. S. Dawes, with the aid of a recently announced long term grant from the Medical Research Council.

AGRICULTURAL ENGINEERING

Automation and the Cows

ULTRASONICALLY guided tractors, shakers for harvesting fruit and computers in the milking parlour are some of the innovations on trial at the National Institute of Agricultural Engineering, near Bedford. These and a host of other developments are described in the institute's latest annual report (price £1). The futuristic nature of some of the projects in progress is explained by the director, Mr C. J. Moss, who points out that the work of the institute is not so much concerned with the problems of farming today as with those which will develop from the middle of the next decade onwards.

One of the big problems for farmers in the future is going to be loss of manpower, which puts a premium on the development of automatic methods. An ultrasonic echo ranging device has been used successfully to guide tractors along artificial furrows. The difficulty of using the same method on soil, however, has led to proposals to use optical methods for the same purpose.

Self propelled vibrating equipment has been built for shaking the stems of blackberry bushes. It successfully removed the fruit, and trials are to continue with similar shakers. Harvest by shaking also promises to increase productivity for growers of apples. The damage to fruit caused by shaking the trunks and branches has been found to depend to some extent on the shape of the tree. Both methods, however, were deemed to be promising, and trials have been extended to plum trees.

The liability to damage which such methods could bring may have inspired the testing of resistance to mechanical injury in fruit and vegetables. The relative susceptibilities of conference pears and Cox's orange pippin apples have been investigated by puncturing and compressing them when picked and during storage. The relative hardness of fruit picked early persisted through storage, with the maximum resistance to damage occurring about a week after picking. Compression tests on tomatoes indicated that they could be stored in piles up to five feet deep without damage by pressure. Most lettuce leaves are apparently very easily damaged by pressure from fingers, but the outermost leaves are so resilient that the plants can safely be thrown some distance into collecting bins.

The introduction of a computer to control operations in the milking parlour is intended to relieve the stockman of various tasks. A project has been set up in cooperation with the National Institute for Research in Dairying, and the first step will be to calculate and feed the appropriate ration to each cow as it is automatically identified in its stall. The end of milking will be indicated for each cow and the computer will record each animal's yield. It will then calculate the feed ration for the next milking. When the project has progressed this far, other factors affecting the cow's performance, such as weight, health, and prices of feed and milk, will be included in the calculations.

NEW WORLD

Perverse Reaction to Pornography Report

by our Washington Correspondent

NOTHING hurts like the truth, the President's Commissioners on Obscenity and Pornography must have been murmuring to each other last week as they read some of the smuttier attacks on their personal motives and competence occasioned by the publication of the commission's report. From dissenting colleagues to outraged Congressmen there were few who had a kind word for the commission's two years and $2 million worth of labour. "This outrageously permissive commission shows how far this nation has travelled down the road of moral decadence," said Senator Byrd of West Virginia. "The most clearcut evidence about the slipshod, sometimes questionable and sometimes unscientific procedures of the commission has come from a witness . . . (in whose opinion) the commission's report is so grossly inadequate that one cannot help suspect that it was written with a predetermined conclusion in mind," Senator Dodd of Connecticut insinuated. Perhaps the most outspoken attack so far has been made by Mr Charles H. Keating, the only member of the commission to be appointed by President Nixon, who seems not to have participated in many of the commission's meetings. His reasons are made clear in a 150-page dissenting report, which gives an unusual amount of space to the opinions of St Paul of Tarsus but also contains remarks such as "In the case of the Commission on Obscenity and Pornography, with men such as Jack Valenti and Abe Fortas in key advisory roles in the Johnson Administration, it was more likely than not that the orientation of the commission for permissiveness would be exactly the opposite to the orientation intended by Congress, namely, for moral discipline and responsibility." It is true that with the approaching congressional elections there is little advantage for public figures in praising the commission's politically controversial findings. Nevertheless, the way in which White House spokesmen repudiated in advance the reports of both the pornography commission and, to a lesser extent, of the recent Scranton commission on campus unrest, is a disturbing token of the Administration's respect for the rationale of disinterested inquiry on which such commissions are predicated.

What did the commission say that has so shocked respectable citizens? It called for a repeal of laws banning the sale of pornography to consenting adults, on the one hand, and tighter regulations against sale of sexual materials to young persons on the other. The principal reasons for the former recommendation were, first, that "empirical research designed to clarify the question has found no evidence to date that exposure to explicit sexual materials plays a significant part in the causation of delinquent or criminal behaviour among youth or adults". Second, that the commission believes it is "exceedingly unwise for government to attempt to legislate individual moral values and standards" and that there is no warrant for continued government interference with the full freedom of adults to "read, obtain or view whatever material they wish". Third, the commission found no consensus among the population at large that explicit sexual materials (the commission's sanitized word for "pornography") should be banned and, moreover, a sizable fraction of the population—87 per cent of adult men and 70 per cent of adult women—have been exposed at some time in their lives to such material, in most cases of their own accord.

These opinions are backed by a formidable quantity of empirical research which, when it is finally printed and given to the world, will occupy ten volumes of data. The bulk of the research consists of 39 studies ordered by the commission into themes ranging from "reactions to viewing films or erotically realistic heterosexual behaviour" to "the sociological and psychological attitudes, experiences and behaviour of the consumer population of the San Francisco marketplace for erotic literature and films".

The commission in its investigations has uncovered much curious information and has come out of its underworld burrowings with no lack of wisdom to offer—"There are no great fortunes to be made in stag film production" is one of the products of this experience. But the hard core of the commission's probings is the research projects designed by its effects panel under Dr W. Cody Wilson. From early on in its work the panel addressed itself to studying three specific problems, the relationship between pornography and crime, the comparative experiences with pornography of the normal population and of juvenile and adult sex offenders, and controlled experiments into the effect of pornography on sexual behaviour, attitudes and emotions.

On the first point, the connexion between pornography and sex crimes, the verdict is that analyses of crime rates in the United States "do not support the thesis of a causal connexion between the availability of erotica and sex crimes among either juveniles or adults", although because of the limitations of method the data cannot be said absolutely to disprove such a connexion. Much argument has taken place about whether the repeal of pornography laws in Denmark has been accompanied by a decrease in sex crimes; the commission's clear conclusion is that there has been a decrease, after all differences of reporting and legislation have been taken into account.

Turning to delinquency, the commission concludes first that most American youth is acquainted with erotic materials and, second, that there is no significant difference between the experiences of delinquents and non-delinquents. The experimental studies to determine the psychosexual effects of pornography were published long before the commission finalized its report (see *Nature*, **227**, 772; 1970). The main findings here are that people's established patterns of sexual

behaviour are very stable and are not altered substantially by exposure to pornographic material. "Straight" forms of pornography are more stimulating than the perverse varieties and "while males tend to judge erotic stimuli as more arousing than females (*sc.* than females do), the sexes do not differ substantially in physiological–sexual response to these stimuli"—meaning, presumably, that women and men are equally aroused by explicit sexual material but the former are less inclined to say so.

COOPERATION

Europe, DOD and the Shuttle

by our Astronomy Correspondent

In Washington as in Paris, views are crystallizing around the space tug concept as the possible European contribution to the next-but-one phase of NASA's manned spaceflight programme, the space shuttle project which NASA expects to be concentrating on once the Skylab programme is over. A European contribution of not more than $1,000 million is being talked of, which would still be enough to give Europe the ten to fifteen per cent share in the entire programme which, it is felt in Paris, would give Europe a say in the programme. About $6,000 million spread over six years is the anticipated United States expenditure if the shuttle is to be working by 1977 or 1978. This year $80 million has been requested for vehicle and engine definition and preliminary design, and $35 million for technology studies. In June, contracts were awarded to McDonnell Douglas and to North American Rockwell to produce preliminary designs for the vehicle, and to Aerojet General, Rocketdyne and Pratt and Whitney for designs of the main engines. Lockheed has a contract to study the impact of the shuttle on payload design and costs.

But an additional variable in the European negotiations revealed last week by Mr Dale D. Myers, associate administrator for manned space flight, is that the Department of Defense is also interested in taking part in the shuttle activities. A joint committee with representatives from NASA and the Air Force representing the Department of Defense has been meeting since last spring. The Air Force, of course, has always longed to consider manned space flight as a logical extension of its activities, until its hopes were finally dashed by the cancellation of the Manned Orbiting Laboratory last year. What the implications of the DOD's involvement are for European participation is not yet known. Would European payloads be allowed to sit in the cargo bay of the shuttle next to military equipment, for example, and would the DOD expect to have the use of the space tug? It is understood, however, that the Department of State does not see any problem in the DOD involvement in the shuttle, although the question was not mentioned during last month's talks with representatives of the European Space Conference. It seems to be generally assumed that the DOD would be free to use any part of the space transportation system.

NASA is not saying how large a European contribution they would like to see, but several ways in which Europe could participate have been drawn up by the previous administrator of NASA, Dr Thomas O. Paine, and Dr H. A. Kissinger, assistant to the President for national security affairs. For example, there could be a multinational agreement to build something like the space tug, in which case the project would be managed by the European space agency which is expected to arise phoenix-like out of the ashes of ESRO and ELDO. As an alternative, European companies could work as subcontractors for the main United States contractors, but without any transfer of United States dollars across the Atlantic so that the European companies would be supported by their governments. A third possibility is direct participation by European governments through their national laboratories. Dr W. von Braun has pointed out that the cyclic temperature facility developed at the Royal Aircraft Establishment for work on Concorde could be applied to the heating problems of the shuttle, for example.

ELDO has placed two study contracts on the space tug with groups of European companies, and according to NASA a phase A study—a preliminary analysis for feasible concepts—might be started in eighteen months or so. Like the lunar module in the Apollo programme,

Priorities in Space

by our Astronomy Correspondent

With disenchantment at NASA's future programme running high, more than usual interest surrounds the next report of the Space Science Board of the National Academy of Sciences. The report, which is unlikely to be published before the beginning of December, will review relative priorities and levels of support for space science activities for the next ten years. Areas covered will include lunar and planetary science, astronomy, and the life sciences. It is believed that the report now being drafted will give first priority to the High Energy Astronomy Observatory, an unmanned satellite now being considered by NASA for the mid-seventies which would be devoted to cosmic ray, X-ray and gamma ray observations. In the planetary sciences, the report will probably recommend that preference be given to in-depth studies of the planet Jupiter rather than to the two grand tour missions due to be launched in 1977 and 1979. As well as worries about developing the technology of the grand tour spacecraft, the feeling is that only scant information will be returned from the close approaches to the outer planets. The report will probably say that if a choice has to be made it would recommend more detailed studies of a single planet, almost certainly Jupiter which is coming to be regarded in much the same way as the Moon used to be as the Rosetta Stone for the solar system. It is true that to cancel the grand tour missions would be to let a rare chance arrangement of the planets go by, but the attitude of the Space Science Board would probably be that nuclear rockets that can make the journey without gravitational boosts will be available by the time knowledge of the outer planets becomes necessary. The report, "Priorities in Space", will be based on a three week conference at Woods Hole, Massachusetts, organized by the Space Science Board and with H. Friedman in the chair.

the space tug would only be able to operate in space, and it would be a general purpose ferry able to move from orbit to orbit, transfer material from the shuttle in near Earth orbit to a geostationary orbit, and possibly be equipped to land on the Moon. NASA is, of course, hard pressed for funds, but says that the European contribution is to be considered as an addition to the NASA budget rather than a substitute for part of it, although, of course, there is nothing wrong with sharing the cost of the space effort. Others have remarked that a helping of European participation will make the programme harder for Congress to cancel, but people with longer memories will say that foreign interest did not help Skybolt.

NASA continues its efforts to sell the shuttle and space station projects to the scientists, but it is problematical whether last month's conference at NASA's Ames Research Center had the desired effect. The kindest thing that some people can find to say is that the shuttle is a solution looking for a problem. Forty-five flights each year into near Earth orbits is taken as the starting point for the calculation of the economics of the programme, and on this basis the shuttle is expected to break even six or seven years after coming into service. Each shuttle will be used a hundred times. But it seems that this rate of almost one flight each week can only be justified if there is going to be a space station in the 1980s that will need regular servicing. NASA is at present working on the design of a twelve-man space station that would cost $6,000–$7,000 million for an operating life of ten years. Many people remain unconvinced that the scientific payoff from manned space stations and shuttles would be worth the effort, however, and in any case the cost of launching satellites is frequently not the chief expense.

ENERGY

Science and Power help Each Other

by our Washington Correspondent

THE Office of Science and Technology has cast itself as peacemaker and problem solver between the electric power companies and the environmentalists. This week Dr Edward David, the newly appointed President's science adviser and director of the Office of Science and Technology, released the report of a study group that aims to balance the conflicting needs of building more power plants and protecting the environment. The report's chief recommendations are that proposed sites for power plants should be announced by the utility companies at least five years in advance so as to allow public debate and approval by the relevant agencies. The pace of research in the electric power industry should be stepped up considerably in the fields of treatment of material and heat wastes, underground transmission and new forms of generation.

In his first press conference since succeeding Dr Lee Dubridge, Dr David chose to use the report as a platform for extolling the practical benefits of scientific research. "After two decades of technological advances in defence and space—many of which have not affected the everyday life of the general public directly—we are now calling upon scientists and engineers to improve the electric power system that serves our daily needs. . . . It is not too much to hope that the ingenuity and scholarship that goes with a fine research and development programme can in the future remove these issues from the need for public debate." Dr David added that "scientists all over the country have made clear their desire to work on these problems".

The President's science adviser could not have chosen a more topical issue with which to dramatize the contribution science can make to soothing the nation's domestic ills. It only took an unusually hot day last month to cause power reductions or "brown-outs" across much of the east coast. In the electric power interconnexion serving Pennsylvania, New Jersey and Maryland, seven generating units were out of action before the heat wave started and another three gave out in the course of the day, resulting in a 30 per cent reduction of the system's full operating capacity. Although unreliable equipment and shortage of suitable fuels are the chief bottlenecks in power supply at present, a shortage of generating capacity is looming large for the future. Demand for electric power is expected to triple or quadruple over the next twenty years, but environmentalists' protests are growing in measure.

In view of this impending crisis the OST study group finds it anomalous that the electric power industry collected operating revenues of $16,500 million in 1968 but invested only 0·23 per cent of this revenue in research and development. The OST report recommends a vigorous programme to iron out some of the snags in present methods of power generation and to develop new methods. A minimum of $250 million should be spent over the next seven years or so on methods of treating the various pollutants emitted by power plants, including sulphur dioxide, nitrogen oxides, tritium and other radioactive substances, and waste heat. Over the same period at least another $50 million should be devoted to research on underground transmission technology, the chief benefit of which would be to relieve the present narrow options of where to site power plants. The practicality of forced circulation or direct cooling of high power cables should be investigated along with the age old pipe dream of superconducting cable systems.

Turning to new forms of generation, the OST group would like its bets to be placed as follows. The liquid metal cooled fast breeder reactor, which received $125 million of research funds last year ($100 million of it from federal sources), should be pushed to the tune of $150 million annually over the next 15 to 20 years. It is worth investing a total sum of $500 million over 15 years on research into magnetohydrodynamics, of which the most promising application for power generation at present uses hot gas from combustion of fossil fuel. Combined gas turbine–steam turbine cycles merit a total research investment of $100 million over the next 5 to 10 years; fuel cells, because of their high efficiencies in small units, merit a similar sum over a rather longer period; and the hopes of controlled thermonuclear fusion should be given substance with expenditures rising to $200 million annually over the next 20 years. Finally, $100,000 a year should be invested in studying means of harnessing solar energy.

The OST report recommends that the National Academy of Sciences and the National Academy of Engineering should set up panels to study the long term effects of power plant emissions, both conventional and radioactive, on the atmosphere and ecosystems.

ENVIRONMENT

Oceans Off Limits to Dumpers

by our Washington Correspondent

THE United States should take the lead in prohibiting or severely restricting the use of the oceans as a dumping ground, says a report of the recently constituted Council on Environmental Quality*. The recommendation has been endorsed by President Nixon in a message to Congress in which he promises to introduce legislation implementing the council's report.

The council has found that ocean dumping does not present much of a problem at present but is likely to become rapidly more serious in the future if not checked. To the extent that this is true, there should be little opposition to the council's recommendations and much praise of its sagacity in forestalling another area of pollution. In 1968 about 48 million tons of waste was dumped at distances between 4 and 125 miles off the coasts of the United States, 80 per cent of which was dredge spoils, 10 per cent industrial wastes, 9 per cent sewage sludge and the remainder construction debris, solid waste and explosives. The council recommends that all forms of ocean dumping should be prohibited or phased out as soon as possible with the exception of certain radioactive wastes when there is no alternative form of disposal offering less harm to man or the environment. These recommendations also apply to dumping in the estuaries and Great Lakes.

Dredge spoils represent a hazard when taken from areas contaminated with the sediments of industrial wastes; about a third of the 38 million of tons of dredgings dumped off US coasts in 1968 were polluted, often with toxic heavy metals such as cadmium, chromium, lead and nickel. Industrial wastes dumped directly in the sea include acids, and the wastes from refineries, pesticide plants and paper mills. The ocean has long been used as a graveyard for obsolete munitions and, since 1963, the navy under Operation Chase has sent to the bottom nineteen liberty ships, four of them loaded with chemical warfare agents and their carriers, and the rest with explosives. Detonation of the explosives, the report says, introduces trace amounts of lead, nickel and bronze into the water.

Little radioactive material is now dumped at sea because in 1960 the Atomic Energy Commission placed a moratorium on new licences for this kind of disposal, and since 1962 the AEC's major contractors have not made use of ocean dumping.

Excluding dredge spoils and explosives, the council reckons that about 2 million tons of wastes were dumped each year in the early 1950s, increasing to 6 million tons at the beginning of the 1960s and 10 million in 1968.

The council's report recommends that having put its own house in order, the United States should take the initiative towards achieving international co-operation on ocean dumping. The government should develop proposals to this end for presentation before the United Nations Conference on the Human Environment to be held in Stockholm in 1972.

Until such time as all dumping is phased out, people who wish to discard their trash in the ocean should be required to seek a permit from the Administrator of the Environmental Protection Agency (EPA), the report says. The agency, along with the National Oceanic and Atmospheric Administration (NOAA), came into existence early this month. The administrator has not yet been appointed, but one among the several names that have been mooted is that of Mr Russell Train, the chairman of the Council on Environmental Quality. His appointment to the post would neatly resolve—or irreversibly confuse—the possible overlap in the roles of the council and the agency. The reorganization plan establishing the EPA says that the duties to be transferred to it include "so much of the functions of the Council on Environmental Quality . . . as pertains to ecological systems". In the act establishing the council these functions are defined as "to conduct investigations, studies, surveys, research and analyses relating to ecological systems and environmental quality".

* *Ocean Dumping, A National Policy* (US Government Printing Office, 55c).

DEMOGRAPHY

1984 the Era of Young Marrieds

by our Washington Correspondent

AT least one of the world's population bombs has been deftly defused and rendered innocuous. In a speech given in New York last week, Dr George H. Brown, director of the Bureau of the Census, gave his own estimates of the state of the nation 15 years ahead. Choosing George Orwell's "1984" as a point of departure—a date Orwell is said to have appointed by no more serious a method than permuting the last two digits of the year in which he was writing—Dr Brown sketched out an apocalypse strangely similar to the present. The basis of this prediction is the prospect of a stable population in the light of the bureau's downward revision of its estimates of population growth.

Until last year, the bureau was predicting an increase of from 80 to 160 million people by the year 2000. But in the face of fertility rates that have steadily dipped from 3·35 children per woman in the 1950s to 2·45 in the late 1960s, the bureau is now only betting on an increase of 35 to 45 million people over the present level of 204 million by 1984, Dr Brown said. "However, fertility rates could well drop below current levels in view of the current concern with the effect of population growth on the environment, and possible changes in the laws on abortion." Population growth rate will inevitably rise for the next decade because the proportion of women in childbearing ages will increase. "On the basis of present information, however, the population growth rate could well return to its present levels in the eighties or, perhaps, slip below it."

The age structure of the population predicted for 1984 differs from that of the present since a third of the expected increase will occur in the 25 to 34 year age group. There will be virtually no change in the number of people between 45 and 64. "The most significant fact about the expected change in age composition is the very sharp increase in the number of persons in their 20s and early 30s. The meaning is clear: the next 15 years is the era of the young married."

Such an age structure could mean a shortage of experienced older men for positions of leadership in government, industrial management and politics. There could be pressure on some older men to postpone retirement.

NEWS AND VIEWS

Another One for the Book

PUNTERS betting on the molecular biology handicap, where scientific reputations are at stake, have taken a trouncing in the past few months. First the central dogma, or, to be more precise, the popular misconception of the central dogma, was shown to be an oversimplification. Now another firm favourite has been shaken. On page 227 of this issue of *Nature*, Chamberlin and his colleagues report, against all the betting, that when the bacteriophage T7 infects *E. coli*, the phage genome specifies a complete RNA polymerase molecule, rather than a sigma factor with the capacity to subvert the pre-existing RNA polymerase of the bacterium to the transcription of the phage genome.

In *E. coli*, RNA polymerase comprises the so-called core enzyme, which catalyses the formation of phosphodiester bonds between successive nucleotides of an RNA molecule, and a sigma factor, which programmes the core enzyme to transcribe only certain genes. The sigma factor transiently associates with the core and the complex is able to recognize the initiation sequences of certain genes. Once the initiation of transcription has been achieved, the sigma factor dissociates and is free to programme another core molecule, while the core, once it has completed the round of transcription, is free to associate with a different sigma factor. This mechanism seems to be an economical way of providing RNA polymerase with specificity, and, assuming a battery of different sigma factors and a set of core molecules, it is easy enough to devise schemes to account for the regulation of gene expression.

As soon as the first sigma factor of *E. coli* had been discovered, there was speculation that bacteriophages might bring about the transcription of the phage genome, at the expense of the transcription of the bacterial genome, by specifying one or more species of sigma factors which are able to initiate the transcription of phage DNA but not bacterial DNA. This scheme has two prerequisites: first the phage sigma factor must be able to displace host sigma factors and, second, the phage gene which specifies the first species of phage sigma factor must itself be recognized by the core enzyme complexed with a bacterial sigma factor.

Chiefly as a result of the work of Travers and his collaborators and Bautz and his colleagues, we know that the transcription of phage T4 genomes in *E. coli* follows this pattern. One of the first products of transcription and translation of the phage genome is a phage sigma factor which then allows the transcription of other phage genes. Furthermore, in the later stages of the infection, a second phage protein, which may well be another sigma factor, is made and this permits the expression of the late genes of the phage. With the precedent of T4 in mind it was not surprising that Summers and Siegel (*Nature*, **223**, 1111; 1969) assumed they had discovered yet another sigma factor when they found that the protein specified by gene 1 of phage T7 is made early in infection and seems to be required for the transcription of the T7 genome. What made this interpretation particularly seducing was the finding that *in vitro* the gene 1 protein transcribed the DNA of phage T7 to a much greater extent in the presence of *E. coli* RNA polymerase than in its absence.

But there were two flies in this balm: Summers and Siegel found that, *in vitro* at least, the product of gene 1 of T7 phage was unable to displace host sigma factor from core, and, more importantly, T7 infections were found to be insensitive to rifamycin, a drug which inhibits *E. coli* RNA polymerase. At that time the first of these findings seemed unimportant because, although the putative T7 sigma factor failed to displace host sigma factor, the product of gene 1 seemed to bind to core enzyme of *E. coli*. The second observation raised a more awkward question: how do T7 infections proceed normally in the presence of rifamycin if the core enzyme of *E. coli*, which is sensitive to this drug, is involved in the transcription of the phage genome? Summers glossed over this objection by arguing that because T7 messenger RNAs are unusually stable, and assuming that the whole of the T7 genome is transcribed very soon after infection, the presence of rifamycin rapidly becomes immaterial.

Clearly Chamberlin, with perspicacity, was unconvinced, believing that the failure of rifamycin to block T7 infections was highly significant. He and his colleagues isolated the product of gene 1 of T7 and found that it was a complete RNA polymerase capable of transcribing the T7 genome, and physically and biochemically quite distinct from the host enzyme. Furthermore, the T7 enzyme is resistant to rifamycin. With hindsight it is easy to see why Summers and Siegel, along with most people including some of the mandarins, were led astray, especially at a time when sigma factors were being invoked to explain every and any example of controlled gene expression. It was their misfortune to be dealing with a protein which has one particularly unusual property. T7 RNA polymerase loses its activity in dilute solutions unless it is stabilized by some other protein. This stabilization, as Chamberlin *et al.* have shown, is not specific; bovine serum albumin, for example, does the job perfectly well, and Summers and Siegel's experiments prove that *E. coli* RNA polymerase is also effective. When they found that the product of gene 1 of T7 transcribes T7 DNA *in vitro* in the presence but not in the absence of *E. coli* RNA polymerase, they were detecting this stabilization phenomenon and not, as they suggested, the association of a T7 sigma factor with the core enzyme of *E. coli*.

If the transcription of the genomes of T7 and T4 is so different, speculations about the role of sigma factors in eukaryotic cells will have to be taken with packets, not pinches, of salt.

Solutions Not Yet Polarized

THEORIES to explain polarity in bacterial operons, which is the topic of a contribution from Imamoto this week (page 232), have abounded since the phenomenon was first described in 1963. "Polarity" describes the observation that a nonsense mutation in one gene of a bacterial operon may not only inactivate the protein which that gene specifies, but may also reduce the amounts synthesized of the (normal) proteins coded by the subsequent genes in the operon. There is a gradient of polarity along the gene because the magnitude of the polar effect depends on the position of the mutation: the greater its distance from the end of the gene, the greater the reduction in synthesis of the subsequent proteins. Because polarity is caused only by nonsense codons—the signals for termination of protein synthesis—the most obvious explanation is that the premature termination of protein synthesis in one gene can in some way affect the ability of ribosomes to translate the messenger RNA corresponding to subsequent genes. "In some way" has led to complicated theories to explain how ribosomes translate polycistronic messengers, and although there is not yet any complete explanation, many polar effects can be explained at the level of translation.

But a bizarre effect which is more difficult to explain is how polar mutants interfere with messenger RNA synthesis. Strongly polar mutants of the tryptophan operon of *E. coli* (which has been extensively used to study polarity because of the development of a hybridization assay to measure its mRNA) contain *trp* messengers which are shorter than usual; these seem to contain only the RNA sequences corresponding to the region of the operon before the mutant site.

This is difficult to explain, because it implies that nonsense codons can affect transcription as well as translation. But two types of theory have been proposed to account for this. Morse and Yanofsky (*Nature*, **224**, 329; 1969) have suggested that the RNA corresponding to the more distant regions of the operon is made, but is not usually detected because it is very unstable. They supported this contention by demonstrating that very short-lived *trp* mRNAs could be detected by hybridization only by using very short pulse doses of radioactivity. They suggested that its rapid degradation might be caused by the dissociation of ribosomes from the messenger at the nonsense mutation, which would leave the far regions of the messenger unprotected against nucleases. The gradient of polarity could be accounted for if ribosomes were able to reattach at the next cistron after the mutation; the probability of degradation would then depend on the distance unprotected between mutation and the end of the cistron.

An alternative idea is that transcription as well as translation come to an untimely end at the nonsense codon, and Imamoto presents evidence to support this concept (page 232). He too observes that polar mutants contain rapidly degraded *trp* mRNA, but differs from Morse and Yanofsky in finding that it represents the early part of the operon before the mutant site; mRNA for the genes beyond the mutation does not seem to be synthesized at all.

If transcription does stop at or near the nonsense mutation, the RNA synthesized so far could be released from the DNA prematurely, and might therefore become available to nucleolytic attack. But why should transcription stop at a nonsense codon? Imamoto suggests that RNA polymerase continues past the mutant site, but because the RNA which it synthesizes is not translated, enzyme and product may become entangled, jamming the polymerase in position on the DNA. This would prevent passage of any other polymerase molecules, and stop transcription. The probability of this interaction taking place would depend on the length of messenger left before the next cistron where it could be reloaded with ribosomes; this accounts for the gradient of polarity.

There is no obvious way to reconcile the different results obtained by Morse and Yanofsky and by Imamoto—they have, after all, been working with the same system. One possible way out is that they use different methods to de-repress the tryptophan operon; another is that there are slight differences in their hybridization assays and the ways in which the results are worked out. But for the while, at any rate, you reads your paper and you picks your theory.

ENZYMES

Dehydrogenases Disclosed

from our Molecular Biology Correspondent

THE dehydrogenases are a ubiquitous family of enzymes which have in common their association with NAD or NADP as co-factors, and have provided a secure livelihood for countless biochemists over the years. More recently, as a consequence of some spectacular progress in their enzymology, sequences and crystallographic structure, they have formed a major growth point. In structural terms most is now known about glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH). Harris and his colleagues have determined the sequences of GAPDH from pig and from lobster muscle—no small task, for they contain 332 and 333 residues per chain, there being four identical chains in each native enzyme.

Now Jörnvall (*Europ. J. Biochem.*, **16**, 25; 1970) reports the sequence of the even longer chain of a horse liver alcohol dehydrogenase (LADH), which has 374 residues. The result establishes that the enzyme, which is a dimer, has identical subunits. There is perceptible homology with the GAPDH sequence only towards the N-terminal end of the chain. The essential, highly reactive, cysteine is residue 46, whereas in GAPDH it is at 149 (where indeed it also is in LDH). Positions 149 in LDH and 46 in GAPDH are both occupied by tyrosines, and it is an interesting speculation, if per-

haps a long one, that these residues are interchangeably juxtaposed at the active site. The histidines are mostly in the N-terminal region, and Jörnvall suggests that it may in fact house most of the enzymatically important elements. Some support for this notion comes from his second paper (*ibid.*, 41): LADH is present in the liver as a set of three isozymes, made up from two types of subunit, E and S. The sequenced species is EE. Examination of tryptic fingerprints of the SS form reveals only six differences, five of which occur in the N-terminal half of the sequence. The isozymes show differences in specificity, and the results suggest, if they do not prove, that some at least of these residues participate in the catalytic function. The suggestion of active site residues predominantly in the N-terminal portion is not at all inconsistent with the situation in LDH, as revealed by the X-ray work of Rossmann's group. The crystallography of LADH is progressing in Sweden, and a comparison with LDH should be highly instructive.

The reactive thiol group is evidently not a universal feature of the dehydrogenases. Malate dehydrogenase, for example, is not inactivated by thiol reagents until nearly all the many cysteines have reacted. Anderton (*ibid.*, 562) has shown that reaction with iodoacetamide at a level of two moles per mole of dimeric enzyme does cause inactivation, but its site of action is a histidine. Anderton and Rabin (*ibid.*, 568) find, further, that the reaction is strongly inhibited by NADH, less so by NAD, and strongly by NAD and the inhibitor, oxaloacetate, together. This indicates the formation of an abortive ternary complex, a phenomenon also observed in other dehydrogenases.

Cooperative ligand binding by dehydrogenases is a far from neglected topic. Some of the enzymes show marked cooperativity, others do not, and yeast GAPDH is interesting in that it can behave in both ways, according to the temperature. Its NAD-binding profiles, and the kinetics of the binding process could, it was concluded, be described in terms of a two-state, all-or-none binding scheme, and not by a sequential model. Last words, however, are rarely uttered in this area; for there is, it sometimes seems, a terrible purgatory for the protagonists, who are forced to wander for all eternity through the labyrinths of positive and negative cooperativity. Cook and Koshland (*Biochemistry*, **9**, 3337; 1970), using four parameters to fit the saturation curves, assert that the best fit is to be found in a mixture of positive and negative cooperativity, that is to say, the binding at one site may promote or hinder that at another. The first binding constant is small, the second larger, the third larger yet, but the fourth is small. They base the necessity for such a scheme principally on a break in the Hill plot—though the negative interaction is not clearly reflected in the magnitude of the slope after the break. Readers must judge for themselves whether the better fit to the observed points is worth the price of two extra parameters.

CELL COMPONENTS

Sequences and Particles

from our Nucleic Acids Correspondent

THE suggestion that uncharged tRNAs have tertiary structures different from those of tRNAs charged with amino-acids has been pursued by Danchin and Grunberg-Manago (*FEBS Letters*, **9**, 327; 1970) working on oligo-C binding by tRNA. Oligonucleotides are known to bind complementary sequences not already involved in base pairing, and Danchin and Grunberg-Manago have examined whether there are differences in the availability of such sequences (in this case, runs of G) between charged and uncharged tRNAs, by comparing their binding constants with oligo-C. These were determined rapidly because labelled oligomers bound to the tRNA (at physiological *p*H) can then be co-precipitated with the tRNA and retained on Millipore filters, while unbound material is washed through. The validity of the method was checked by equilibrium dialysis. In the cases of mixed *E. coli* tRNA, pure *E. coli* $tRNA^{val}$ and pure yeast $tRNA^{phe}$, the binding constant of the charged tRNA for $(Cp)_4$ was five to ten times greater than that of the uncharged tRNA. The magnitude of the binding constant was similar to that calculated by other workers for two adjacent GC pairs. They therefore suggest that a sequence of two G residues is exposed when the tRNAs are charged. This sequence is normally unavailable when the tRNAs are uncharged. Because the mixed tRNA behaved in the same way as the purified species, the sequence involved must be common to most tRNAs, and Danchin and Grunberg-Manago speculate that the two G residues in the dihydroU loop are responsible.

In the past few years, several models of ribosomal subunit structure have been proposed, in most cases suggesting that much of the ribosomal RNA is on the surface of the sub-particles. These are now increasingly disbelieved, and a further jolt to the wire and golf-balls has been administered by Serdyuk, Smirnov, Ptitsyn and Federov (*FEBS Letters*, **9**, 324; 1970), who have studied the internal density distribution of 50S ribosomal sub-particles from *E. coli*. They estimated the radius of gyration of the particle by means of small angle X-ray scattering, and also measured its diffusion coefficient. For spherical particles of uniform density, there is a simple relation between the radius of gyration and the diffusion coefficient. But the diffusion coefficient measured in this case leads to a value for the radius of gyration 20 per cent higher than that observed. Serdyuk *et al.* exclude the possibility that this arises from imperfections in the sphere, such as hollows on the surface, after work with ebonite models. The particles must therefore have a non-uniform density, with a core denser than the peripheral areas. They suggest that this could arise if the RNA component was, on average, nearer the centre of the particle than the protein.

Further information about the fine structure of the ribosome will certainly be obtained with the electrophoretic techniques being used to fractionate ribosomal proteins by Kaltschmidt and Wittmann (*Anal. Biochem.*, **36**, 401; 1970). They have described their method of two dimensional electrophoresis through polyacrylamide gel, which permits the fractionation of as many as fifty ribosomal proteins. The electrophoresis is carried out first in a tube of gel (8 per cent, *p*H 9·6) and then in a slab (18 per cent, *p*H 4·6). The method can be applied to the proteins of the 70S ribosome of *E. coli*, and also to the 30S and 50S subunit proteins. If the fingerprints of the subunit proteins are superimposed, a picture identical to the 70S protein fingerprint is happily obtained. The versatility of the method is also demonstrated by fingerprinting ribosomal

proteins from the strains B and K12 of *E. coli*. In confirmation of previous results, no differences are detected between the proteins from the 50S subunits, but two changes are evident between the 30S subunit proteins.

LACTOSE OPERON

Release of Repression

from a Correspondent

PEOPLE who thought that they knew everything about the lactose operon of *E. coli* must stand confounded by reports that its transcription is controlled in two ways. According to traditional wisdom, a repressor protein binds to the operator, preventing RNA polymerase (which binds at the preceding promotor site) from proceeding into the operon to synthesize messenger RNA. The operon is switched on when this repressor is released from the DNA by β-galactoside inducer (the substrate for the enzymes coded by the operon).

But releasing the repression, although necessary, is not sufficient for full expression of the operon. This requires another control system, which was discovered by studying the effect of glucose in depressing the synthesis of many inducible enzymes in bacteria. Glucose has this effect because it reduces the amount of cyclic AMP in the cell. Reasoning that this nucleotide is unlikely to interact directly with the components of the operon, Zubay, Schwartz and Beckwith sought a protein factor which might mediate its effect.

They report (*Proc. US Nat. Acad. Sci.*, **66**, 104; 1970) the isolation of a mutant unable to grow on lactose or arabinose—a characteristic of a mutant lacking the protein factor, because both the lactose and arabinose operons are susceptible to the glucose effect. Using a DNA-dependent β-galactosidase synthesizing system, they could not synthesize this product of the lactose operon *in vitro* when the supernatant components were derived from the mutants, but they could do so with the corresponding extracts from wild type cells. They have purified an activity that seems to reside in a protein of molecular weight 45,000.

Starting from a biochemical instead of a genetic point of view, Pastan and his colleagues sought a protein that could bind cyclic AMP. They, too, found a protein of molecular weight about 40,000 (*Proc. US Nat. Acad. Sci.*, **66**, 480; 1970). Mutants unable to respond to cyclic AMP had a protein with an altered affinity for the nucleotide. The only significant difference between the two groups apart from the name ascribed to the protein (CAP to Zubay *et al.*, CR to Pastan *et al.*) is a two order magnitude difference in the binding constant for cyclic AMP. Although cyclic GMP competes with cyclic AMP for the protein, no other nucleotide binds.

The cyclic AMP binding protein probably acts at the promotor, for mutations at this site affect the response of the lactose to cyclic AMP (*Proc. US Nat. Acad. Sci.*, **66**, 773; 1970), and this genetic analysis is nicely complemented by the finding that the amount of messenger RNA synthesized by the lactose operon correlates with the level of β-galactosidase produced in response to cyclic AMP (*J. Biol. Chem.*, **245**, 2259; 1970). This implies that, as a result of its interaction with cyclic AMP, the binding protein helps the initiation of transcription at the promotor of the lactose and other operons.

Surprises are also in store so far as the interaction of the repressor and operator itself is concerned. Riggs *et al.* (*J. Mol. Biol.*, **48**, 67; and **51**, 303; 1970) have shown that nitrocellulose in the form of membrane filters may be as powerful a tool as nitrocellulose in the form of centrifuge tubes. They have taken advantage of the way in which repressor protein sticks to these filters but double stranded DNA does not. This means that only the DNA that interacts with the protein is trapped on the filter.

DNA trapped on the filter can be released by addition of IPTG (an inducer of the lactose operon). The rate of dissociation of the complex can be measured by following the disappearance of radioactive DNA counts from the filter when unlabelled DNA is added after a radioactive complex has been formed. An unexpected result was obtained by changing the concentration of IPTG; the inducer increased greatly the rate of dissociation of the complex. Another small molecule, nitrophenyl-fucoside, which opposes the effect of IPTG *in vivo*, decreased the rate of dissociation of the complex.

One of the popular models for the action of the repressor is ruled out by the finding that these small molecules directly affect the release of repressor from the operator. This is the conventional allosteric model which suggests that there is a rapid equilibrium between free and bound repressor, and inducers (or anti-inducers) bind only to the free repressor, thus blocking its DNA binding site. The scheme would predict that the rate of dissociation of repressor from DNA would not be affected by inducer concentration. But the inducer seems to pry the repressor off the DNA, probably by binding to an independent site on the repressor. Another surprise revealed by this technique is that glucose, hitherto thought to exert its effect on repression only by altering the amount of cyclic AMP, seems to interact directly with the repressor as an anti-inducer. But the significance of this effect *in vivo* may be limited, for it takes place *in vitro* only at unphysiologically high levels of glucose.

TRANSCRIPTION

Approaching the *in Vivo* System

from our Nucleic Acids Correspondent

RECENTLY, molecular biologists have come to realize that the instant transcription or replication of DNA offered by the use of purified enzymes *in vitro* may differ significantly from the way in which these processes take place within the cell. The present fashion is to regard these activities as catalysed by cell organelles rather than individual enzymes, and accordingly, two articles in the current issue of the *Journal of Molecular Biology* report the isolation of structural complexes from the cell which contain all the components necessary for transcription or replication.

Pettijohn *et al.* (*J. Mol. Biol.*, **52**, 281; 1970) have established an experimental system for studying the transcription of ribosomal RNA cistrons *in vitro* from *E. coli* or *Bacillus megaterium*, which must at least approximate to the real situation. *In vivo*, the rate of transcription of the ribosomal RNA cistrons is as much as 100 times greater than mRNA copying in bacteria. Pettijohn *et al.* have been able to purify a DNA-protein

complex *in vitro*, which contains RNA polymerase as the principal protein attached to the DNA. When radioactive nucleoside triphosphates were added to the complex, synthesis of RNA was observed. This radioactive RNA had the high G : U ratio characteristic of ribosomal RNA, and hybridized efficiently to bulk DNA when the input level of RNA was low. Unlabelled 16S and 23S rRNAs competed efficiently with the synthesized material in hybridization. The unsurprising conclusion was that this DNA-protein complex was engaged chiefly in rRNA synthesis. Pettijohn *et al.* also referred to unpublished results showing that the RNAs released in the course of the synthesis had sedimentation coefficients of 23S and 17S. From the amount of rRNA synthesized in the time the reaction continued, they calculate that as many as 100 RNA polymerase molecules are attached to each 23S RNA cistron. This is close to the packing limit. Although these findings explain the very high rate of rRNA synthesis, the central problem is still the cause of the high rate of initiation of transcription of tRNA cistrons, by comparison with mRNAs such as the tryptophan operon.

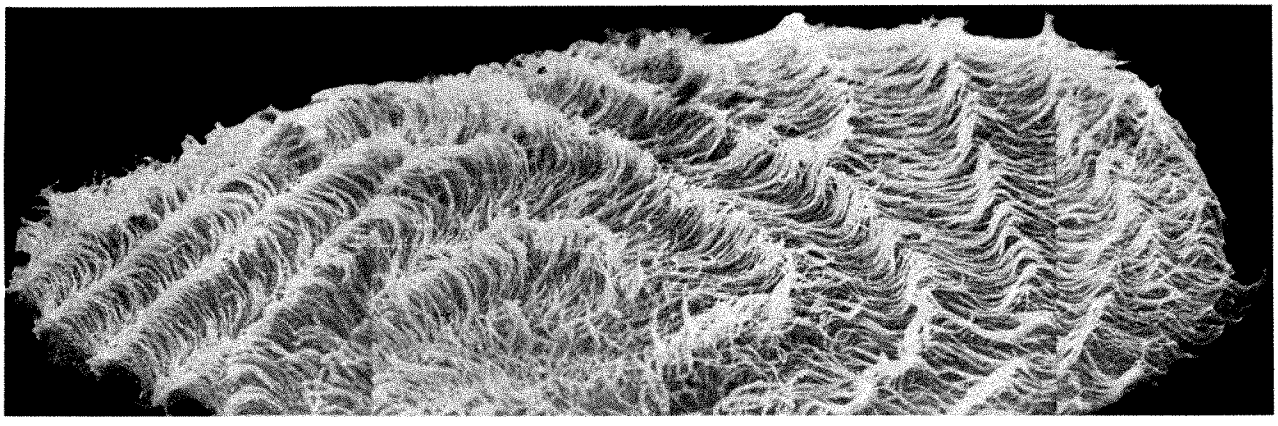
Fuchs and Hanawalt (*J. Mol. Biol.*, **52**, 301; 1970) have described the isolation of a DNA-protein complex from *E. coli*. They believe that this is the growing point, or fork, which arises in the semi-conservative replication of DNA, where the two strands of the chromosomal DNA are parted, enabling the complementary strand to be synthesized on each of the original strands. Their secret lay in lysing the bacterial cells with a non-ionic detergent which did not dissociate the DNA-protein complex. The newly synthesized DNA in the neighbourhood of the growing points was specifically labelled with brief (10–30 s) pulses of radioactive thymine or thymidine immediately before lysis. The lysates were sonicated, and the labelled DNA-protein complex could be purified from the bulk DNA about 100-fold by successive sucrose gradient centrifugation.

They investigated the complex by treating it with various enzymes, among other things. Both micrococcal nuclease and pronase caused rapid dissolution of the complex, as expected. Phospholipase, however, had no effect on stability. This is interesting, for it is commonly thought that the growing point is attached to the membrane of the bacterial cell. Nevertheless, it is possible that the growing point regions isolated by Fuchs and Hanawalt were detached from the membranes during lysis, while still retaining some structural integrity.

Perhaps their most significant finding was the negative result that there is virtually no DNA polymerase activity in the complex proteins, as judged by a standard assay for this enzyme. Furthermore, a mutant of *E. coli* described by De Lucia and Cairns which lacks active DNA polymerase nevertheless yields the same amount of the labelled complex as the wild type. It is difficult to avoid the conclusion already suggested by the viability of such mutants, that the DNA polymerase known and loved is not the enzyme of replication.

PROTOZOA

Waves Caught by the Camera



This is not a bath mat, but a composite electron micrograph of the protozoan *Opalina*, which has been fixed instantaneously and dried by the critical point method. The preparation of S. Tamm and G. Horridge (*Proc. Roy. Soc.*, **175**, 219; 1970) has preserved waves of movement passing through the cilia (×3,000).

FIBRES

Spinning a Yarn

from a Correspondent

DEVELOPMENTS in the fibre and textile industry seem to be coming thick and fast. New flame resistant textiles, fibres for reinforcing composites, and fibres from films were some of the topics for discussion at an international seminar, organized by the Shirley Institute in Manchester from September 29 to October 1.

Contributions concerned with new developments emphasized two approaches: the modification of existing fibres and the development of new fibres. Cotton and wool, two of the oldest fibres, can be modified to such an extent now that in some of their finished forms they must be regarded as "new" textiles. Dr H. R. Hardy (Courtaulds Ltd) described how viscose rayon, one of the oldest man-made fibres, has been developed into a range of new products. Among the most important of

these viscoses are the new "high wet modulus" fibres, which have much improved mechanical properties and dimensional stability, especially when wet.

Dr J. W. S. Hearle (University of Manchester Institute of Science and Technology) summarized the diverse views held on the structure of fibres, and Dr J. D. Owen (Shirley Institute) showed that the "handle" and "feel" of fabrics are at last beginning to be understood in terms of measurable physical properties. A good example of the application of basic research to fibre development was provided by Dr C. Mazzolini (Chatillon), describing the development and properties of a new syndiotactic PVC fibre. This fibre, in which the chlorine atoms are arranged symmetrically with respect to the polymer chain, is much more resistant to heat and dry cleaning solvents than the ordinary atactic PVC fibre, and is thus suitable for a wider range of applications.

The full scope of progress in the textile industry was demonstrated most effectively by the contributors who described new methods of producing yarns and fabrics. In the "melded" (melt-welded) technique for making fabrics, described by Mr K. West (ICI Fibres Ltd), a special sheath-core bicomponent fibre is used; the sheath has a lower melting point than the core. The fibres are formed into a web-like fabric structure, and then heated to a temperature hot enough to melt the sheath components and cause fusion at the points of contact between fibres; with cooling an excellent fibre bond is obtained. Mr. J. E. Ford (Shirley Institute) and Mr O-B. Rasmussen (Rasmussen Ingeniorfirma) discussed new methods by which polymer film can be converted into fibres. The films are first stretched to orient the molecular structure, and then subjected to lateral mechanical forces, which act on the natural tendency of oriented film to fibrillate, and thus convert the film into a fine network of fibres. Contributions on spun bonded fibres, from Dr O. L. Shealy (Du Pont), and on the Bobtex ICS spinning technology, from Dr A. J. Bobkowicz (Bobtex Corporation), completed the indication of things to come.

METALLURGY

Splat-cooling and Kindred Trades

from our Materials Science Correspondent

THE "new technique" of splat-cooling stole the limelight at the international conference on metastable metallic alloys in Brela, Yugoslavia, at the end of September. The conference, held on the initiative of the Institute of Physics of Zagreb University, and with the support of IUPAP and the European Physical Society, was devoted almost solely to the novel phases created by ultra-rapid quenching of alloys, direct from the molten state, by impact of drops against a cold metallic substrate—splat-cooling.

By means of splat-cooling a new family of metastably extended solid solutions, new phases that do not appear in the equilibrium diagram, and amorphous phases can be generated. Their interest lies not only in the regularities governing their appearance but also in their physical and mechanical properties, their applications and how they set about the approach to equilibrium when they are annealed. All these matters were discussed at Brela, and so was the complementary technique of coevaporation of metals on to a cold substrate, which is even more effective in creating metastable phases but has been less widely used.

Professor P. Duwez (California Institute of Technology), the inventor of the technique, surveyed metastable phases obtained by splat-cooling. His account of new developments included Fe–P–C ternary alloys and other transition metal–phosphorus alloys, all of which display amorphous phases. a-$Fe_{75}P_{15}C_{10}$—the prefix indicates an amorphous alloy—is a soft ferromagnet which stays amorphous up to its Curie temperature of 310° C. Duwez also demonstrated a Vegard's law for a-alloys; instead of the lattice parameter, the nearest neighbour distance deduced from diffuse X-ray patterns is plotted against solute fraction and gives a straight line.

Variants on the standard splat-cooling procedures included a description by Dr B. Leontić (Zagreb) of a method of spraying liquid alloy at synchronous speed into the gap between two rapidly rotating rollers, to make long strips of metastable alloy. Professor N. J. Grant (Massachusetts Institute of Technology) emphasized that interparticle spacing in a two-phase alloy is determined by dendrite spacing or grain size, which in turn are determined by cooling rate (so that a log–log plot of these variables is a straight line over many decades). Dendrite or grain size varies from about 1,000 μm at 10^{-3} deg C/s to about 0·01 μm at 10^{8} deg C/s. Grant has utilized this knowledge, manufacturing alloys by atomizing the melt and spraying it against a copper wheel. The resulting alloys, if concentrated enough, are supersaturated but also two-phase, and have outstanding mechanical properties. This applies both to aluminium-based alloys of various kinds and to Cu–Zr alloys which combine high conductivity with great strength. The mechanical properties of an a-alloy were surveyed by Professor R. Maddin (University of Oxford) and Dr T. Masumoto. Referring entirely to Pd-20 per cent Si alloys, they demonstrated the very large hardening, without total loss of ductility, entailed in the formation of a concentrated amorphous solid solution.

Several people pointed out the need for a metalloid or non-metallic constituent (such as Si, Ge, Te or P). Professor A. Guinier (Paris) believes that this indicates that a-alloys depend on a kind of covalent bond network, analogous to oxide glasses. This comparison was also made by Professor R. W. Cahn (University of Sussex), surveying the structural and kinetic theories concerning the formation ranges of oxide glasses, and the way these relate to a-alloys. Duwez enunciated the "confusion principle": in ternaries, amorphous alloys form more easily than in binaries, *ceteris paribus*. Expressed crudely, this is because two solutes form logjams with each other and with the solvent atoms. This kind of notion has also been successfully applied to the rationalization of vapour-deposited a-alloys (which, however, do not seem to require the presence of covalent bonds). Still on a-alloys, Guinier distinguished, more clearly than has been possible before, between truly amorphous materials, exemplified by Ni–P, and microcrystalline ones, such as Pt–C; in each case, X-ray scattering was the essential tool.

Several contributions were concerned with the breakdown of supersaturated Al–Fe alloys and the assorted property changes. Dr R. Ricci Bitti *et al.* (Paris) showed that flash-coevaporation of N–Pb films depended on the formation of alloy "molecules" in flight. Other contributors dealt with electrical properties of

splat-cooled or coevaporated films (including the contentious question of whether resistivity ratios can be used to assess reliably the extent of metastable solubility).

GAMMA RAYS

Confidence Restored

by our Astronomy Correspondent

ONE may be forgiven for thinking that gamma ray astronomy is proving the least rewarding of the new astronomies, but at last quite a solid body of evidence is building up to support the claim that an extraterrestrial source of gamma rays is being observed. The breakthrough was provided by a detector in the third Orbiting Solar Observatory, which picked up high energy gamma rays (greater than 100 MeV) coming from the galactic plane, and especially strongly from the galactic centre (Clark, Garmire and Kraushaar, *Astrophys. J. Lett.*, **153**, L203; 1968). But understandable reserve has prevented hats being thrown in the air until verification was produced, especially after balloon experiments by the Case Western Reserve–University of Melbourne group cast doubt on the reality of the OSO 3 source (Frye *et al.*, *Nature*, **223**, 1320; 1969). Confusion over the OSO 3 calibration which finally resulted in a downgrading by a factor of three in the claimed intensity has not helped to inspire confidence. A report from Fichtel's group at the Goddard Space Flight Center should set the seal on the 1968 result, however (*Astrophys. J. Lett.*, **161**, L157; 1970).

During a balloon flight of a spark chamber that was being developed for use in the Small Astronomy Satellite, Kniffen and Fichtel picked up gamma rays coming from the galactic centre at an intensity corresponding to the revised OSO 3 estimate. The indications are that the origin of the gamma rays is in high energy collisions between cosmic ray protons and protons in the interstellar gas. Neutral pions produced in the collisions decay into gamma ray photons, having a flattish spectrum at these energies, a spectrum which seems to be indicated by a comparison of the intensities measured by Kniffen and Fichtel above 50 MeV and above 100 MeV. According to theoretical calculations there should also be a small contribution from bremsstrahlung by cosmic ray electrons. But it is too early yet to rule out the alternative explanation that cosmic ray electrons produce Compton scattering of the anomalously high infrared background radiation that has been recorded—questionably—during the past few years.

It is disappointing that Kniffen and Fichtel found no sign of the point source of gamma rays in Sagittarius reported by Frye *et al.* last year, but people will not be mortified. The evidence was never as solid as for the galactic source, and in any case, now that X-ray stars have been found to come and go it is no longer bad form to get round the problem by suggesting that gamma ray sources are also variable. The non-detection of the OSO 3 galactic source by Frye *et al.* remains a problem, however.

But the greatest disappointment remains the failure to detect gamma rays from the Crab Nebula—astronomy's *Escherichia coli*—against expectations that there should be a measurable source. The flux from the galactic centre, on the other hand, has turned out to be a few times more than predicted. From a balloon experiment such as that by Fichtel's group in which a large background of atmospheric radiation has to be subtracted, next to nothing can be deciphered about the second component reported in 1968 by Clark, Garmire and Kraushaar, an isotropic flux of gamma rays which seems to be present in the OSO 3 data. More satellite experiments will have to be carried out before the nature and indeed the existence of the isotropic background can be made more definite. With gamma ray astronomy now in the post-natal state, what are needed are detectors with large surface areas to be exposed for long times. This is a goal for the large lifting capability now available to the Americans.

EARTH'S MANTLE

Gravity and Global Tectonics

from our Geomagnetism Correspondent

MANY people have remarked on the correlations between certain anomalies in the geoid and surface features of the Earth—large negative anomalies, for example, seem to be associated with the deeper parts of the ocean, especially the ocean margin trenches. The problem with this type of generalization is that the low order harmonics of the geoid, which are the strongest, are much more likely to be associated with features in the lower mantle than tectonic features close to the Earth's surface. Thus unless very high harmonics are included in the geoid determination, the significance of the correlations is bound to remain in dispute.

But recently, Gaposhkin and Lambeck have made an improved determination of the Earth's gravity field (so far unpublished) from satellite data and terrestrial gravimetry, which contains spherical harmonic components of the potential complete to degree 16, and which thus gives a resolution of about 11° or 1,200 km. This has enabled Kaula (*Science*, **169**, 982; 1970) to show the Earth's gravity anomalies in much finer detail than before and to relate them more closely to the Earth's tectonic features and processes in the upper mantle.

The most interesting point to emerge from Kaula's analysis is that both ocean rises and island arc-trench systems are clearly associated with high positive gravity anomalies, which means that both types of region contain excess mass. Because excess mass anomalies can only be created by mass transfer, it seems natural to look for the source of the anomalies in terms of upper mantle convection processes—movement of the asthenosphere and the resulting lithospheric motion. But if, as seems to be the case, asthenospheric flow is essentially steady state and the moving material incompressible, it follows that any mass transfer cannot be a once-and-for-all phenomenon. What is required is a process which maintains a mass excess in particular regions of the overall flowing pattern.

Kaula lists five ways in which this could occur—the accumulation of material at the surface, the replacement of less dense by more dense material at an interior interface, transition to a denser phase, thermal contraction and petrological fractionation by which a less dense component is separated from the material before it enters the relevant region. As Kaula points out, however, the precise relationship between gravity anomalies and the flow system depends critically on the boundary conditions. If the upper boundary is fixed, an upwelling current such as that at a mid-oceanic ridge will produce a negative gravity anomaly because

of its lower density. Because the anomalies over ridges are positive, the boundary there must be free so that the effect of the mass pushed up at the surface outweighs the effect of reduced density. In an arc-trench system, on the other hand, the situation is reversed. The material approaches a fixed boundary so that the density increase has greater effect than the removal of mass from the surface.

In contrast with the rise and arc-trench systems, the gravity anomalies over the ocean basins are negative, especially close to the rise flanks. The immediate cause of this phenomenon must be that the crust carried along by the sea floor spreading is thicker than would be expected, on isostatic grounds, from the depths of the basins. But why should this be so? And why should there be a drop of several kilometres between a rise and a basin? Kaula offers alternative suggestions—either there is insufficient asthenospheric material from the rise to match the rate of spread of the lithosphere or there is a settling out of a denser component—but he does not commit himself to either.

Finally, there is a large negative anomaly over Antarctica which is more than three times too large to be attributable to glaciation, and a positive anomaly over Hawaii which is far from any oceanic rise. Although Antarctica is more than 80 per cent surrounded by oceanic rises, it is not associated with the seismicity to be expected from descending lithosphere. The rises must therefore be moving away from the continent. The negative anomaly here could also be explained by insufficient asthenospheric material. Hawaii, on the other hand, is unique in being the only land region in the world more than 5,000 kilometres from an oceanic rise. Kaula suggests that the positive anomaly there may be due to partial upwelling not directly related to the world wide ridge system.

In general, however, the newly determined gravity field is compatible with the new global tectonics and allows much more sensitive correlations with surface and near-surface features.

INORGANIC COMPOUNDS

Phosphorus Looks Ahead

from a Correspondent

THERE seems to be a bright future for phosphorus–nitrogen compounds, according to trends discussed in Prague recently. These substances drew the most attention at a symposium on inorganic phosphorus compounds held in the Czech capital between September 14 and 17.

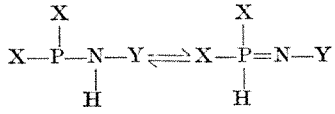
Dealing with the cyclophosphazenes, Professor R. A. Shaw (Birkbeck College, London) drew attention to the fairly wide occurrence of long range virtual coupling in the proton nuclear magnetic resonance spectra of these compounds. Recent work on their basicity has made it possible to use pK'_a measurements in structure determination, and evaluation of substituent constants has facilitated the prediction of the basicities of many compounds based on the cyclotriphosphazatriene skeleton. Novel ring opening and closing reactions of $N_3P_3Cl_6$ and $N_4P_4Cl_8$ with organometallic reagents were reported.

Dr H. W. Roesky and co-workers (University of Göttingen) reported some novel developments in the controlled stepwise synthesis of acyclic phosphazene units, for example,

$$R{-}NH_2 \xrightarrow{PCl_5} R{-}N{=}PCl_3 \xrightarrow{(Me_3Si)_2NH} R{-}N{=}PCl_2{-}NHSiMe_3$$
$$\xrightarrow{PCl_5} R{-}N{=}PCl_2{-}N{=}PCl_3, \text{ and so on.}$$

R represents, for example, $N_3P_3F_5$.

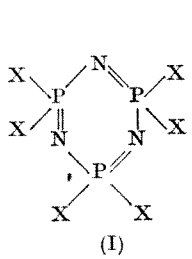
Drs A. Schmidpeter and K. Schumann (University of Munich) threw light on the prototropic behaviour in aminophosphazanes–iminophosphazenes, and showed how the position of this equilibrium was dependent on the nature of the substituents, both on the phosphorus and on the nitrogen atoms.



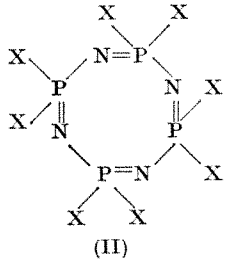
The detailed replacement patterns of $N_3P_3Cl_6$, with nucleophilic reagents, which Shaw discussed, were supplemented by preliminary data of similar reactions with $N_3P_3F_6$ from Drs E. Niecke, H. Thamm and O. Glemser (University of Göttingen). Data obtained so far suggest that patterns similar to those of the chloride pertain also with the fluoride (although the leaving group is different), but that there are some significant differences, for example, when the nucleophile is a thiolate ion.

Drs G. Wunsch (BASF, Ludwigshafen) and J. Emsley and P. B. Udy (King's College, London) described phosphorus-31 nuclear magnetic resonance studies which throw light on the mechanism of the formation of the homologous series of chlorocyclophosphazenes $(NPCl_2)_n$ ($n=3$, 4, 5 and so on). The most recent findings suggest that in controlled conditions the formation of a given ring system, such as the trimer, $N_3P_3Cl_6$, is favoured to such an extent as to make it the sole or almost the sole product of the reaction. This would represent a significant advance in the industrial use of this type of compound, for previously mixtures of the whole homologous series were obtained.

Dr G. Wunsch and Dr W. Wanék (Pedagogical Faculty, Ústí nad Labem) forecast major industrial uses for compounds based on phosphazenes. Wanék dealt with the specific application of aminophosphazenes $[NP(NH_2)_2]_n$ ($n=3$, 4, 5 and so on) as highly concentrated fertilizers. Recent legislation in the United States and United Kingdom, and other countries, concerning the degree of flammability permitted in fibres and other materials, has stimulated research on flameproofing agents. Alkoxyphosphazenes based on structures such as I and II (X = OAlk) have recently been developed as most successful flame-retarding agents for viscous rayon.



(I)



(II)

Even quite modest extrapolations of the future use in this field suggest that large tonnages would be required. Other applications of phosphazenes as plasticizers and as additives to hydraulic fluids and lubricants were also forecast.

Science Policy and Science Policy Research

by
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This assessment of the value of science policy research in the making of policies is based on a lecture given at a meeting of the Sociology of Science Study Group of the British Sociological Association last March. The thoughts it contains are of course those of Dr Blume himself, and not of the Department of Education and Science or the Council for Scientific Policy.

In this article I want to say something about science policy research, and its relations with the process of formulating national science policy. I shall start by giving what seems to me to be a useful definition of science policy, taken from the first Report of the Council for Scientific Policy[1].

"Science policy does not (therefore) direct the advance of scientific knowledge, though it may well be concerned to encourage or to direct the application of the results of scientific advances. The tasks of science policy are of another kind: to maintain the environment necessary for scientific discovery; to ensure the provision of a sufficient share of the total national resources; to ensure that there is balance between fields and that others are not avoidably neglected; to provide opportunities for inter-fertilization between fields, and between the scientific programmes of nations."

Conscious government involvement with the organization and finance of scientific research on a scale necessitating any talk of "a share of the total national resources" dates only from the Second World War—this is as true in the United States as in the United Kingdom. On the other hand, abstract discussion of the problems of science policy by scientists, philosophers, and philosopher-scientists is rather old (consider Francis Bacon for example), and there are many classic 19th century texts, such as Babbage's "Decline of Science in England" and James Hole's Essay on Mechanics' Institutes. Later almost all governments became increasingly preoccupied both with ensuring that indigenous science flourishes and with the demands its growth makes on the national purse. During this time it seems to me that the intellectual debate has rather lost touch with reality, and now pursues a logically independent course of its own. A famous contribution to the intellectual debate is the collection of papers on "criteria for scientific choice" published in *Minerva*[2], and which includes contributions by men of the calibre of Michael Polanyi, Stephen Toulmin and Alvin Weinberg. Harvey Brooks has succinctly put some of the questions with which the debate has dealt[3]:

"To what extent should planning be left to the internal working of the scientific system ?

"Who should be involved in the setting of scientific goals at various levels of detail?

"What are the categories of classification of scientific activities for which it is appropriate to plan?"

To be contentious, I think that this debate derives more from Babbage and Hole than from an understanding of contemporary policy making. It is not what I mean by science policy research. One day it might properly be seen as a contribution to the philosophy of science. As I understand it, science policy research has to follow a narrow middle way between this *a priori* generalism and pure data-gathering—and I shall define it in this way. Fortunately I can claim as precedent Harold Lasswell's description of the objective of policy sciences in general, in his essay on the policy orientation[4]. Perhaps because they have been less worried about inducing neuroses in the scientific system, I think that economists might have contributed more to these issues of planning. One has only to compare the *Minerva* debate with the passage quoted here[5]:

"Where social costs are high but private costs lower than private benefits, regulation is often essential (e.g., pollution); or where social benefits are high but private benefits lower than private costs, there will be a tendency to underinvestment, as in the case of fundamental research itself."

Just as there may be severe limitations on the fruitfulness of the kind of *a priori* philosophical argument I have mentioned, so there may be limits to the usefulness of the attempt which they make to define and describe science policies in terms of their objectives. I should like to suggest an alternative conceptual framework, using the two dimensions instruments and influences—although some would call this "one dimensional thought".

Constraints on Policy Making

Any truth still inherent in Polanyi's dictum that science is a self-governing system probably derives less from the unshakable commitment of the scientific community to a set of norms than from the overall inertia of the system. A better image than the city state would be the fly wheel! This inertia limits what the policy maker can do, and one can probably say that policy making can function only in practice at the margin, as economists have it. For example, suppose it were thought that the ratio of scientists to engineers in the labour force were too large. All that can be done in most countries is to make relatively more engineering places available in universities, and clearly it will be many years before the composition of the work force is affected significantly. Part of the duty of the science policy maker, whether government official or, as in the United States, member of a relevant Congressional committee, is to agree on a budget for his agency with the principal agency concerned with economic regulation—or the Appropriations committee. As in any allocation process this is largely determined by previous commitment, and debate needs must hinge on determination of the most appropriate rate of growth. Again, as in other allocation processes, much of the additional subvention is absorbed by inflation of costs in existing projects (or "sophistication"), and frequently—as a consequence—new developments suffer most. In the United Kingdom as in other countries mechanisms have been devised partially to compensate for this, but new developments are, of course, of rather special significance to science. It could be argued that their curtailment affects the health of science more than the provision of less extra places affects that of higher education, or the provision of less extra hospital beds affects that of the health service.

These two examples are intended to illustrate the point that the executive instruments available to the policy

maker are rather limited. They are also crude to the extent that he cannot be entirely sure of their effect. For example, he can make available more engineering places at universities, but cannot be sure that they will be taken up. What is more, there are instruments which are not accessible to him: the market for skills in another part of the world might have implications for the agency with which he is concerned. A more sophisticated apparatus would include a feedback loop, so that some indication of the effects of a new measure could rapidly be obtained. This is largely lacking.

The system which I have described, of small uncertain steps, can be designated, to borrow Kuhn's terminology, "normal" policy making. There are, of course, occasional periods of "crisis" when more far reaching changes are made. Such "crises" may follow severe budgetary restrictions, or a review of the institutional structure of the national scientific effort such as that carried out in Britain by the Trend Committee in 1963[6]. As with scientific crises, it is not always clear which factors have been primarily responsible for their precipitation, other than the realization that an accumulation of *ad hoc* changes to an existing institutional structure has reached a stage where more fundamental changes are needed.

Although science policy is constrained by the relatively unsophisticated executive instruments at its disposal, it is also fashioned by a creative tension between the various groups to which it is of interest. This is, of course, a characteristic of policy making in any democratic polity. I need hardly point out that much recent discussion of "science and society" has been concerned with this process, though in a fashion which has been exhortative rather than analytic or prescriptive. I shall discuss some of the factors which shape national scientific policies. It is as one of these that I suggest science policy research can fruitfully be viewed, and it is in periods of crisis that it might be most relevant.

To discuss the relative power of the various interest groups would require a sophisticated political sociology of science which we cannot at present provide. At present discussion of relative power must be very specific and left to the historian. I wish only to describe the groups involved and to say something about their relationships with those who must make decisions.

Influence of Government Advisory Boards

Most advanced countries probably now possess a formally constituted standing advisory body on science policy, composed principally of eminent scientists. In addition, in many countries the national scientific academy (for example the Royal Society, National Academy of Sciences, Swedish Academy of Sciences) has some advisory role. As far as I know only Australia has in recent years rejected the formation of the former on the grounds that the latter was totally competent. Of course the functions of both types of body vary between countries, as do their relations with the variety of organs of government which exist everywhere. Beneath the highest level policy committees is usually a hierarchy of committees with more limited and more technical mandates. Sociologists have pointed out that membership of the highest committees is frequently restricted to the most eminent among the scientific community. Although this is partly because of their proven intellectual abilities, it has been suggested that their prestige is important because it vindicates their speaking "for" their discipline or some wider "electorate" (for example ref. 7). Social and political scientists, such as Don K. Price, have also suggested that in addition to the value of the advice they offer, the advisory committee of scientists has an additional value to the policy maker; because their statements are validated by the prestige of the scientific community the weight of this prestige can be used to balance the prestige of other advisory groups, for example military and economic.

Basically, then, it is by such a system of committees that governments take the advice and opinions of the scientific community on such issues as seem to them important. Although the relationship between executive and adviser obtaining in any country at a given time must be a combination of formal and informal (or individual) factors, it is of course only the former which is subject to sociological generalization.

The Legislature

The influence of any legislative body on scientific policy must depend ultimately on three factors: the constitutional limitations on legislative control; the availability of information and qualified assistance; and the interest and concern of members. As far as the first is concerned, there is no need for detailed exposition of the difference in role between, for example, the Houses of Parliament, Congress, and the Assemblée Nationale. Clearly discussion such as would be required by any real comparison demands the talents of a specialized political scientist. It is well known that US Congressional committees (such as the Joint Committee on Atomic Energy) frequently act as "protectors" of the agency under their surveillance. In Britain it is accepted that the Select Committee of the House of Commons, though having a very different mandate, performs a most valuable service. Parliamentarians commonly receive information through three sources—library services, informal contacts and "parliamentary and scientific committees", such as that in Britain, which now exist in several countries. The importance of this information requirement has been much discussed in debates on "science and society", and emphasized more formally by the OECD/Council of Europe report on Science and Parliament[8]. Interest, of course, cannot be legislated for. Although very few parliamentarians, to my knowledge in any country, have a background in pure science, there are more (though still few) with a background in applied science. On the whole, interest is in the application of science to welfare and economic aspects of social change, rather than in pure science for its own sake. Suitable indicators of interest might be membership of Parliamentary and Scientific Committees, or questions asked in the House (which has been used by Vig[9]).

The Wider Community

I would include both the scientific community and the population at large in the wider community. Clearly both have some concern with issues of science policy, though perhaps with different emphases. For such concern to manifest itself, three things are required: the latent interest, the information and a mechanism. The provision of information is partly the obligation of the media; the popular scientific press on the one hand and the mass information media on the other. It would probably be true to say that in the United Kingdom we are fortunate in the extent to which the information media meet these obligations. The Government too can encourage proper and informed public discussion, as, for example, it has

done recently with its White Paper on Public Expenditure[10] and with its Green Paper on Government Research[11], both of which are in their different ways relevant to science policy issues.

When both interest and information are in evidence, some institutional means of crystallizing and presenting opinion is required, if influence is to result from interest. Again, journals (such as *Nature* and *Science*) and the press can do this in part, but far more influence would be likely to accrue to a formally constituted pressure group (whether specially constituted or resulting from the initiative of a pre-existing group). The scientific community is, of course, highly organized—primarily on a disciplinary basis. Such scientific societies, or institutes, by virtue of their representative character, can frequently legitimately claim access to or even representation on decision-making bodies. In order truly to serve as representatives, however, it could be argued that they would have to discuss policy issues, so that officers were partly elected according to their attitudes to such issues. Price has argued that scientific bodies are exceedingly unsophisticated politically (for example ref. 12). Other means of influence are also open to them, such as lobbying the legislature and preparing published reports—such as those produced by the chemistry and physics panels of the National Academy of Sciences[13]. The social structure of science also contains a few smaller bodies formed specifically for influencing public opinion, such as the old Federation of Atomic Scientists and the British and American Societies for Social Responsibility in Science, but because of their lack of representativeness these must carry less weight and would tend to use different means.

So far as the community at large is concerned the institutional forms are on the whole lacking. Britain, the United States and Australasia do have Associations for the Advancement of Science, but these now more rarely than formerly take issue on matters of public policy. (It is interesting, however, to note the rather sudden "repoliticization" which seems to have taken place at the recent Boston meeting of the American Association for the Advancement of Science.) There also exist quite large numbers of organizations with a limited interest, though frequently in an issue bearing in some way on science and technology policy, and formed specifically for lobbying. In this category could be included the various anti-pollution bodies, as representative of organizations which do seem to have a better understanding of the instruments at their disposal than do many bodies in this area. The overall impression, however, is that the community is far less well organized for influencing scientific policy making than it is for influencing, say, economic policy making or than it would be for influencing regional planning if the recommendations of the Skeffington Report[14] were to be implemented.

Statisticians and Science Policy Researches

The production of statistics has considerable influence on policy making even though this may not be the chief objective of those who produce them. As the political scientist Robert E. Lane has pertinently observed, facts can exert a certain tyranny upon the decision-maker by their mere existence as facts: some kinds of knowledge are inevitable "pressures for policy change"[15]. Comparative statistics, in whose production international organizations such as OECD and UNESCO specialize, have a particular power. Tables on research and development expenditure as a percentage of gross national product for various countries are treated very seriously, and such comparison can be a powerful incentive to increase effort or divert resources. For example, the French Comité Consultatif recently stated that it[16]:

> "continues to estimate that the rate (of expenditure) should pass 2·5 % of GNP in 1970, to 3·0 % in 1975 and 3·5 % in 1980—representing a growth of 0·1 % per annum in the part of the product given over to R & D. . . .
>
> . . . To attain in 1980 a level of expenditure on R & D equal to 3·5 % GNP implies, in effect, that France consecrates in 12 years a percentage of national revenue approximately equal to that hitherto spent by the US".

The consideration of the role of statistics in policy making suggests that one must differentiate in some measure between the influence of the tool (the statistics) and of the worker who wields it (whoever he may be). This is a point I shall take up later.

The framework within which I propose to discuss science policy research should now be clear. In the final section of the paper I shall suggest that science policy research is only differentiated from, as it may be, sociology, political science, operations research or economics by its potential, or real, relations with the policy making process. Thus it is not possible to consider it in substantive intellectual terms as one might consider, for example, political science. I suggested earlier that research in science policy has to be distinguished from, on the one hand, *a priori* philosophizing about the social relations of science, and on the other the collection of statistical data. It does, of course, share, with the statistical information that I have discussed, a certain potential "tyranny of knowledge", and once again it is necessary to distinguish between the power of the instrument and that of the man who wields it. Furthermore, it is necessary to point out that policy research can have a critical as well as an instrumental function. That is to say, not only can it clarify the options of the policy maker, by examining such matters as "the utilization of highly qualified manpower" or "sophistication in expenditure", but it should be able to clarify and criticize the fundamental assumptions (both explicit and implicit) on which policy making is based. I should like to suggest that this must be true of any policy research, but that in other fields of decision making this duality of purpose has at least a *de facto* recognition.

Role of Research in Policy Formulation

Ben-David[17] has suggested that to increase the relevance of fundamental research to the innovation process requires not the artificial direction of such research down obviously useful channels, but that those responsible for the management of innovation be aware of what is being done and constantly assess and reassess its relevance to their own objectives. Applied research includes all fundamental research which turns out to be useful. So it is with science policy research—policy research is what turns out, or what resonably could turn out, to be relevant.

Two famous examples of what can be called science policy research are the projects Hindsight and TRACES, carried out respectively by the RAND Corporation for the Department of Defense, and Illinois Institute of Technology for the NSF. Hindsight[18] involved twenty post-war American weapons systems (innovations), seeking to assess the importance of fundamental and applied research and development in their production. It concluded that of the relevant research and development "events" leading up to the various innovations, only 0·3 per cent were fundamental research, and 92 per cent

were development. The project only included events after 1940, and neglected all that was known before this. The conclusion drawn was that, given the Defense Department's objectives, it might not be worth supporting fundamental research. TRACES[19] was a slightly later study, commissioned by the National Science Foundation (the principal mandate of which is to support fundamental research) which used a similar methodology to look at five non-military innovations (including oral contraceptives and electron microscopes)—a major difference being that the Illinois investigators looked at relevant "events" right back to the nineteenth century. Of 341 events identified, 70 per cent were fundamental research and only 10 per cent development. Apart from the superficial contradiction, two things are noteworthy in these two studies: (i) they deal with a fundamental issue of science policy, and (ii) although rather large studies they are readily reducible to very simple conclusions. For both these reasons they are easily used, and have become a part of the folk mythology of science policy. Their easy assimilation suggests a difficulty. On most issues as central as that treated there are already firmly entrenched positions; thus the influence of a piece of research may well depend on the preconceptions and influence of those who see it, as well as on its intellectual significance. (This need not horrify—it is quite analogous to the demonstration of Barber and Merton of a resistance to discovery in the scientific community.) I shall suggest later that if those who do such research had an independent organization, an independence, if this were possible, and yet the capability to do such work, there could be interesting repercussions.

But who does do science policy research? Perhaps the most famous performers of all are the American research corporations, like RAND, the Institute for Defense Analysis and the Hudson Institute. They originated towards the end of the Second World War, concerning themselves exclusively at that time with defence policy, first of a tactical and then of a strategic kind. RAND's "biographer" has given the following reasons for their appearance[20]. First, the type of scientist needed would not be attracted into the civil service because neither salaries nor conditions were at that time attractive; second, long-range thinking is difficult to cultivate in the hectic atmosphere of a policy making agency—especially "heretical" thought; third, universities seemed inappropriate because of the difficulties at that time of establishing interdisciplinary institutes; and fourth, industry was inappropriate because of conflicts of interest. These corporations frequently work entirely for the government, on a broad range of problems, and their approach includes (in Smith's words) "the broader systems approach which examines fundamental assumptions of strategy and overall defence policy". Many intellectuals, however, have criticized the scope of their critiques, on the grounds that they too are formulated on far too limited a range of assumptions. Perhaps theirs is a kind of "critical-instrumental" research. Although the RAND Corporation is situated some 3,000 miles from the Pentagon, its influence is said to be significant; it is certainly large, affluent, and prestigious. In 1964 *Science* tried to determine its influence, and was able to report no more than that in some decisions its work had been influential, in others ignored[21]. Such influence as there was clearly depended on the capacity and willingness of the sponsor to utilize the work, and *Science* pointed out that this had resulted in the growth of a group of specialists, almost mirroring RAND, in the Air Force headquarters. What is more, it is argued that an important source of RAND influence has been through the alumnii who have subsequently entered government service. If this is so, then ideas move with men—a familiar concept.

If RAND and its fellows have been criticized for the instrumentality of their criticism, can a different kind of policy research be found in the universities? Science policy research is done in universities, in America of course, and in Europe too (for example Heidelberg, Lund and Sussex) although its necessary interdisciplinarity has made its accommodation difficult in some countries. Of course, sociologists would argue that attention to the immediate problems of policy makers can yield no professional reward, no recognition. So perhaps academic research must be of the most critical kind. Studying the attitudes and interests of both officials and academics concerned with science policy, La Porte[22] found a clear incongruity of interest between the two groups, which derived their interests in very different ways, one from theory, the other from practice. To put his findings into the language of this article, it could be said that the policy makers to whom he spoke were only interested in obtaining relatively limited instrumental research, and the academics were only interested in doing what would have been critical research if they had understood the policy making process adequately. La Porte suggests that academics should be allowed to spend periods of a few months working in relevant government agencies. Would they want to?

Where then do we stand? I have suggested that an instrumental kind of policy research can be influential and useful, provided it is taken up by some pre-existing interest group or, perhaps, if it is done by certain institutes with high prestige and their own special links to the decision-making structure. Academics are loath to do this kind of work. The same limitations apply to more fundamentally critical research, which must pose very difficult problems, and in this case the academics with both the interest and the understanding to do it are limited in number. If research of this kind is to be influential a good deal of thought must be given both to its organization and to ways in which it might be applied.

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Interferon stimulated by Double Stranded RNA

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Interferon represents an interesting approach to the control of viral disease in man and his domestic animals, as this review shows, for it is a part of the animal's own recovery processes during naturally occurring infection and active against most viruses that cause acute infection.

INTERFERON has become a topic of great medical and biological interest, for it is possible that careful analysis of this defence mechanism against viruses could be utilized to prevent the full expression of virus disease. In the decade since the discovery of interferon[1] by Isaacs and Lindemann many microbes and microbial extracts other than viruses have been observed to stimulate the production of this protein. But it was the polyanionic interferon inducers, particularly the most potent of them, double stranded nucleic acids, which raised the possibility of a modern molecular pharmacological approach to the utilization of interferon in human and animal disease. Although the double stranded RNAs are potentially economically feasible as drugs, their toxicity or other limitations may be prohibitive. This article, rather than being an exhaustive review of the rapidly growing literature on this topic, aims to evaluate this latter question.

Hilleman led the group at Merck Sharpe and Dohme that discovered the capacity of double stranded RNA to stimulate interferon[2-5]. This group and other groups have also pursued the other principal approach to the clinical utilization of the interferon mechanism—the preparation and purification of exogenous interferon. Because of the specificity of interferon, a preparation derived from primates would be required for humans. The short half-life of its activity in the blood means that large, presently impractical quantities of material derived from tissue culture or explants would be required. The instability of the biological activity of human interferon[6] has made this approach even more formidable. But important collaborative trials are in progress to determine the threshold amount of exogenous interferon required in man for the prophylaxis of several diseases[7].

There are some interesting implications of the potency of double stranded nucleic acid in the stimulation of the interferon. For example, it seems reasonable to expect that investigations of the action of these materials will lead to a better understanding of the natural mechanism by which viruses stimulate interferon in cells and whole animals. Also, this is a further function of nucleic acids, and the structural basis of this activity may have implications in nucleic acid structure and genetic function. Increasing knowledge of the genetic role of nucleic acids in the past two decades has produced an immense amount of chemical information about their structure and synthesis. The stimulation of interferon might be one way by which this information will be brought directly to bear on problems of human disease. In contrast to microorganisms or their extracts, double stranded synthetic nucleic acids have appeal for clinical use because they are not pathogenic not do they contain potentially dangerous adventitious contaminants. They are highly active, and so the small doses which must be used are not likely to be immunogenic. The fundamental question remaining is whether or not their side effects will limit their systemic use in man. There is a real need for a broad spectrum approach to viral control in the respiratory tract because of the vast number of agents causing disease in this region of the body. Because of the selectivity of action of most small molecule antiviral agents and the highly specific action of vaccines, the use of interferon, which is active against most of the viruses infecting this region[8], is particularly attractive. Here, treatment could be started or prophylaxis given before the specific infecting virus strain were identified.

Structural Requirements

The property of interferon stimulation is shared, although to a differing degree, by many synthetic and naturally occurring polyanions, including polyribonucleotides[2-5], plastics[9-12] and polysaccharides[13,14]. Similarities in the secondary structure of such diverse materials are suggested by the recent report that a sulphated seaweed polysaccharide which can stimulate interferon has a double helical structure, revealed by X-ray analysis[15]. A resemblance of these polyanions to viral nucleic acids is suggested by the finding that two of the active plastics, dextran sulphate and polyvinyl sulphate, as well as various nucleotide homopolymers can nucleate the assembly of the capsid proteins of plant viruses into particles closely resembling the original virus when examined under the electron microscope[16].

Double stranded ribonucleic acids with a high potency for interferon stimulation have been obtained from many sources, including fungal[2], bacterial[5], animal[4] and plant viruses. It is interesting that several preparations derived from *Penicillium*, which have previously shown antiviral activities in pharmaceutical screening programmes, owe much of their activity to their content of one or more serologically unrelated[17] double stranded RNA-containing mycophages[2,18]. A synthetic nucleic acid, that is the homopolymer pair (poly I : C) derived from annealing polycytidylic acid with polyinosinic acid, is among the most active[3] and readily available from several commercial sources. It is therefore the most widely studied compound in the series. But some twenty-seven other synthetic nucleic acids have also been studied for their ability to stimulate interferon or cellular resistance to virus infection, and so certain general conclusions are possible about structural requirements. RNA is much more active than any DNA[3,14,19], and double stranded molecules formed through complementary base pairing are much more active than one, three or four stranded molecules[3,14,19]. Double stranded complexes may form between two complementary homopolymers or through self-annealing of an alternating copolymer. Similar observations have

been made with naturally occurring RNA; for example, in contrast to the high activity of the double stranded replicative complex of MS–2 bacteriophage, the single stranded progeny virus is inactive[5].

Those homopolymer types which tend to form stable multi-stranded complexes have some activity in a highly sensitive tissue culture assay system[20]. These are polyinosinic acid, polyguanylic acid and polyxanthylic acid, and such aggregation can also be promoted with polyadenylic acid and polycytidylic acid by acidic or high magnesium conditions, which makes them somewhat active in tissue culture. There also seems to be requirement for a stable secondary structure of the double stranded complex because polymers with a low thermal stability seem to be less active than those with a higher thermal stability, that is a Tm greater than 60° C as measured in buffer containing 0·15 M sodium ion (review in ref. 14). For example, chemical modification like N_7 methylation, which lowers the stability of the double stranded complex found between polyinosinic and polycytidylic acids, decreases antiviral activity, whereas C_7 halogenation which does not effect stability does not influence activity (Gresser, I., personal communication).

It is not yet clear at what level this requirement for multi-strandedness and stable secondary structure acts; that is, it may be important in regulating the uptake of the RNA or in its interaction with a receptor site on or within the cell. But, as I shall discuss later, the most likely possibility is that multi-strandedness promotes the resistance of polymers to the ribonuclease in the cell as it does in the test tube. Double stranded RNA may account for the production of interferon by all mammalian viruses, for such material has been isolated from cells infected with either RNA or DNA virus[21,22], and even a virus irradiated with ultraviolet light has been reported to produce double stranded RNA as it stimulates interferon production[23]. It is possible, however, that the natural stimulation of viral interferon involves the synthesis or the location of single stranded RNA[24] at a critical site within the cell beyond the level at which the requirement for double strandedness acts.

The Stimulation Process

Radioactive materials have been used to investigate the sequence of interactions between interferon-stimulating RNA and cells[19,25]. The first step is adsorption to the cell surface. This is followed by a "recognition" step occurring at 37° C, which is a metabolic step within the cell, dependent on either the bulk of the RNA which is associated with membranes on the cell surface, or on the minute quantity of RNA which penetrates the cell[25], presumably in phagocytic vacuoles. The RNA is known ultimately to be degraded by the cells, and its components re-utilized in host RNA synthesis[25], but the recognition step is followed immediately by the production of interferon. The effects of metabolic inhibitors on this interferon production are quite complex; certain doses of agents inhibiting RNA or protein synthesis actually increase the amount of interferon produced by cells[26,27]. Such findings have led to the postulation of complex mechanisms of control, including a two-stage "synthesis and activation" mechanism[28] or a mechanism involving the synthesis of an inhibitor of interferon formation[29] within the cells concerned. Whether the production of interferon is through *de novo* synthesis or release of preformed material is a point of controversy requiring further study. Cells producing interferon are resistant to virus infection, and after the production step, there is a resistance to re-stimulation ("tolerance")[28] which may be related to the mechanism which turns off the initial response[29,30]. Both the protective effects and this tolerance phenomenon are transient in cells, both *in vitro* and *in vivo* lasting only a few days.

Potentiation of RNA Stimulation

Certain cell lines do not produce interferon when exposed to synthetic nucleic acids alone; they must be exposed to polycations at the same time, particularly DEAE-dextran[31] or neomycin[32]. These polybasic substances have been observed to increase the uptake of RNA by cells and also, by forming complexes, they may cause resistance to ribonuclease degradation within cells[25,33]. They have been reported[32] to act with single homopolymer species as well as double stranded complexes. Unfortunately, this type of potentiation is not consistently effective *in vivo*[34,35].

Awareness of the mechanism of interaction of cells with RNA has led to the development of several other methods for potentiating the interferon stimulating activity of RNA (Table 1). I have mentioned certain metabolic inhibitors which potentiate interferon levels *in vivo* or *in vitro* when dose and timing of administration are carefully controlled. A complex of fungal polysaccharide and RNA has been isolated which is said to contain only single stranded RNA[36]. It also seems to be an example of RNA potentiation, for the components of the complex are inactive alone and the complex appears to be resistant to degradation by ribonuclease in contrast to its susceptible RNA component.

Table 1. APPROACHES TO POTENTIATION OF INTERFERON STIMULATING ABILITY OF POLYNUCLEOTIDES

	Activity shown	Suggested mechanism
Polycations (DEAE-dextran, neomycin)	*In vitro* and inconsistently *in vivo*	Increased binding or ribonuclease resistance through complex formation
RNA-polysaccharide complexes	*In vitro* and *in vivo*	Protection from ribonuclease
Thiophosphate analogues	*In vitro* and *in vivo*	Protection from ribonuclease
Heating of alternating copolymers to 38° C	*In vitro* only	Protection from ribonuclease
Freund's adjuvant	*In vivo* only	Increased uptake of polynucleotide by interferon producing cells
Cycloheximide or actinomycin	*In vitro* and *in vivo*	Interferes with turnoff of interferon production mechanism

Two other methods for potentiating the production of interferon have been described. Both are apparently a consequence of increasing resistance of RNA to nuclease. One of these methods is the replacement of phosphate with thiophosphate in the backbone of the RNA molecule, which causes an increase of several orders of magnitude in the resistance to nuclease and the capacity to produce interferon[37]. This potentiation can be demonstrated both *in vivo* and in tissue culture. It may be of clinical significance as a useful molecular manipulation, particularly in the light of a report of an activity in animal sera which destroys the biological activity of poly I:C[38] but does not destroy the thiophosphate analogues as rapidly (unpublished work of De Clercq, E., Eckstein, F., Sternbach, H., and myself). In the second of these approaches to potentiation, nuclease resistance is achieved by a heat-induced configurational change in polymers in which two complementary bases alternate with each other[39]. A highly branched base paired complex seems to be converted into a linear fully base paired double stranded molecule, which removes sites for potential degradation by endonucleases. This potentiation can be shown best in tissue culture (and only to a much smaller extent *in vivo*), and is interesting primarily because it reinforces the idea, suggested by the

thiophosphate RNA analogues, that cellular nucleases compete with the interferon triggering site for polymers.

Another method potentiating the production of interferon involves the use of Freund's adjuvant (unpublished work of De Clercq, E., Nuwer, M., and myself). This mixture of mycobacterial extract and oil increases interferon after *in vivo* injection either of endotoxin or of the double stranded nucleic acids. The mechanism of this increase is obscure, however, although it is likely to involve increased reticuloendothelial function in processing and uptake of the RNA. Another method for potentiation of interferon production by endotoxin *in vivo* involves the injection of lead acetate, but unfortunately there is a parallel enhancement of the lethal effects of endotoxin (Stinebring, W. R., and Absher, M., personal communication).

Polyanion Effects in Animals

Knowledge of the interferon stimulating capacities of these double stranded nucleic acids has increased interest in their adjuvant effect in priming antibody production[40,41] (Table 2). They have also been reported to promote phagocytosis[42] and cell mediated immunity[43,44]. In addition to this broad range of effects on host defences, this class of compounds has been reported to have various side effects disturbingly similar to those seen with bacterial endotoxin[45], including pyrogenicity[46] and embryotoxic effects[47] as well as tumour necrotic effects[48] (which will be discussed later). A condition resembling runt disease has also been produced in mice with several types of double stranded RNA, including polyinosinic-polycytidylic acid[49].

Table 2. RANGE OF EFFECTS OF POLYANIONS

Agents against which polyanions are active	Diverse biological effects of polyanions
Numerous RNA or DNA viruses (both cytocidal and oncogenic)	Interferon stimulation
Bedsonia (trachoma)	Adjuvant effect on antibody production
Protozoa (*P. berghei*, *T. gondi* and *L. donovani*)	Accelerated cell-mediated immunity
Bacteria (*L. monocytogenes*, *E. coli*, etc.)	Macrophage "activation"
Fungus (*C. neoformans*)	Anti-complement activity
Tumours	Pyrogenicity

Several animal model virus infections have been explored with naturally occurring or synthetic double stranded RNA (poly I : C) prophylaxis. In most cases the results have been favourable. Notable exceptions have been the slow virus, scrapie[50], which was not influenced by a fungal double stranded RNA preparation and the so-called NZB disease of mice (an autoimmune disease thought to be initiated or intensified by a virus) which seems to be worsened by treatment with poly I : C [51]. Many favourable results have been reported in animal model systems which resemble human disease: for example, influenza, vaccinia and Columbia SK (caused by a polio-like virus) in the mouse[52,53] and rabies[54] (also B. Janis and K. Habel, personal communication) and herpes simplex[55] in the rabbit. In the latter infection and in an encephalitis in mice due to vesicular stomatitis virus (VSV)[56] favourable results have been reported even if the administration of RNA is delayed until after virus replication has reached a peak in the target organ. These results give some hope that therapeutic effects will be achieved in man. In all these models the challenge doses may be much greater than in man, but the routes of infection and the pathogenesis of the disease seem to resemble their human counterparts. It is encouraging that there was a 200-fold margin of difference between the lowest protective dose and the toxic dose in the mouse-VSV models[56]. The timing, dosage and route of inoculation of the RNA are critical in determining the results and must be adjusted, depending on the particular virus model under study.

Inhibitory effects with polyanionic interferon stimulators have also been seen in *in vivo* models of protozoa[57] and trachoma[58] infections. Such results are not surprising, for interferon is active against these agents. On the other hand, in very few animal model systems has interferon been directly implicated in the protective effect. When the blood concentrations of interferon produced by intravenous poly I : C are stimulated with passively administered interferon obtained in tissue culture, protective effects can be observed in the mouse-VSV model similar to those seen with the corresponding dose of poly I : C (unpublished work of De Clercq, E., Nuwer, M., and myself).

Protective effects with double stranded RNA have also been reported against bacteria and fungi (refs. 59 and 60 and Weinstein, M., Waitz, J., and Came, P., personal communication), as well as certain tumours[48,61] not known to be dependent on continued virus replication, including both chemically and viral induced standard laboratory tumour lines, as well as certain spontaneously acquired neoplasms. Here, it is likely that other effects of the polyanions mediate the inhibitory action, including the endotoxin-like side effects, as well as the stimulatory effect on cell-mediated immunity, although crude interferon has suppressed certain non-viral tumours[62]. Interferon itself seems to be most potent against RNA virus tumours such as the mouse leukaemias[63,64] where the continued reinfection of cells may be critical in maintaining the progression of the tumour. Lesser effects, however, are seen with the DNA tumour viruses where transformed cell growth[65,66] seems critical rather than continued rounds of infection and transformation. It is significant that single stranded nucleic acids have been observed to have anti-tumour effects, which suggests that other, most likely immunological, effects of polyanions are involved[67].

Potential for Human Diseases

The principal rationale for the use of interferon in human virus infections comes from the fact that viruses which stimulate interferon suppress the development of certain virus infections in man[68–70]. These results, however, have been obtained in prophylactic rather than therapeutic studies, and because the bulk of virus replication occurs early in the disease process in most virus infections, it is clear that interferon, like all types of antiviral agents, is more effective in prophylaxis than therapy.

A major unanswered question is whether tolerance to repeated interferon stimulation will be a problem. As the requirement for prophylaxis might be quite prolonged, continued stimulation of the system would be desirable. Within a narrowly defined range of "partial" stimulation, this is apparently possible[28,71]. In contrast to the lack of complications with local therapy, systemic administration of double stranded RNA to animals has produced various toxic effects. Many such effects, however, have been reported without concomitant examination of antiviral activities or any physical characterization of the polynucleotide preparations used. So far, trials with humans have indicated that interferon can be stimulated in the absence of striking toxic effects, although fever and slight changes in liver, haematopoietic and blood clotting parameters have been observed.

Clinical studies have been conducted to evaluate the anti-tumour properties of these compounds[72,73], and it

is not clear what their effects will be on humans with serious acute systemic infection. There may be some cross-tolerance with the infecting virus so that little or no further augmentation of the host interferon response is obtained. It is apparent therefore that after a proper period of experimentation with animals, the questions involving poly I : C efficacy against human virus infections must be answered in carefully conducted clinical trials in humans with virus infections. Ultimately, we must know the therapeutic ratio (the ratio of antiviral activity to the toxic activity) in man, for marked differences in toxicities have been observed from species to species. This ratio also depends on the nature and stage of the infection under treatment, as well as the route of drug administration. It is likely that, as usual with clinical investigation, work will begin with the serious diseases where the patient has the most to gain from any useful effects to balance the dangers inherent in the new drug. Humans with cancer will be early candidates for this material, in spite of the lack of evidence of permanent tumour eradication in any of the animal models. On the other hand, local treatment of the respiratory tract has the obvious advantage of minimal systemic side effects, and encouraging prophylactic antiviral effects have been noted against a rhinovirus in human volunteers[72]. But once the infectious process has developed in the respiratory tract, delivery of the inducer to the involved local areas for therapy seems to be difficult, and so uses seem to be limited to prophylaxis. New interferon stimulators under consideration as clinically useful alternatives to double stranded RNA include polyanions derived from partially oxidized starch[74] and the small synthetic molecule, DEAE-fluoronone, which is active orally[75]. But at this point it seems that only further empirical investigation will show whether the interferon stimulating capacity or other effects on host defences of the double stranded RNAs can be used in the control of human infections (see also reviews[76–84]).

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DNA Chain Growth: *In Vivo* and *in Vitro* Synthesis in a DNA Polymerase-negative Mutant of *E. coli*

by

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The enzyme involved in the discontinuous replication of DNA seems not to be the Kornberg polymerase but to be associated with the membrane.

STUDIES in this and other laboratories[1–18] support the idea that one or both strands of DNA are synthesized *in vivo* in a discontinuous fashion, whereby short DNA segments are synthesized in the 5′→3′ direction and subsequently connected by ligase. In the T4 system, both strands seem to be synthesized discontinuously[4].

It was originally postulated that the short DNA chains may be synthesized by DNA polymerase[2]. Although the exclusive synthesis of the short chains in the 5′→3′ direction (refs. 5, 17 and 18 and A. S. and R. O., manuscripts in preparation) conforms to this notion, certain other aspects of *in vivo* DNA synthesis disclosed by our studies are difficult to reconcile with the known properties of DNA polymerase. (1) Whereas two strand-discontinuous replication needs frequent initiation of synthesis of new chains along two template strands, DNA polymerase cannot carry out *de novo* chain initiation[19]. (2) The rate of elongation of the T4 short chains is estimated to be a few thousand nucleotides per second at 37° C (refs. 17 and 18, A. S. and R. O., manuscripts in preparation). The chain elongation rate in the *in vitro* DNA polymerase reaction, however, is at most 10 to 20 nucleotides per second at 37° C (ref. 20). (3) There is no indication that DNA polymerase synthesizes DNA chains of discrete sizes similar to the *in vivo* nascent short DNA chains.

These considerations led us to begin a serious search for a DNA synthesizing activity distinct from that of DNA polymerase. Initial work with *E. coli* ER22 (endonuclease I⁻) showed that the "membrane fraction" isolated by gentle lysis[21] synthesizes "short DNA chains" similar to the *in vivo* nascent short DNA chains. The isolation of a mutant of *E. coli* (P3478) which has little DNA polymerase activity[22] prompted us to examine this organism for DNA synthesizing activities, particularly the *in vitro* activity apparently associated with the membrane. A spontaneous *thy*⁺ revertant from *E. coli* P3478 *thy*⁻ (*polA*1), referred to as *polA*1′, and the parent strain W3110 *thy*⁻, referred to as *polA*1⁺, were used. The results obtained suggest strongly that a DNA synthesizing activity which is distinct from DNA polymerase and which is associated with the membrane carries out synthesis of the short DNA chains in the discontinuous mode of replication. Since the completion of this work there have been two reports of *in vitro* DNA synthesizing systems in *polA*1 or its derivative[23,24].

Discontinuous Synthesis of DNA by *Pol*A1

We first examined *polA*1′ for the newly synthesized short DNA chains that are found in wild-type cells.

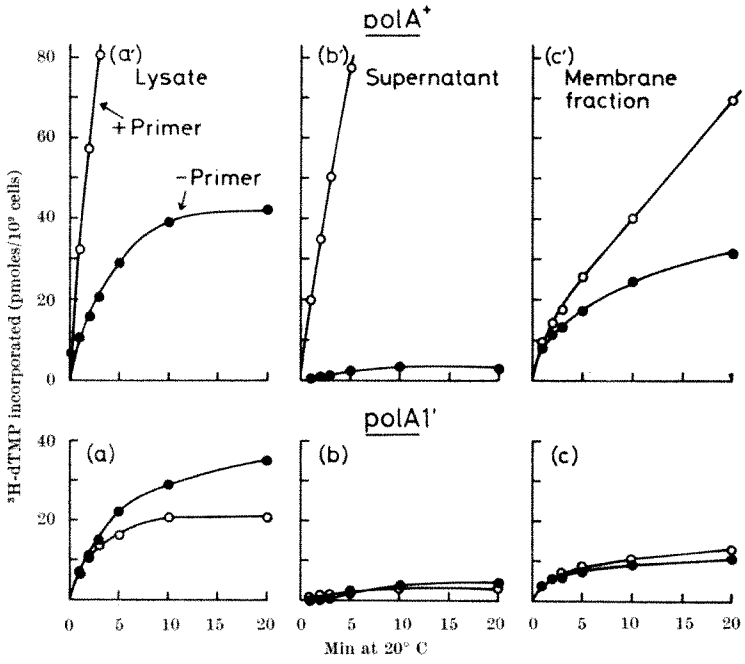


Fig. 1. DNA synthesis by "lysate", "supernatant" and "membrane fraction" of *polA*⁺ and *polA*1′. "Lysates" were prepared from a 40 ml. culture of *polA*⁺ and *polA*1′ grown in tryptose broth at 37° C to 6×10^8/ml. Cells were harvested by centrifugation, washed with 0·01 M Tris (*p*H 8·0) and suspended in 0·8 ml. of 25 per cent sucrose–10 mM Tris (*p*H 8·0)–1 mM EDTA. After addition of 0·2 ml. of a lysozyme solution (2 mg/ml. in 0·25 M Tris (*p*H 8·0)) the suspension was incubated at 0° C for 1 min, mixed with 1 ml. of a lytic mixture consisting of 1 per cent 'Brij 58', 2 mM Tris (*p*H 7·3) and 30 mM $MgSO_4$ and immersed in an ice bath for 5 min. The "lysate" was centrifuged at 6,000*g* for 5 min to yield the "supernatant". To isolate the "membrane fraction", 7 ml. of 20 mM Tris (*p*H 8·0)–15 mM $MgSO_4$ were added to 2 ml. of lysate. The mixture was placed on 0·2 ml. of 20 per cent sucrose–20 mM Tris (*p*H 8·0)–15 mM $MgSO_4$ and centrifuged at 6,000*g* for 5 min. The pellet was suspended in 2 ml. of 20 mM Tris (*p*H 8·0)–15 mM $MgSO_4$. Reaction mixtures (200 μl.) for DNA synthesis contained 17 mM Tris (*p*H 8·0), 12 mM $MgSO_4$, 200 μM rATP, 100 μM dATP, dGTP and dCTP, 10 μM ³H-dTTP (0·3 Ci/mmole), 0·13 mM yeast tRNA and 100 μl. of lysate, supernatant or membrane fraction. Where indicated 50 mM "activated" and denatured salmon sperm DNA[25] was added as "primer". The reaction was carried out at 20° C and terminated by cold TCA. Acid-insoluble radioactivity was counted.

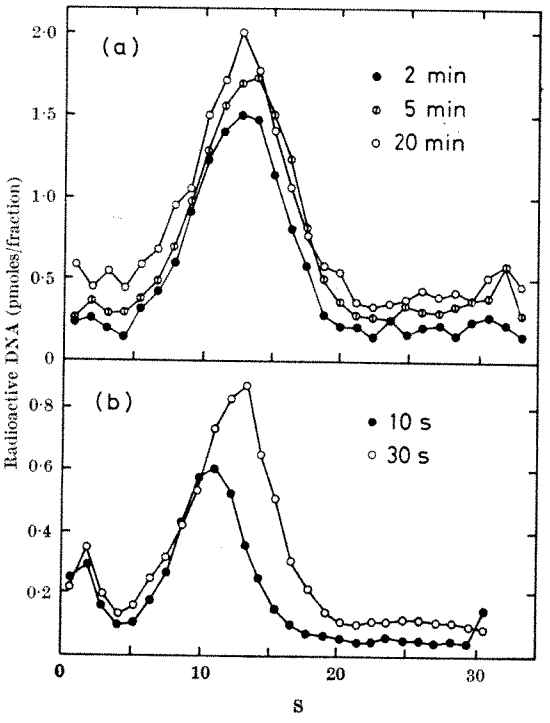


Fig. 2. Alkaline sucrose gradient sedimentation of DNA synthesized by the *polA1′* membrane fraction. The reaction mixtures (200 μl.) were as in Fig. 1 except that 10 μM ^{3}H-dATP (1·6 Ci/mmole) and 10 μM ^{3}H-dTTP (12 Ci/mmole) were used as labelled substrate in *a* and *b* respectively and the other deoxynucleoside triphosphates added at 100 μM. No primer DNA was added. Incubations at 20° C were terminated by chilling, and the addition of 0·2 ml. of 0·4 N NaOH–40 mM EDTA–1 per cent sodium N-lauroyl sarcosinate. The mixtures were incubated at 37° C for 20 min and sedimented through alkaline sucrose gradients for 21 h at 27,000 r.p.m. and 4° C in a Spinco SW27 rotor. The amount of radioactive DNA, in pmoles of total nucleotide residues, was calculated assuming that the mole fraction of A and T is 0·25 respectively.

Growing cells of *polA1′* were exposed to ^{3}H-thymidine at 30° C for various periods and the labelled DNA was analysed by alkaline sucrose gradient sedimentation. A large portion of DNA labelled by short pulses (5 to 12 s) sedimented at an average rate of 10 to 11S. Increasing the pulse time caused increases in the sedimentation rate of the labelled DNA. After neutral sucrose gradient sedimentation of the labelled DNA that had not been subjected to denaturation, 47, 21 and 19 per cent of the radioactivity was found in a DNA component sedimenting at about 15S after 5, 12 and 30 s pulses, respectively. Furthermore, comparable portions of the labelled DNA (45, 34 and 22 per cent after 5, 12 and 30 s pulses, respectively) were susceptible to *E. coli* exonuclease I. All these results are similar to those obtained previously using other *E. coli* strains[1,2]. Thus *polA1′* seems to synthesize DNA *in vivo* by the same discontinuous mechanism as wild-type *E. coli*.

Membrane and Supernatant DNA Synthesis

Sonic extracts of *polA1′* had almost no DNA polymerase activity (less than 1 per cent of the activity found in *polA*$^+$ extracts) when assayed using various primers. Lysates prepared from *polA1′* cells, however, by treatment with lysozyme–EDTA and 'Brij 58' (ref. 21), as well as lysates of *polA*$^+$, had an activity that catalyses the incorporation of the radioactivity from ^{3}H-dTTP or ^{3}H-dATP into DNA in the presence of the other three deoxynucleoside triphosphates and rATP (Fig. 1*a*, *a′*). In the absence of added primer DNA, the lysates of *polA1′* and *polA*$^+$ exhibited almost the same level of incorporation. The rate of incorporation in the lysates of both strains declined quickly during incubation. The addition of primer DNA greatly enhanced the incorporation in the *polA*$^+$ lysate but slightly inhibited the incorporation catalysed by the *polA1′* lysate.

When the "supernatant" and the "membrane fraction" were prepared from the *polA1′* lysate by low-speed centrifugation, about half of the activity (as well as more than 95 per cent of the nascent and bulk DNA) was recovered in the membrane fraction (Fig. 1*c*). About 10 per cent of the activity was found in the supernatant (Fig. 1*b*). A large portion of the activity of the *polA*$^+$ lysate, apparent only on addition of primer, was recovered in the supernatant (Fig. 1*b′*). The rest of the activity of *polA*$^+$ lysate was recovered in the membrane fraction and was weakly stimulated by the primer presumably because of the contaminating supernatant activity (Fig. 1*c′*). In the absence of added primer, more activity was found in the membrane fraction than in the supernatant as in *polA1′*.

The DNA synthesizing activity of our *polA1′* membrane fraction is similar in its general properties to that of the membrane-associated DNA synthesizing systems of Smith *et al.*[23] and Knippers and Strätling[24], which were prepared differently.

In our *polA1′* membrane fraction system the reaction levelled off as quickly as the reaction catalysed by the whole lysate (Fig. 1*c*) and did not require exogenous DNA. It was abolished by omission of any of the four deoxynucleoside triphosphates and was halved by omission of rATP. It was little affected by replacement of the three unlabelled deoxynucleoside triphosphates with the corresponding diphosphates. Pyrophosphate (100 mM) but not orthophosphate (10 mM) inhibited the reaction, however, suggesting that the triphosphates are the direct precursor of DNA. The incorporation of ^{3}H-dTTP was greatly reduced by the addition of a ten-fold excess of unlabelled dTTP or dTDP but was not affected by unlabelled dTMP or thymidine. The reaction was not inhibited by either KCN (4 mM) or nalidixic acid (50 μg/ml.), but was inhibited almost completely by *p*-chloromercuribenzoate (10^{-5} M), which has little effect on *E. coli* DNA polymerase.

Short DNA Chains of a Discrete Size

The radioactive DNA synthesized by the *polA1′* membrane fraction pelleted along with the membrane when the reaction mixture was centrifuged at 6,000*g* for 5 min, indicating that the product DNA associates with the "membrane". If the reaction mixture incubated for 1 min at 20° C was treated with sodium N-lauroyl sarcosinate and then centrifuged, the labelled DNA was recovered in the supernatant. Whereas about 60 per cent of the radioactive DNA sedimented rapidly (more than 80S) in a neutral sucrose gradient containing SSC, about 40 per cent of the DNA was recovered in a component sedimenting at about 20S. Thirteen per cent of the synthesized DNA was susceptible to exonuclease I after extraction by the Thomas procedure. These properties of the DNA synthesized by the membrane fraction resemble those of the *in vivo* nascent DNA.

Fig. 2 shows the profile of an alkaline sucrose gradient sedimentation of DNA which was synthesized by the *polA1′* membrane fraction during various periods of incubation. After 10 s incubation at 20° C, most of the labelled DNA sedimented at an average rate of 10·5S. The DNA synthesized during an incubation of 30 s mostly sedimented as a band with an average sedimentation coefficient of 12·5S. Although the synthesis did continue after 30 s, increasing the incubation time beyond 30 s did not change the size of the DNA. When the membrane fraction prepared from cells which had been exposed to ^{3}H-thymidine for 15 s at 20° C or 10 min at 37° C was incubated with unlabelled substrates for a further 20 min, virtually no change was found in the size and amount of the labelled DNA. These results suggest that these *in vitro* short chains are not products of endonucleolytic

degradation. The *polA*1′ membrane fraction seems to have an ability to synthesize short DNA chains of a discrete size (about 12·5S). This is close to but a little larger than the size of the *in vivo* nascent short DNA chains of *E. coli* (10 to 11S), which may represent an average size of growing and completed short chains in the steady-state conditions. The number of short chains produced by the *polA*1′ membrane fraction *in vitro* during a 2 min incubation at 20° C is about ten per cell if *de novo* synthesis of these chains is assumed.

DNA Synthesis in T4 infected *polA*1′

DNA synthesizing activity associated with the membrane fraction of *polA*1′ disappeared rapidly on infection of the cell with T4 *am* B22 (gene 43), a DNA polymerase mutant (Fig. 3*a*). The disappearance of the activity of the membrane fraction was also observed after infection with another DO mutant, T4 *am* N82 (gene 44), which induces phage DNA polymerase (Fig. 3*b*). On infection with T4 *r*II (EM7) as well as T4D (wild type), the DNA synthesizing activity of the membrane fraction also decreased, but this was followed by a sharp increase (Fig. 3*c*). Thus after T4 infection, the DNA synthesizing ability of the *polA*1′ membrane fraction changes in parallel with the DNA synthesis *in vivo*.

The activity associated with the membrane fraction of T4D or T4 *r*II-infected cells was basically similar to that of the membrane fraction from uninfected cells. The reaction (assayed by ^{3}H-dTTP incorporation), requiring the presence of four deoxynucleoside triphosphates, was slightly stimulated by rATP, levelled off shortly after onset, and was not affected by the addition of thymidine, dTMP, KCN or NaN_3. The synthesized DNA was of a discrete size (8 to 9S), but was distinctly smaller than the short DNA chains synthesized by the membrane fraction from uninfected cells (12·5S). This is similar to the size difference between *in vivo* nascent short DNA chains of T4 (8 to 9S) and *E. coli* (10 to 11S) (refs. 2, 3, 6). It is worth noting that there is little indication of endonucleolytic degradation of these short chains during the period between the first and tenth minute of incubation. T4 *r*II was used in these experiments, for the *r*II mutation presumably suppresses the synthesis or the function of a T4 endonuclease. Similar results were obtained with T4D.

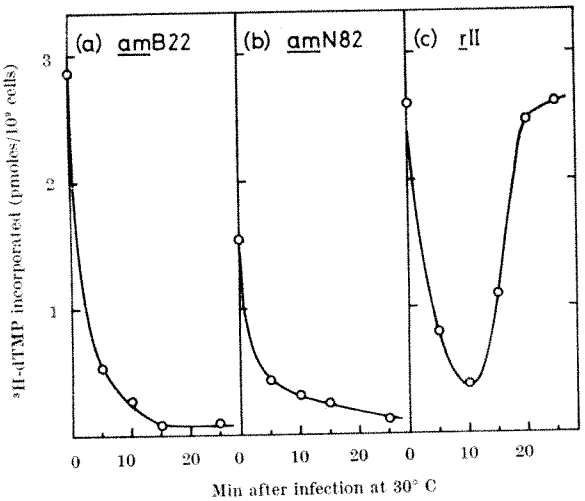


Fig. 3. Changes of DNA synthesizing activity of the *polA*1′ membrane fraction after T4 infection. Cells were grown to 6×10^8/ml. at 30° C in medium *A* (ref. 2) supplemented with 0·5 per cent casamino-acids and infected with the indicated strains of T4 (m.o.i. = 10). Samples (20 ml.) were taken at various times after infection and cells were collected by centrifugation. The "lysate" was prepared as described in Fig. 1 except that cells were not washed before lysozyme treatment. The "membrane fraction" was prepared from the lysate as described in Fig. 1 and washed once in 20 mM Tris (*p*H 8·0)–15 mM $MgSO_4$. The reaction was carried out in a 250-μl. incubation mixture as in Fig. 1 for 2 min at 20° C with 160 μl. of the membrane fraction and ^{3}H-dTTP with a specific activity of 0·15 Ci/mmole.

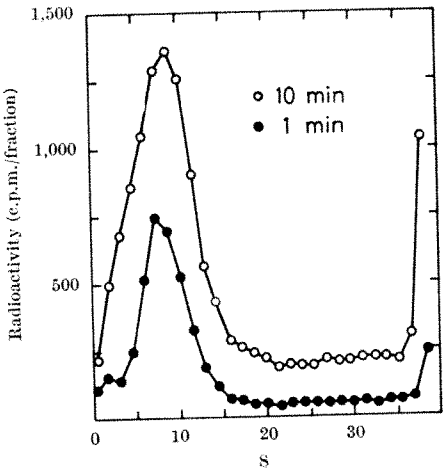


Fig. 4. Alkaline sucrose gradient sedimentation of DNA synthesized by the membrane fraction of T4 *r*II-infected *polA*1′. The membrane fraction was prepared as described in Fig. 3 from cells at 25 min after infection at 30° C. The reaction was carried out as in Fig. 2 using 160 μl. of the membrane fraction and ^{3}H-dTTP with a specific activity of 12 Ci/mmole. An equal volume of 0·4 M NaOH–40 mM EDTA–1 per cent sodium N-lauroyl sarcosinate was added and the mixture was incubated at 37° C for 20 min and sedimented through alkaline sucrose gradients for 22 h at 25,000 r.p.m. and 5° C in the 'SW25·3' rotor.

The ^{3}H-DNA synthesized by the membrane fraction of T4 *r*II-infected *polA*1′ annealed efficiently with T4 DNA, whereas little hybridization was observed with *E. coli* DNA. Thus in a typical experiment, 78·8 per cent of the input ^{3}H-DNA annealed with T4 DNA (4 μg), whereas only 7·4 per cent annealed with *E. coli* DNA (20 μg). The ^{3}H-DNA, like *in vivo* T4 nascent short DNA chains[4], annealed equally to both of the separated complementary strands of T4.

DNA Chain Synthesis *de novo*

It is possible that the reaction catalysed by the membrane fraction represents only the addition of some nucleotides to the 3′ termini of the *in vivo* nascent short chains present in the membrane complex. To test this the following experiment was performed. The *polA*1′ membrane fraction was allowed to incorporate ^{3}H-dTTP for 1 min at 20° C in the standard reaction mixture. DNA was extracted and sedimented on alkaline sucrose gradients. The labelled 12·5S DNA was then subjected to successive treatment with spleen deoxyribonuclease II and spleen phosphodiesterase followed by 'Sephadex' gel filtration. This procedure[17], which will be described in detail elsewhere, permits the selective isolation of the 5′-phosphoryl terminal portion of DNA chains as large oligonucleotide fragments. The results shown in Fig. 5*a* indicate that approximately five per cent of the radioactivity of the DNA was recovered in the oligonucleotide fragments, which were eluted from the column with the void volume. If this material was treated directly with spleen phosphodiesterase a second time, almost no degradation was observed (Fig. 5*b*). The same material was degraded to mononucleotides, however, if it was treated with bacterial alkaline phosphatase before the treatment with spleen phosphodiesterase (Fig. 5*c*). ^{3}H label must therefore be in the 5′-phosphoryl terminal portion of the 12·5S DNA. It is thus unlikely that the incorporation catalysed by the membrane fraction represents only the addition of nucleotides to the 3′ termini of preformed *in vivo* nascent short DNA chains of nearly completed sizes. The results suggest, though do not prove, that the *polA*1′ membrane fraction may carry out *de novo* synthesis of these short chains. The possibility that the synthesis might proceed in the 3′→5′ direction has not been excluded, although this seems remote, because *in vivo* DNA synthesis (as

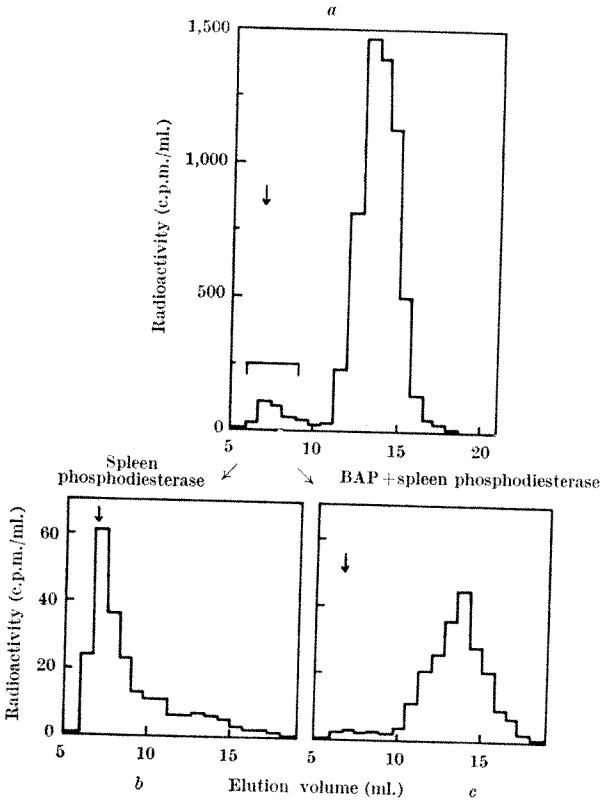


Fig. 5. Isolation of the 5′-phosphoryl termini of ^{3}H-labelled DNA synthesized by the membrane fraction as radioactive oligonucleotide fragments by treatment with spleen deoxyribonuclease II and phosphodiesterase followed by 'Sephadex' gel filtration. The *polA1′* membrane fraction (1·6 ml.) was incubated with ^{3}H-dTTP (12 Ci/mmole) in the standard conditions at 20° C for 1 min. The labelled DNA was pelleted along with the membrane by centrifugation at 7,000*g* for 10 min, extracted by the modified Thomas method[3], treated with 50 μg/ml. pancreatic ribonuclease in SSC, denatured in 0·1 M NaOH–10 mM EDTA, and sedimented through an alkaline sucrose gradient. (*a*) The peak fractions (average 12·5S) were pooled and treated with hog spleen deoxyribonuclease II at 37° C for 30 min in a 1·8 ml. reaction mixture containing 0·1 M sodium formate (*p*H 4·5) and 40 units of enzyme. After heating at 80° C for 10 min, the DNA was further digested with bovine spleen phosphodiesterase at 37° C in a 2·4 ml. reaction mixture containing 0·1 M potassium phosphate (*p*H 7·6), 100 μM d–pTp and 9 units of enzyme. After 6 h, 9 units of enzyme were added and the incubation continued for 6 h. The sample was applied together with blue dextran 2,000 to a 'Sephadex G-25' column (0·8 × 35 cm) and eluted with 0·1 M NaCl–1 mM Tris (*p*H 8·2). The arrow indicates the void volume. (*b*) The bracketed fractions of (*a*) were combined, concentrated, added to a 'Sephadex G-25' column and eluted with 0·01 M NaCl–1 mM Tris (*p*H 8·2). One portion of the sample was treated with spleen phosphodiesterase as in (*a*) in a 0·9 ml. reaction mixture with 4 units of enzyme and subjected to 'Sephadex G-25' gel filtration. (*c*) The other portion of the excluded material was treated with bacterial alkaline phosphatase (BAP) at 37° C in a 1·1 ml. reaction mixture, containing 10 mM Tris (*p*H 8·2) and 10 μg of enzyme. After 30 min 10 μg of enzyme was added and incubation was continued for 30 min. The BAP-treated sample was then digested with spleen phosphodiesterase as in (*a*) in a 1·3 ml. reaction mixture with 6 units of enzyme and subjected to 'Sephadex G-25' filtration.

well as all the known enzymic reactions for polynucleotide synthesis) occurs in the 5′→3′ direction. Experiments to clarify this point are in progress.

When duplex DNA with nicks is used as primer, *E. coli* DNA polymerase catalyses "nick translation", by concurrent 5′→3′ exonucleolytic degradation and 5′→3′ chain elongation[19]. The possibility that the reaction catalysed by the membrane fraction is of this type was considered seriously, for discontinuities exist in the nascent portion of DNA which is presumably attached to the membrane[26,27] and residual DNA polymerase might also be there. Two observations suggest, however, that this is not the case. The membrane fraction was isolated from *polA1′* cells which had been exposed to ^{3}H-thymidine (12 s at 30° C) to label nascent DNA chains totally and incubated in standard conditions for DNA synthesis with unlabelled deoxynucleoside triphosphates. During 1 to 5 min of incubation virtually no degradation of the labelled nascent chains (less than 1 per cent of the synthesis) was found. The *polA1′* membrane fraction was incubated in a standard reaction mixture with ^{3}H-dTTP for 30 s at 20° C. Further synthesis in the presence of a fifty-fold excess of unlabelled dTTP did not decrease the label in the short DNA chains.

DNA Synthesis by the Lysate

About a half (30–60 per cent) of the activity in the lysate of *polA1′* was recovered in the membrane fraction and a small portion (about 10 per cent) in the supernatant (Fig. 1). The unfractionated lysate may contain additional activity compared with the membrane fraction. After incubation for 1 min with the *polA1′* lysate, two sizes of products were found. In addition to a slowly sedimenting component comparable in size with that produced by the membrane fraction (13S in alkali), there was a component sedimenting at an average rate of 31S. Pulse-chase experiments indicated that the 13S component is not a precursor of the 31S component. But in similar experiments with lysates of T4 *r*II-infected *polA1′* most of the incorporated label was found in an 8 to 9S component and there was no indication of a fast sedimenting product with a discrete size. These results might be related to the recent findings suggesting that in bacteria only one of the two DNA strands may be synthesized discontinuously. Nascent short DNA chains of *B. subtilis* anneal almost exclusively to the "L" strand[28,29]. Iyer and Lark[30] found that a half of the pulse label incorporated into *E. coli* DNA was present at the 3′ ends of long DNA chains.

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New RNA Polymerase from *Escherichia coli* infected with Bacteriophage T7

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A new T7-specific RNA polymerase is found in T7 phage-infected cells. It is the product of T7 gene 1 and is physically and biochemically distinct from the host cell RNA polymerase. The synthesis of T7 RNA polymerase provides a direct explanation for the pleiotropic control of late T7 functions exerted by gene 1.

THE growth of a bacteriophage in a bacterial cell provides a useful system for the study of the regulation of gene expression. In the case of several bacteriophages of *Escherichia coli*, regulation of gene expression seems to occur at the level of transcription; only messenger RNA species containing information for early bacteriophage proteins are formed early in infection. At the appropriate time, one or more "switching" events occur which trigger the synthesis of messenger RNA for late bacteriophage genes (for reviews, see refs. 1, 2) and which terminate the transcription of some early bacteriophage genes. It has been proposed that the change in transcription specificity leading to synthesis of late messenger RNA is provided in bacteriophages T4[3] and T7[4] by synthesis of a new protein component, which functions in place of the sigma subunit of *E. coli* RNA polymerase and which allows the host cell RNA polymerase to initiate RNA synthesis at promoter sites for late bacteriophage genes, where it otherwise would not function. We show here that bacteriophage T7 induces the formation of a new RNA polymerase activity in infected *E. coli*. The T7 RNA polymerase is distinct from *E. coli* RNA polymerase and seems to be the protein product of gene 1 of T7 bacteriophage. Synthesis of messenger RNA for late bacteriophage functions seems to be mediated by this phage-induced RNA polymerase and not by an alteration of the host RNA polymerase.

Procedures

^{3}H-labelled and unlabelled nucleotides were obtained from Schwarz Bioresearch; α-^{32}P-ATP was prepared by the method of Symons[5]. DNA from T2, T7 and λ bacteriophages was prepared according to Thomas and Abelson[6]. dAT copolymer and dG·dC homopolymer pairs were prepared according to Schachman *et al.*[7] and Radding *et al.*[8], respectively. DNA concentrations are expressed in terms of nucleotide equivalents. Rifamycin was the gift of Ciba Pharmaceuticals, and streptolydigin was donated by the Upjohn Co. Gel electrophoresis was carried out according to Shapiro *et al.*[9] using *E. coli* RNA polymerase to provide molecular weight standards[10]. *E. coli* RNA polymerase was purified by the procedure of Berg *et al.*[11] and was fraction V enzyme. Strains of T7 bacteriophage containing amber mutations and *E. coli* strains B and O11′ were obtained from Dr F. W. Studier and were grown according to Studier[12]. T7 342-15, which contains a mutation in gene 1 rendering the growth of the phage temperature sensitive, was obtained from Dr W. C. Summers[4]. *E. coli* infected with T7 bacteriophage were grown in a "Biogen" (American Sterilizer Co.). *E. coli* B/1 were grown to a density of 3×10^9 cells per ml. with maximum aeration using M9 medium[12] supplemented with additional ammonium chloride and 5 g/l. of glucose. T7 bacteriophage were added to give a multiplicity of 3 and after 12·5 min the cells were rapidly chilled and harvested by continuous flow centrifugation. Infected cells were stored at −15° C until use.

RNA polymerase was purified from T7-infected cells by two methods. Procedure I was carried out essentially as described for isolation of *E. coli* RNA polymerase from uninfected cells[11] using T7-infected cells. RNA polymerase assays done during this procedure were done as described by Berg *et al.*[11] using T7 DNA as template. Procedure II was devised to obtain T7 specific RNA polymerase (Table 1). RNA polymerase assays for this enzyme

Table 1. SUMMARY OF PURIFICATION PROCEDURE II

Fraction	Volume (ml.)	Total activity (units $\times 10^{-3}$)	Protein (mg/ml.)	Specific activity (U/mg)	Percentage total activity
A. Extract	47	570	20	600	100
B. Streptomycin supernatant fluid	40	370	15	630	65
C. $(NH_4)_2SO_4$ extract fraction	9	202	14·5	1,500	35
D. DEAE-cellulose pooled fractions	90	200	0·25	8,800	35
E. Phosphocellulose pooled fractions	33	125	(0·03)	(120,000)	22
F. Concentrated phosphocellulose fractions	3·1	87	0·23	120,000	15

All operations were carried out at 0°–4° C and all centrifugations were carried out for 30 min at 15,000 r.p.m. in the 'SS 34' rotor of the Sorvall 'RC IIB' centrifuge except where specified otherwise. T7-infected cells (13 g) were mixed with 26 g of cold, acid-washed alumina powder and 0·4 ml. of 0·05 M Tris-HCl (*p*H 8)–0·01 M $MgCl_2$–0·0001 M dithiothreitol (DTT)–5 per cent glycerol (buffer A) and the mixture was ground in a mortar for 12 min. Buffer A (30 ml.) was added and the viscous mixture was mixed thoroughly and was centrifuged for 1 h. The supernatant fluid was diluted to a protein concentration of 20 mg/ml. (fraction A, 47 ml.). To fraction A was added 3·3 ml. of 10 per cent streptomycin sulphate and the mixture was stirred for 15 min and then centrifuged. The supernatant fluid (fraction B, 39 ml.) was decanted and 10·8 g of ammonium sulphate added. After 30 min of gently stirring the precipitate was collected by centrifugation and extracted in a 50 ml. centrifuge tube with 8 ml. of 1·2 M ammonium sulphate in 0·01 M Tris-HCl (*p*H 8)–0·0001 M dithiothreitol–DTT 5 per cent glycerol (buffer I), using a tightly fitting pestle. After 30 min the suspension was centrifuged. The supernatant fluid was saved and the pellet extracted again exactly as before to give a second supernatant fluid. The two supernatant fluid fractions were pooled (volume 16 ml.) and 2·06 g of ammonium sulphate added. After 30 min the precipitate was collected by centrifugation and dissolved in buffer I (fraction C, 9 ml.). Fraction C was dialysed for 3 h against two changes of 500 ml. each of buffer I in a rapid dialysis apparatus[13] and then diluted to 3 mg/ml. protein concentration. The enzyme was adsorbed to a column of DEAE-cellulose (20 cm length × 3 cm² cross section) equilibrated with buffer I. Elution was carried out with a linear gradient from 0·05–0·4 M KCl in buffer I (total volume 400 ml.). Fractions containing a high specific activity of T7 RNA polymerase were pooled (fraction D, 90 ml.) and immediately passed through a column of phosphocellulose (Whatman 'P-11'; 20 cm length × 3 cm²) previously equilibrated with buffer I containing 0·15 M KCl. A flow rate of about 1 ml./min is maintained. The enzyme is eluted from the column with a linear gradient from 0·15–1·0 M KCl in buffer I (total volume 300 ml.). Fractions containing a high specific activity of T7 RNA polymerase and showing only a single protein band on sodium dodecyl sulphate SDS-acrylamide gel electrophoresis were pooled (fraction E, 22 ml.) and concentrated by pervaporation[14] against buffer I to give a final protein concentration of more than 0·25 mg/ml. (fraction F, 3·1 ml.). These fractions were stored at 0° C or, after addition of an equal volume of glycerol, at −15° C.

were carried out as in procedure I with the following modifications. The enzyme was diluted in a solution containing 0·05 M Tris-HCl (*p*H 8), 0·01 M 2-mercaptoethanol and 2 mg/ml. of bovine serum albumin (BSA). Reaction mixtures contained 4 μmoles of Tris-HCl (*p*H 8), 2 μmoles of $MgCl_2$, 1 nmole of 2-mercaptoethanol, 100 μg of BSA, 36 nmole of T7 DNA, 40 nmole of ATP, CTP, GTP and UTP, and 0·5–10 units of enzyme in a final volume of 0·1 ml. One of the nucleoside triphosphates was labelled with α-^{32}P or ^{3}H at a specific activity of 2,000–20,000 c.p.m. per nmole. The rate of nucleotide incorporation with T7 RNA polymerase was constant for at least 20 min at 37° C with infected cell extracts or with the purified enzyme. Standard assays were terminated after 10 min incubation at 37° C and the amount of acid-insoluble radioactivity was determined as before. The amount of nucleotide incorporation was directly proportional to the amount of enzyme added to the reaction over the range of activity given above. One unit of activity is the amount of enzyme which gives a rate of 1 nmole of AMP incorporation in 1 h at 37° C in the standard assay. The activity of *E. coli* RNA polymerase holoenzyme is essentially the same in the two different assay procedures.

Two RNA Polymerase Activities

As a result of the studies of Summers and Siegel[4] it seemed likely that *E. coli* RNA polymerase in cells infected with T7 would be modified by replacement of the sigma subunit. To study the properties of such a modified polymerase we chose procedure I, which should allow isolation of the virtually intact enzyme. During the purification there were extensive losses in RNA polymerase activity. The missing activity did not appear in discarded fractions, so it was assumed that the activity was unstable, although the *E. coli* RNA polymerase is quite stable in similar conditions. Gradient elution of the enzymatic activity from DEAE-cellulose resolved two separate peaks of RNA polymerase activity, one in the range of 0·15 M KCl (fraction 5A), and the second in the range of 0·25 M KCl (fraction 5B). RNA polymerase in uninfected cells elutes at about 0·25 M KCl, in a position similar to fraction 5B. Fractions 5A and 5B contained 1·5 and 3 per cent, respectively, of the initial RNA polymerase activity measured in the original extract.

Characterization of these two fractions revealed that fraction 5A was highly active with T7 DNA as template, but did not use dAT copolymer whereas fraction 5B was active with both templates but most active with dAT copolymer (Table 2). The template specificity of fraction 5A was remarkable, for *E. coli* RNA polymerase is highly active with dAT copolymer as template whether or not it contains the sigma subunit (see Table 2). From the specific activities of the two fractions and the known specific activity of the *E. coli* RNA polymerase it was estimated that the fractions were probably about 30 per cent pure, that is, one-third of the protein in each fraction

Table 2. ACTIVITIES OF RNA POLYMERASE FRACTIONS FROM T7-INFECTED *E. coli*

Fraction	Source	Specific activity T7 DNA (U/mg)	Specific activity dAT copolymer (U/mg)	Ratio T7/dAT
5A	T7-infected *E. coli*	2,500	64	39
5B	T7-infected *E. coli*	1,700	4,000	0·4
RNA polymerase holoenzyme	*E. coli*	8,100	20,000	0·4
Core polymerase	*E. coli*	880	16.000	0·06

Data for *E. coli* polymerases are from Berg *et al.*[11]. Where indicated, 20 nmoles of dAT copolymer replaced T7 DNA as template.

would be RNA polymerase. Analysis of the two fractions by electrophoresis in SDS-acrylamide gels indicated that fraction 5B contained an appreciable amount of a protein component having the size of the subunits β' and β of the *E. coli* RNA polymerase[15,10]. Fraction 5A contained no detectable β bands, however, suggesting that the enzymatic activity was not associated with these subunits of the host RNA polymerase. We tested this possibility further by measuring the sensitivity of the two fractions to inhibitors of *E. coli* RNA polymerase: rifamycin[17], streptolydigin[18], and a purified γ-globulin fraction prepared from a high titre antiserum to *E. coli* RNA polymerase (Table 3). It is clear that fraction 5B behaves as would be expected if most of its RNA polymerase activity were *E. coli* polymerase*. By contrast, fraction 5A is insensitive to both drugs and its activity is not affected by antibody to *E. coli* RNA polymerase. This suggested that T7 bacteriophage might induce a new RNA polymerase protein in infected cells.

Table 3. INHIBITION OF T7 RNA POLYMERASE FRACTIONS BY RIFAMYCIN, STREPTOLYDIGIN AND ANTIBODY TO *E. coli* RNA POLYMERASE

Enzyme	Additions	Activity for T7 RNA synthesis (per cent)
E. coli B RNA polymerase	—	100
	Streptolydigin	4
	Rifamycin	< 1
	Antibody	10
T7 RNA polymerase, fraction 5A	—	100
	Streptolydigin	100
	Rifamycin	100
	Antibody	91
T7 RNA polymerase, fraction 5B	—	100
	Streptolydigin	22 (7)
	Rifamycin	18 (3)
	Antibody	40 (31)

Incorporation of ^{14}C-AMP into T7 RNA was followed as before with the addition of 2×10^{-4} M streptolydigin, 20 μg/ml. rifamycin or 0·02 A_{280} unit of purified antibody to *E. coli* RNA polymerase where noted. With *E. coli* RNA polymerase, T7 RNA polymerase fraction 5A, and 5B, 1·2, 1·1 and 1·5 nmoles of ^{14}C-AMP were incorporated, respectively, in the absence of inhibitors. Values given in parentheses for T7 RNA polymerase fraction 5B are those obtained with dAT copolymer as template.

Purification and Characterization of T7 RNA Polymerase

Preliminary characterization of the T7 RNA polymerase using fraction 5A led to the modification of the assay conditions. In particular, the activity—both in extracts and in purified fractions—is lost if the enzyme is diluted for assay in solutions without high concentrations of BSA. Considering other differences between the T7 RNA polymerase and the *E. coli* enzyme, we believe that this factor accounts for the extensive losses of enzyme activity incurred during purification by procedure I and also for the failure of previous workers to identify T7 RNA polymerase. Using the modified RNA polymerase assay it was found that RNA polymerase activity increases at least thirty-fold after infection by T7. This activity is stable in cell-free extracts for at least 1 week when the extracts are kept at 0° C. A purification for the T7 RNA polymerase was devised using the assay described and adding 20 μg/ml. of rifamycin to all assays. In these conditions the *E. coli* RNA polymerase is not active and one can fractionate selectively for the T7 RNA polymerase activity. Procedure II gives about a 130-fold purification of the initial T7 RNA polymerase activity with a reasonable recovery of total activity. Analysis

* The residual activity seen with fraction 5B in the presence of rifamycin or streptolydigin arises from trace contamination of this fraction by T7 RNA polymerase as shown by the lack of rifamycin resistant activity with dAT as template. The activity of fraction 5B with dAT was also more than 90 per cent sensitive to anti-*E. coli* RNA polymerase antibody when larger amounts of antibody were added.

of the enzyme fractions obtained after chromatography on phosphocellulose using SDS-acrylamide gel electrophoresis (Fig. 1) shows that tubes with the highest enzyme activity contain a single major protein component which has a molecular weight of about 107,000 (Fig. 1). When large amounts of protein from T7 polymerase fraction F are analysed, traces of other proteins are detected (Fig. 1). But fraction F T7 RNA polymerase consists of at least 80 per cent of this single protein component. Protein components which could be subunits of *E. coli* RNA polymerase are present in only trace amounts if at all. The final specific activity of T7 RNA polymerase is extremely high. Although protein determinations by the Lowry procedure are made difficult by the low concentrations of protein in fraction E and by the fact that buffer I interferes with the determination, it is clear that the specific activity of these fractions is about fifteen times higher than that of the *E. coli* RNA polymerase acting with T7 DNA template.

As found for fraction 5A, the purified T7 RNA polymerase is not inhibited by high concentrations of rifamycin (100 μg/ml.), streptolydigin (2×10^{-3} M), or of antibody to *E. coli* RNA polymerase. The enzyme requires Mg^{2+} as a divalent metal ion and is not active when Mn^{2+} replaces Mg^{2+}. T7 RNA polymerase shows optimal activity at 2×10^{-2} M Mg^{2+} as compared with 8×10^{-3} M Mg^{2+} for the *E. coli* enzyme[19]. T7 RNA polymerase is strongly inhibited by KCl at concentrations exceeding 0·05 M and is essentially inactive at 0·15 M KCl. The T7 enzyme shows a remarkable template specificity. Whereas *E. coli* RNA polymerase shows equivalent activities with T7 and T2 DNAs and is highly active with dAT copolymer, T7 RNA polymerase is active only with T7 DNA (Table 4). Denatured T7 DNA is active but much less so, and T2 DNA, λ DNA and dAT copolymer are almost inactive as templates. The homopolymer pair dG·dC is highly active for poly rG synthesis but does not direct poly rC synthesis at all with T7 RNA polymerase. This suggests that the T7 enzyme is highly specific in its recognition of initiation sites on DNA; although such sites occur on T7 DNA, they are lacking on any of the other DNAs we have tested and are not replaced by dAT copolymer. The activity of T7 RNA polymerase on dG·dC suggests that the promoter sites recognized by this enzyme may be C-rich regions of the DNA. Such regions are known to occur in T7 DNA[20].

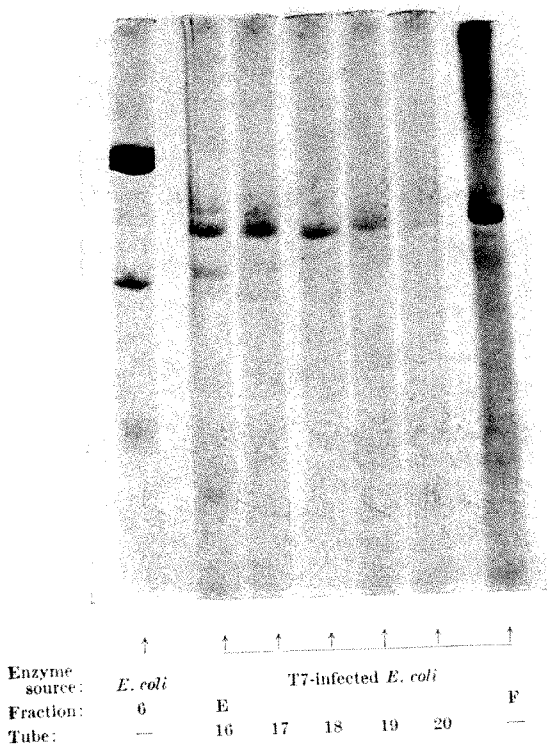


Fig. 1. Analysis of T7 RNA polymerase fractions in SDS-acrylamide gels. Samples contained 8 μg of *E. coli* RNA polymerase fraction 6, 1·5–3 μg of T7 RNA polymerase fractions from phosphocellulose chromatography (fractions E 16–20) or 11 μg of T7 RNA polymerase fraction F. The protein was analysed by gel electrophoresis in SDS-acrylamide gels and was stained with coomassie blue. The peak of T7 RNA polymerase activity eluting from phosphocellulose was found in fractions 17–19 which contained enzyme with a constant specific activity of 120,000 U/mg. Protein in these fractions was concentrated for gel analysis by precipitation with 10 per cent trichloroacetic acid.

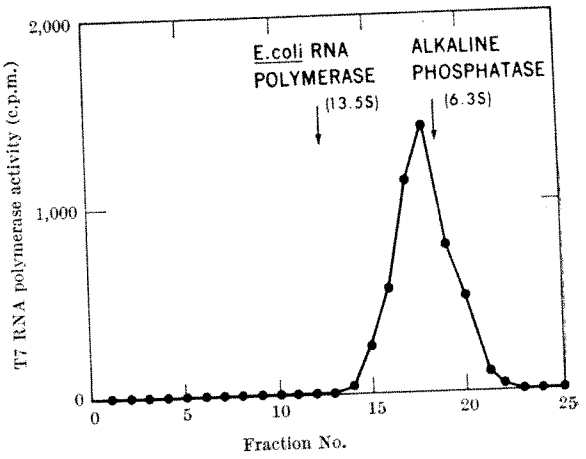


Fig. 2. Zone sedimentation of T7 RNA polymerase in a glycerol gradient. T7 RNA polymerase (33 U), fraction 5A, was layered on a 10–30 per cent glycerol gradient and sedimented at 40,000 r.p.m. for 11·5 h. All solutions contained 1 mg/ml. BSA, 0·2 M KCl and buffer I. Fractions were collected and were analysed for T7 RNA polymerase activity. Sedimentation standards, *E. coli* RNA polymerase fraction 5 and *E. coli* alkaline phosphatase, were run in a duplicate tube during the same run. The arrows indicate the peak position of each.

Table 4. COMPARISON OF ACTIVITIES OF *E. coli* RNA POLYMERASE AND T7 RNA POLYMERASE WITH DIFFERENT DNA TEMPLATES

Template	Specific activity (U/mg) *E. coli* polymerase	T7 polymerase
dAT copolymer	15,000	140
dG·dC	17,000 (^{3}H-GTP)	40,000 (^{3}H-GTP)
	8,700 (^{3}H-CTP)	< 90 (^{3}H-CTP)
T7 DNA	7,700	68,000
Denatured T7 DNA	950	24,000
T2 DNA	6,000	< 60
λ DNA	1,700	300

Where indicated, 40 nmoles of the indicated DNA or 20 nmoles of dAT or dG·dC replaced T7 DNA in the standard assay for T7 RNA polymerase. When dG·dC was used as template, only ^{3}H-CTP and GTP or ^{3}H-GTP and CTP were added as nucleotide substrates. Assays were carried out using 0·15 μg of fraction F T7 RNA polymerase or 0·14 μg of fraction 5 *E. coli* RNA polymerase.

To test the specificity of the T7 RNA polymerase further we determined the strand specificity of the T7 RNA formed by T7 RNA polymerase with native T7 DNA as template. *In vivo*, all T7 RNA seems to be transcribed from the r-strand of T7 DNA[21], so that synthesis of l-strand specific RNA would signal that non-specific transcription was occurring. More than 95 per cent of the T7 RNA formed by T7 RNA polymerase is r-strand specific, indicating that T7 RNA polymerase does initiate RNA chains at specific sites on the T7 template (Table 5).

Analysis of T7 RNA polymerase by sedimentation through a glycerol gradient showed a single peak of enzymatic activity (Fig. 2). Calibration of the gradient[22], using *E. coli* RNA polymerase ($s_{20,w} = 13·5S$[10]) and *E. coli* alkaline phosphatase ($s_{20,w} = 6·3S$[23]) as sedimentation standards, gave an estimate of $s_{20,w} = 7·2S$ for the

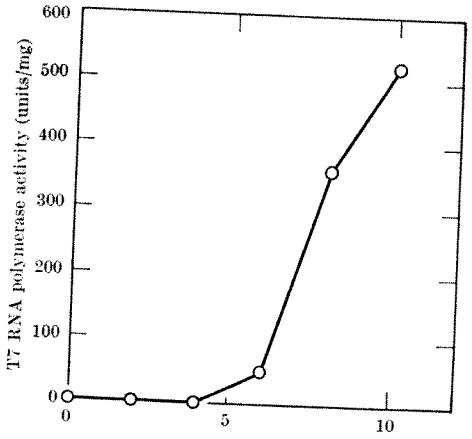


Fig. 3. Induction of T7 RNA polymerase activity after T7 bacteriophage infection. *E. coli* B/1 were grown at 37° C in 1·5 l. of M9 medium containing 5 g/l. of glucose. When cell density reached 1×10^8 cells/ml. the cells were chilled and harvested and were suspended in 28 ml. of M9 medium. An aliquot (2·5 ml.) was taken as an uninfected sample and $7{\cdot}5 \times 10^{13}$ purified T7 phage were added in 0·3 ml. After 10 min at 0° C the cells were diluted into 475 ml. of prewarmed M9 medium which contained 5 g/l. glucose and the culture was shaken vigorously at 30° C. Aliquots (100 ml.) were taken at 2 min intervals and were chilled rapidly by pouring the solution on ice at −15° C. The cells were collected by centrifugation, were suspended in 1 ml. of buffer A, and were disrupted by sonic vibration for 2·5 min at 50 V using a Bronwill sonic vibrator. The extracts were centrifuged and the supernatant fluid was assayed for protein and for T7 RNA polymerase activity. Assay reactions contained 20 μg/ml. of rifamycin and 4×10^{-4} M streptolydigin and used ^{3}H-CTP as the labelled nucleotide. In these conditions *E. coli* RNA polymerase is not active. Uninfected *E. coli* extracts contain about 25 units/mg of RNA polymerase activity with T7 DNA as template, when assayed in similar conditions in the absence of rifamycin.

T7 RNA polymerase. If one assumes that the protein is globular and that $\bar{v} = 0{\cdot}73$, this would correspond to a molecular weight of about 100,000 (ref. 24). This result, taken with the results of SDS-gel analysis, suggests that the native T7 RNA polymerase consists of a single polypeptide chain of molecular weight 100,000–110,000.

Table 5. STRAND SPECIFICITY OF T7 RNA SYNTHESIZED USING T7 RNA POLYMERASE

Enzyme	RNA annealing to T7 DNA (c.p.m.)		Percentage r-strand RNA
	r-strand	l-strand	
E. coli RNA polymerase	1,159	282	80
T7 RNA polymerase	1,045	63	94

T7 RNA was synthesized in a standard T7 RNA polymerase assay scaled up to 1·0 ml. using either 18 μg of fraction 6 *E. coli* RNA polymerase (2 min reaction, 28 nmoles RNA formed) or 4 μg of fraction F T7 RNA polymerase (10 min reaction, 70 nmoles RNA formed). ATP was labelled with ^{32}P at a specific activity of 100,000 c.p.m./nmole. The reaction was terminated with 10 μg of pancreatic deoxyribonuclease, and after 10 min at 37° C the RNA was extracted three times with phenol. Aliquots of the aqueous phase were assayed for acid-insoluble radioactivity and for their ability to anneal to the separated strands of T7 DNA[21] using a modification of the method of Gillespie and Spiegelman[27]. The total amount of RNA annealing to r and l strands was about 30 per cent of that added to the annealing mixture. Similar efficiencies were obtained when annealing either RNA product to T7 DNA which had not been separated into r and l strands.

T7 RNA polymerase did not interact physically with *E. coli* RNA polymerase holoenzyme or core polymerase when the two purified enzymes were mixed and were sedimented together in a glycerol gradient. Furthermore, T7 polymerase did not stimulate the synthetic activity of *E. coli* core RNA polymerase on T7 DNA when the two were mixed in an assay. *E. coli* polymerase holoenzyme did not stimulate synthesis by T7 RNA polymerase with T7 DNA as template. Thus T7 RNA polymerase seems to be a complete polymerase *per se* and neither replaces the *E. coli* RNA polymerase sigma component nor binds to the *E. coli* core polymerase in its stead.

Appearance of T7 RNA Polymerase in Infected Cells

Studier and Maizel[25] have shown that there are two principal classes of T7 induced proteins; early proteins which begin to appear 5–7·5 min after infection at 30° C, and late proteins after about 10 min. To determine to which of these classes the T7 RNA polymerase belongs, *E. coli* B/1 cells were grown in minimal medium with glucose and were infected at 0° C to allow synchronous infection[21]. The infected cells were then diluted into prewarmed medium and samples were taken at intervals to follow the appearance of T7 RNA polymerase activity in extracts. The results (Fig. 3) show that T7 RNA polymerase activity appears 4–6 min after infection. Thus T7 RNA polymerase is an early T7 protein.

Structural Gene for T7 RNA Polymerase

Because only three early genes are currently known for T7 phage[25], it was of interest to determine if any of these might represent the structural gene for T7 RNA polymerase. The size of the T7 polymerase and its ability specifically to transcribe T7 DNA suggested that T7 gene 1 was a likely candidate, because it specifies a protein of about 100,000 molecular weight, the protein is required for the synthesis of late mRNA in T7-infected cells[25,26], and gene 1 mutants are defective in all known late T7 functions. In fact, no other protein of this size is synthesized in T7-infected cells[25]. Because of the availability of a variety of mutant T7 phages[12,4], this possibility was easily tested. Table 6 records the activity of T7 RNA polymerase in extracts of *E. coli* B/1 infected with amber mutants for T7 genes, 1, 2 and 3, and with a T7 mutant which contains a temperature-sensitive mutation in gene 1. Whereas extracts of cells infected with amber mutants for genes 2 and 3 have about half of the normal specific activity of T7 RNA polymerase, the extract from cells infected with an amber mutation in gene 1 has no detectable T7 RNA polymerase activity. When extracts are prepared from cells infected with T7 tsl (342–15) the resulting extract (tsl extract) contains detectable but reduced levels of T7 RNA polymerase. By contrast with the T7 RNA polymerase activity obtained with extracts prepared from cells infected with wild-type T7 (T7+ extract), the activity in a tsl extract was unstable and 50 per cent of the activity was lost after overnight storage. Preliminary experiments showed that the rate of inactivation of T7 RNA polymerase activity in a tsl extract at either 37° C or 45° C was similar to the rate of inactivation found for RNA polymerase from a T7+ extract. The enzymatic activity of T7 RNA polymerase in a tsl extract was, however, strikingly reduced when assays were carried out at 45° C (Table 6). By contrast, the activity of T7 RNA polymerase in a T7+ extract was slightly enhanced when assayed at 45° C relative to 37° C. Thus a temperature-sensitive mutation in gene 1 affects the T7 RNA polymerase protein and seems to lead to reduced enzymatic activity at elevated temperatures, but not to an increased sensitivity to thermal denaturation. These results, taken with the lower initial activity of the enzyme in cells infected with T7 tsl and with the physical and biochemical properties of the T7 RNA polymerase, indicate that the structural gene for T7 RNA polymerase is gene 1.

Table 6. ACTIVITY OF T7 POLYMERASE IN EXTRACTS OF CELLS INFECTED WITH MUTANT T7 PHAGES

Phage	Genotype	T7 polymerase activity (U/mg)	
		37° C	45° C
T7+	Wild type	520	570
T7 tsl (342–15)	Temperature-sensitive, gene 1	48	18
T7 am 23	Gene 1 amber	< 2	—
T7 am 64	Gene 2 amber	290	—
T7 am 29	Gene 3 amber	260	—

100 ml. aliquots of *E. coli* B/1 (a nonpermissive strain for T7 amber mutants) were grown at 30° C in M9 medium containing 5 g/l. glucose to a density of 1×10^8 cells/l. A lysate of T7 phage was added to give a final multiplicity of five and growth was continued for 15 min. Infected cells were harvested, and extracts were prepared and assayed as described in Fig. 3.

Model for Regulation

The identification of T7 gene 1 protein as a new RNA polymerase provides a clear model for the positive control of transcription by T7 phage: the infecting T7 DNA is transcribed initially by the host RNA polymerase giving rise to early T7 mRNA. Translation of this early mRNA leads to T7 RNA polymerase along with two other early proteins of unknown function[25]. The T7 RNA polymerase then carries out the transcription of late T7 mRNA. The question remains how negative control of early T7 genes might occur. Synthesis of gene 1 protein seems to cease about 10 min after infection[25], at a time when late particle proteins are just beginning to appear. Thus T7 may have a mechanism to turn off transcription of early genes. Such a negative control of early transcription could result from modification leading to inactivation of the *E. coli* RNA polymerase by a late phage function, for the host enzyme is no longer needed after synthesis of the T7 RNA polymerase*. Extensive modification of *E. coli* RNA polymerase occurs after infection by T4 phage[28], and may serve a negative control function in the growth of that phage as well.

What implications does the identification of a T7-specific RNA polymerase have for our understanding of the control of transcription in other bacteriophage systems? At least three possible mechanisms have been proposed by which a bacteriophage could initiate transcription of a set of genes which it had not previously transcribed: (1) alteration of the initiation specificity of the host RNA polymerase by modification of the polymerase protein or by the synthesis of a new subunit such as sigma subunit[3]; (2) *de novo* synthesis of a new RNA polymerase having an altered initiation specificity, and (3) synthesis of a protein factor (anti-termination factor) which prevents normal termination of RNA chains and so allows the host RNA polymerase to "read through" to distal genes[29,30]. These mechanisms are not mutually exclusive; in the case of complex bacteriophages such as λ phage and T4 phage, at least three classes of transcriptional units are known, and more than one positive control mechanism may well be used.

Alteration of transcriptional specificity of RNA polymerase by replacement of sigma subunit with a subunit carrying a new initiation specificity has been proposed to occur during growth of T4 and λ phages and during sporulation of *B. subtilis*[31]. Although there is good evidence for modification of bacterial RNA polymerases in some of these systems, there is still no evidence that this modification confers a new transcriptional specificity on the RNA polymerase *in vivo*. In particular, as discussed, negative control mechanisms through which certain genes are turned off are present in all of these systems. It is possible that most modifications of bacterial RNA polymerase are directed to such an end and not toward activation of the enzyme for a new function.

In the case of bacteriophages λ and T4, it seems likely that synthesis of early and delayed early classes of mRNA is mediated directly by the host cell RNA polymerase by a mechanism such as the first or third and not by phage-directed synthesis of a new RNA polymerase. Activation of late transcriptional units in λ and T4 infected cells is, however, more complex. At least one new phage protein is directly required for late transcription in each case, T4 gene 55 (ref. 32) and λ gene Q[33]. Evidence that the host RNA polymerase itself is responsible for late transcription in T4 and λ-infected cells is based primarily on studies which show that phage growth and also mRNA synthesis remain sensitive to rifamycin throughout phage infection[34,35]. By contrast with these results, the growth of T7 phage becomes resistant to rifamycin inhibition after 5 min, the time at which the T7 RNA polymerase appears[4]. One interpretation of the continued sensitivity of late transcription to rifamycin in λ and T4-infected cells is that the host cell RNA polymerase is directly involved in late transcription as proposed by mechanism 1 or 3. Alternatively, our results suggest that late transcription might be carried out by a new phase-specific polymerase as in mechanism 2. Such a late RNA polymerase could be specified by gene 55 and T4 and by gene Q in λ phage. By this hypothesis, the sensitivity of late transcription to rifamycin would be the result of a continued requirement for host cell RNA polymerase for some other phage function. This would result, for example, if a small amount of early or delayed early transcription were continually required for late transcription to continue. In the case of phage λ, some evidence supports this latter possibility, for both N gene function and an active, early r-strand promoter site seem to be continually required for late transcription[36,37], and the N gene product seems to be unstable during growth[38,39]. Thus it is possible that the initiation of transcription of late phage genes by *de novo* synthesis of a bacteriophage specific RNA polymerase may prove to be a general mechanism for the activation of late bacteriophage transcription.

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* T7 RNA polymerase fraction 5B from procedure I seems to be composed of host cell enzyme subunits and is active as an RNA polymerase on both T7 DNA and T2 DNA templates. This does not rule out the possibility that the *E. coli* RNA polymerase is modified or inactivated in T7-infected cells, however, because we cannot yet exclude the possibility that the active polymerase in this fraction is derived entirely from uninfected cells present, in the infected cell population.

Evidence for Premature Termination of Transcription of the Tryptophan Operon in Polarity Mutants of *Escherichia coli*

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The polarity of mutations in the tryptophan operon of *E. coli* may be caused not by an accelerated degradation of untranslated messenger operator-distal to the nonsense codon but by premature termination of transcription at or near the site of mutation and by restriction of RNA polymerase movement beyond it.

A NONSENSE mutation in a gene of a bacterial operon may both inactivate its protein product and decrease the level of synthesis of proteins coded by genes operator-distal to the mutation site. This polarity is associated with a decrease in the amount of mRNA for the operator-distal genes; although some of the mRNA synthesized by the operon corresponds to its whole length, much of it is shorter and corresponds to genes operator-proximal to the mutant site[1,2]. The loss of mRNA from beyond the mutation site could result either from premature termination of transcription at or near the mutation[1,3-6] or from an accelerated degradation of the messenger corresponding to the operator-distal genes[1,6-9].

Previous reports have suggested that strong polar mutations in the first two genes (*E* and *D*) of the tryptophan operon of *Escherichia coli* suffer an accelerated degradation of untranslated messenger distal to the nonsense codon[7,9]. I suggest here, however, that polarity in this operon is caused rather by a premature termination of transcription. First, both strong polar and deletion mutants in the initial region of the first gene of the operon produce labile mRNA which can be detected only by a brief pulse label; this mRNA corresponds only to the region that is operator-proximal to a mutation site. I attribute this to premature termination of transcription near the mutation site. Second, the intragenic initiation of transcription caused by treating bacteria with dinitrophenol[10,11] is less frequent with strong polar than with weak polar mutants. Thus the strong polar mutants must possess a mechanism which limits transcription distal to the mutated site on the operon.

Production of Labile mRNA

I have characterized the labile mRNA synthesized at a steady state of transcription of the *trp* operon in strong polar mutants. It has been reported previously that strong polar mutants such as E9802[7], D9778[7] and D9838[9] produce labile mRNA which is only detectable in a very short period of labelling with ^{3}H-uridine. This possibility was investigated further in this experiment and the results obtained are represented in Fig. 1. A map of the *trp* operon, the nonsense mutations, the deletion mutations and φ80 pt phages used for assay of *trp* mRNA by DNA-RNA hybridization technique[11] is shown in Fig. 2. Pulse-labelling was started at the twentieth minute after derepression to assure a steady state of transcription of the operon. Bacteria were derepressed by transfer from rich medium to minimal medium containing 1·0 per cent glucose and a mixture of nineteen amino-acids at 0·5 mM each, lacking L-tryptophan. They were incubated at 30° C with vigorous shaking[11]. In these conditions, it takes about 6 min to complete a round of transcription of the operon[12].

As shown in Fig. 1, the most pronounced effect of varying the period of pulse labelling on the detectable level of *trp* mRNA is seen with the E mRNA of mutant E9802. The concentration of E mRNA rises as the duration of pulse labelling is decreased, while the levels of E_{distal} mRNA, D mRNA, and C–A mRNA do not. With D9778 and C9941, similar changes in the levels of *trp* mRNA are not observed. The labile mRNA in E9802 does not hybridize with DNA from φ80 pt E_{distal} phage (the *trp* gene segment of which corresponds to the operator-distal 1/3 portion of the E gene[13]) (see also Fig. 2).

Of ten polar mutants of the E gene examined for change of *trp* mRNA levels as a function of length of pulse labelling, six strong polar mutants (E9829, E9851, E10203, E9802, E12242 and E9758) produced detectable amounts of labile E mRNA, while the other mutants did not. Evidently, production of labile mRNA is characteristic of strains with nonsense alterations at the beginning of the operator-proximal gene. Some of these data are presented in Table 1. Short *trp* mRNA of E12242, E9758 and E9903 corresponds to the operator-proximal 45, 53 and 63 per cent of the intact E gene[13]. I have assumed that the molecular size of the short messenger of the polar mutants corresponds to the distance from the beginning of the operon to the site of the mutational alteration[2]. The distance from the mutational site in E12242 or E9758 to the site in E9903 should therefore correspond to only 18 or 10 per cent, respectively, of the E gene. As shown in Table 1, twice as much E12242 and E9758 E mRNA is detectable with very short pulse labellings compared with labelling for 2 min. These labile messengers are not hybridizable with DNA from φ80 pt E_{distal} phage. Thus the bulk of labile mRNA produced by strong polar mutants such as E12242 and E9758 must correspond to the operator-proximal region before the nonsense mutation in the E gene. The other strong polar mutants such as E9851, E9829 and E10203 also produce labile E mRNA which corresponds to the region proximal to the site of the mutational alteration of E9903.

Consistent with this, I have also observed that deletion of the operator-distal half of the E gene does

Table 1. CHANGES IN *trp* mRNA LEVEL IN POLAR MUTANTS AS A FUNCTION OF PERIOD OF PULSE LABELLING

RNA source		Amount of *trp* mRNA synthesized		
Strain	Period of pulse labelling (s)	E mRNA ($\times 10^{-3}$ per cent)	E_{distal} mRNA	Labile E mRNA* ($\times 10^{-3}$ per cent)
E12242	10	413	13	201
	20	404	12	192
	120	212	15	
E9758	10	454	25	230
	20	440	24	216
	120	224	18	
E9903	10	402	54	16
	20	435	60	49
	120	386	62	
E9887	10	448	56	96
	20	434	51	82
	120	352	51	

Tritiated RNA was prepared from bacterial cultures pulse labelled with 200 μCi of ^{3}H-uridine (15 Ci/mmole) for various periods at the tenth minute after derepression. In standard conditions[12], 47–64 μg of E12242 ^{3}H-RNA (specific activity, 2,200–21,900 c.p.m./μg), 49–73 μg of E9758 ^{3}H-RNA (specific activity, 1,700–17,500 c.p.m./μg), 61–76 μg of E9903 ^{3}H-RNA (specific activity, 2,900–19,500 c.p.m./μg) or 58–68 μg of E9887 ^{3}H-RNA (specific activity, 2,100–18,200 c.p.m./μg) were used for each hybridization assay. The other conditions were as described in Fig. 1.

* The *trp* mRNA values of RNA from cultures pulse-labelled for 2 min were subtracted in each case.

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not affect the production of labile messenger by mutant E9829; a double mutant, KAE-44–E9829, which retains the nonsense mutation E9829 operator–proximal to a deletion terminus KAE44 in the E gene (see Fig. 2) produces almost the same amount of labile messenger as E9829. The extent of polarity, in the sense of production of short *trp* mRNA, has been observed to remain as strong in KAE44–E9829 as in the parent strain, E9829 (F. I. and Y. Kano, to be published elsewhere).

The molecular size of *trp* mRNA synthesized during a very short labelling period such as 10 or 20 s was examined with E9802. If the *trp* mRNA synthesized consists of larger molecules than the mRNA detected during a 2 min labelling period because of selective degradation of the messenger segment distal to the nonsense mutation site, the sedimentation profile of these *trp* mRNA preparations should differ. On the contrary, as can be seen in Fig. 3, the sedimentation profiles of the *trp* mRNA isolated following the 10, 20 and 60 s labelling periods were essentially the same. It was not possible therefore to detect the production of larger *trp* mRNA molecules, as the pulse time was decreased.

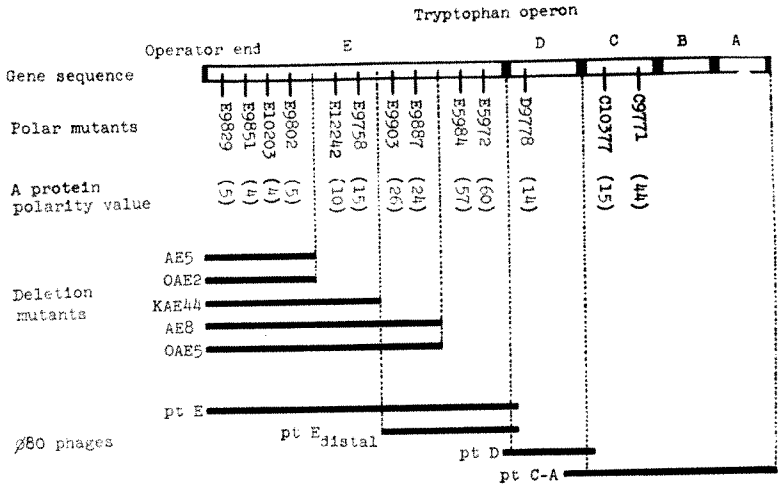


Fig. 2. Genetic map of the *trp* operon and relative polarity values in mutants. The order of mutationally altered sites and the A protein polarity values (in parentheses) were based on previous studies[2,15] and personal communication from C. Yanofsky. A protein polarity value is the percentage of A protein production by the mutant and by the wild type control. *Trp* operon segments carried by the deletion mutant or the φ80 pt are indicated by a solid line. The position of the deletion terminus is based on previous studies[12,19–21]. The relative size of the *trp* genes and distances between the mutant sites are only approximate. Phages were kindly supplied by Drs A. Matsushiro (φ80 and pt E$_{distal}$), S. Deeb and B. Hall (pt C–A), J. P. Gratia (pt E), C. Yanofsky (pt D) and M. Taylor (pt A). Deletion mutants, AE strains, and polar mutants were kindly supplied by Dr C. Yanofsky and deletion mutants, OAE strains, were kindly donated by Dr J. Ito. Deletion mutant, KAE[44], was isolated by Dr Y. Kano.

If transcription of the *trp* operon in strong polar mutants is frequently terminated near the site of the nonsense alteration, the messenger detached prematurely from the operon might be rapidly attacked by nuclease and degraded. When a constant period of pulse labelling is used, the probability of survival of premature messenger might decrease with shortening of the length of the transcribable region. This possibility has been demonstrated in experiments measuring detectable *trp* mRNA as a function of the length of pulse labelling with various deletion mutations of the E gene[13]. The *trp* mRNA produced by the deletion mutant such as AE5, OAE2, AE8 or OAE5 has been determined to correspond to the operator–proximal 33, 40, 63 or 63 per cent, respectively, of the intact E gene[13]. Comparison of the order of the molecular size of the *trp* mRNA from various polar and deletion mutants with the order of the mutational alterations and deletion termini on the genetic map has indicated that *trp* mRNA size increases in relation to the distance from the beginning of the operon to the site of the deletion termini, which indicates that the *trp* mRNA molecules of these deletion mutants have apparently terminated near the site of their deletion termini[13]. Because the nonsense alteration sites of six strong polar E gene mutants that produce labile messenger are located within the operator–proximal 53 per cent of the E gene (to the site of E9758), two deletion mutants, AE5 and OAE2, might also be expected to produce labile messenger. These expectations have been realized: the deletion mutants AE5 and OAE2 exhibit twice or three times as much E mRNA in very brief pulse labelling as in 2 min labelling, and the E mRNA level falls as the length of exposure to ^{3}H-uridine is increased.

The analogous production of labile mRNA in the strong polar and deletion mutants of the E gene suggests that a segment of the *trp* messenger, detached prematurely from the operon in the strong polar mutants as a result of termination of transcription at or near the site of nonsense alteration, is exposed to a critical condition of nucleolytic attack. It is of interest that the stability of messengers detached from the DNA template has been shown in *E. coli* to be lower than those attached to the template[14].

In these experiments, rapid degradation of messenger corresponding to the region proximal to the nonsense alteration occurred only in strong polar mutants with nonsense alterations in the initial region of the E gene. In a previous article[7], Morse and Yanofsky reported accelerated degradation of messenger distal to the nonsense codon of the mutated gene. One E and two D gene polar mutations have been reported to have that effect[7,9].

These contradictory findings can probably be attributed to differences in the two groups of experiments. Morse and Yanofsky used 3-indolyl propionic acid to derepress the *trp* operon in bacteria growing in minimal medium. I have effected derepression by transferring bacteria grown in rich medium to minimal medium lacking trypto-

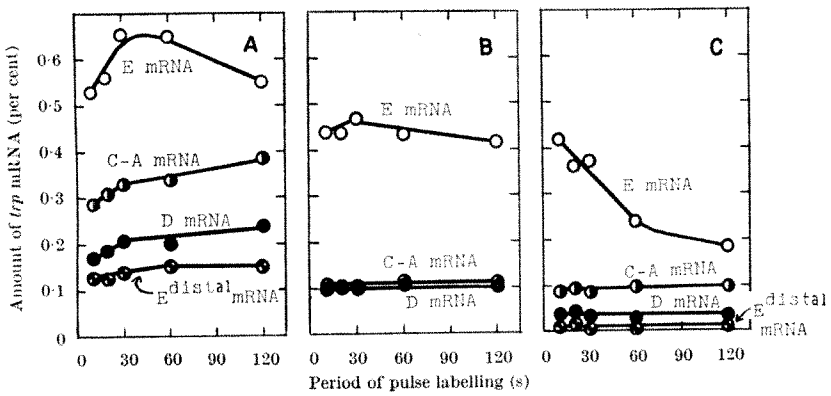


Fig. 1. Changes in *trp* mRNA level as a function of the duration of pulse labelling. Tritiated RNA was prepared from cultures of C9941, a CRM mutant of the C gene (*A*), D9778 (*B*) or E9802 (*C*) pulse labelled with 200 μCi of ^{3}H-uridine (15 Ci/mmole) for various periods indicated at the twentieth minute after derepression. Conditions for derepression are described in the text. In standard conditions[12], 59–65 μg of C9941 ^{3}H-RNA (specific activity, 1,900–12,500 c.p.m./μg), 61–69 μg of D9778 ^{3}H-RNA (specific activity, 2,500–20,500 c.p.m./μg) or 72–93 μg of E9802 ^{3}H-RNA (specific activity, 1,700–11,200 c.p.m./μg) annealed with 5 μg of heat-denatured phage DNA in a 0·25 ml. reaction mixture. The values of *trp* mRNA are represented as percentage of total labelled RNA and are the averages of duplicate determinations. The *trp* mRNA hybridizable specifically with DNA from pt E, pt E$_{distal}$, pt D or pt C–A phages is represented as E mRNA, E$_{distal}$ mRNA, D mRNA or C–A mRNA, respectively. The other conditions of determination and calculation of *trp* mRNA are as reported previously[11,12].

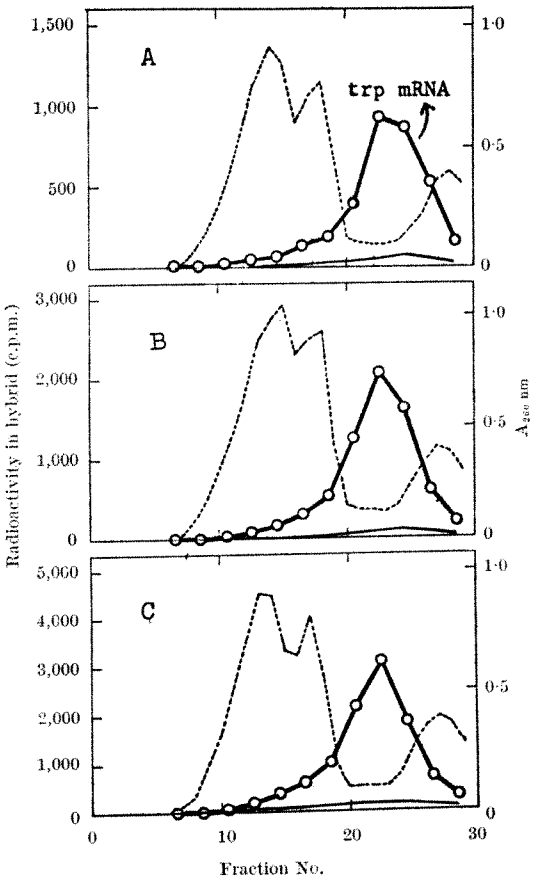


Fig. 3. Comparison of sedimentation profiles of *trp* mRNA from E9802 pulse labelled for various periods at steady state transcription. Pulse labelling was carried out with 500 μCi of ^{3}H-uridine (15 Ci/mmole) for 10 (*A*), 20 (*B*) and 60 (*C*) 20 min after derepression. Tritiated RNA was sedimentated in a 5–30 per cent linear sucrose gradient, containing 0·01 M Tris-HCl (*p*H 7·3) and 0·05 M KCl, for 5 h at 36,000 r.p.m. A three-eighths portion of each fraction was assayed with DNA from pt E phage to determine *trp* mRNA. The other conditions are as described previously[11]. - - - -, Absorbance at 260 nm; ———, hybridization with φ80 DNA (background value); ○—○, difference between hybridization with pt E DNA and φ80 DNA (E mRNA).

phan. In the former conditions, D gene polar mutants have been observed to show rapid degradation of D gene messenger. This effect has never been seen with R^-_{trp} (repressor-deficient) strains carrying D gene polar mutations (personal communication from D. E. Morse), although polarity of this operon has been known to be invariant to the R_{trp} mutation[2,15]. In fact, in the conditions of the present experiments, E9829 and E9758 have been observed to produce labile messenger in the parental R^+_{trp} strains (F. I. and Y. Kano, to be published elsewhere). The disagreement between results might be resolved when further information is obtained about how different techniques of derepressing the *trp* operon affect the degree of polarity.

Differences in *trp* mRNA assay systems might also be responsible for discrepancy in the findings with E9802. Morse and Yanofsky defined $E_{proximal}$ mRNA in terms of the difference between hybridization with DNAs from φ80 pt ED_{25} containing all of the D and E genes, and φ80 pt D_2 containing all of the D and a distal part of the E gene. The *trp* segment of φ80 pt D_2 is known to cover the E gene region distal to the E9851 site (personal communication from C. Yanofsky; refs. 7 and 21). The difference of annealing of RNA to these two DNAs is a small fraction of the annealing with either DNA alone. There are also problems associated with the efficiency of hybridization. Thus these experiments must be regarded as testing the limits of current technical capabilities.

Lower Frequency of Intragenic Initiation

I have investigated the distribution of RNA polymerase molecules over the region of the *trp* region distal to the mutated gene. If bacteria are incubated in derepression conditions with 2,4-dinitrophenol (DNP), which severely but reversibly inhibits transcription of the derepressed operon, the newly synthesized *trp* messengers made after removal of the inhibitor are small in size and contain operator distal information. Transcription of the operon apparently is therefore initiated at sites within the operon[10,11]. These *trp* mRNA molecules are translated with almost normal efficiency[10]. If polarity results from rapid degradation of the segment of the messenger distal to the nonsense alteration which is caused by non-translation of this region of the polycistronic messenger, then after intragenic initiation of transcription in the presence of tryptophan (added to block ordinary initiation at the *trp* promotor), functional messenger could be synthesized from the region distal to the nonsense alteration. On the other hand, if polarity is attributed to premature termination of transcription leading to scanty distribution of polymerase molecules over the distal genes of the operon, then it might be expected that the frequency of intragenic initiation of transcription distal to the nonsense alteration would be diminished assuming that termination involves the dissociation of polymerase from its template.

These predictions were tested in the following experiment: DNP (5×10^{-3} M) was added to a derepressed culture 10 min after derepression (to ensure a steady state distribution of polymerases over the operon) and the culture was further incubated for 45 min at 30° C. During this incubation, *trp* mRNA molecules which had been synthesized before the addition of DNP were detached from the operon and broken down[13]. After the removal of DNP, bacteria were quickly transferred into the pre-warmed standard medium including L-tryptophan[11] and pulse labelled with ^{3}H-uridine for the first minute of incubation. Sedimentation profiles of *trp* mRNA molecules using a CRM mutant (A9952), two strong polar mutants (D9778 and C10377) and a weak polar mutant (C9771) labelled in repression conditions for 1 min immediately after the removal of DNP show that intragenic initiation of transcription has occurred with all these mutants in these conditions[13].

Production of *trp* messenger fragments lacking the operator-proximal segment of the operon, synthesized as a result of intragenic initiation, was determined with five strong polar mutants (E9802, E9829, E9758, D9778 and C10377), two weak polar mutants (E5984 and C9771) and a CRM mutant (C9941). Results (Table 2) show that frequency of intragenic initiations on the operator–distal (C, B and A) genes correlates closely with the extent of polarity: there is the expected reduced amount of messenger distal to the nonsense alteration. Similar experiments

Table 2. COMPARISON OF *trp* mRNA LEVELS OF VARIOUS POLAR MUTANTS IN THE STEADY STATE AND AT THE INITIAL STAGE AFTER INTRAGENIC INITIATION OF TRANSCRIPTION

Strain	Amount of C–A mRNA synthesized	
	In steady state	After intragenic initiation
	($\times 10^{-3}$ per cent)	
E9802	79	68
E9829	77	64
E9758	67	62
E5984	182	210
D9778	77	95
C10377	178	117
C9771	277	210
C9941	332	248

Tritiated RNA was prepared from bacterial cultures pulse labelled with 200 μCi of ^{3}H-uridine (15 Ci/mmole) for 1 min at the twentieth minute after derepression (steady state), or from cultures pulse labelled with 300 μCi of ^{3}H-uridine in conditions of repression during the first minute after the removal of DNP (intragenic initiation)[11]. In intragenic initiation, before the removal of the inhibitor, bacterial cultures were incubated in the presence of DNP (5×10^{-3} M), added at the tenth minute after derepression, for 45 min. In standard conditions[11], 41–60 μg of ^{4}H-RNA (specific activity, 5,800–11,500 c.p.m./μg) (steady state) or 24–38 μg of ^{3}H-RNA (specific activity, 3,300–10,600 c.p.m./μg) (intragenic initiation) was used for one hybridization assay. The other conditions were as described in Fig. 1.

in which only A mRNA was measured (with φ80 pt A DNA) yielded a similar result[13]. Transcription of the operator-distal genes of the *trp* operon is therefore limited in strong polar mutants. It is conceivable that molecules of RNA polymerase wander along the operon without transcribing in the presence of DNP and, upon removal of the inhibitor, resume synthesis of mRNA at any position in the operon. The reduced frequency of intragenic initiation observed with strong polar mutants may be attributed to a reduced number of RNA polymerase molecules on the operator–distal genes of the operon. This possibility is being examined.

Cessation of Transcription

The mechanism proposed to explain premature termination of transcription in the polar mutant demands coupling of transcription and translation[16]. RNA polymerase action is closely tied to ribosome movement, which is halted by a nonsense codon. Transcription is frequently terminated, therefore, when an altered nucleotide sequence in DNA is transcribed as a nonsense codon, making a subsequent region of the messenger untranslatable[3,6]. There is considerable evidence, however, in favour of the presumption that *in vivo* transcription requires neither translation nor ribosome movement[17,18]. It is possible that RNA polymerases progresses beyond the nonsense codon, but that the progress of mRNA synthesis along the region of DNA beyond the nonsense mutation site might be inhibited when the RNA polymerase is enveloped by the internal untranslatable messenger segment free of ribosome[18]. The polymerase molecules entangled with the messenger segments would stick on DNA and jam the region beyond the nonsense codon, thereby inhibiting the progress of the polymerase molecules that follow.

RNA polymerase might perhaps be rescued from messenger envelopment by attachment of ribosomes (or subunits) to the internal messenger segment growing behind the RNA polymerase, even if these ribosomes cannot start to translate the messenger segment because the latter contains no reinitiator signal. The probability that the untranslatable messenger segments overtake the polymerase before the next reinitiator for polypeptide synthesis, where surviving messengers are reloaded, would be proportional to the length of the untranslatable region. The gradient of polarity reflects the frequency with which the polymerase avoids these crises.

Before these hypotheses can be critically examined, the characteristics of normal and abnormal transcription termination should be elucidated in order to arrive at a more complete understanding of the regulatory mechanisms in transcription.

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Chain Termination of Ribosomal RNA Synthesis *in vitro*

by

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An RNA chain termination event in highly purified systems of polymerase and template is as precise as it is *in vivo*. Polyacrylamide gel electrophoresis analysis suggests that other protein factors such as the ρ-factor are not involved in this process.

During the transcription *in vitro* of certain bacteriophage DNAs, the DNA dependent RNA polymerase frequently terminates its synthesis, and in the appropriate conditions releases its RNA product and reinitiates a new cycle of RNA synthesis[1–5]. The chain termination occurs in highly purified systems, apparently without the participation of macromolecules other than the polymerase and its DNA template. It is not clear whether the termination is random on the DNA template or whether it occurs at specific sites similar to those used *in vivo*. When the termination factor ρ is added, the RNA products are of smaller length and are transcripts of more limited regions of the DNA template corresponding to the early and pre-early *in vivo* mRNA species[3,6]. Thus it seems that the ρ-factor mediates a biologically meaningful termination of RNA synthesis. The question remains whether there are other, more basic interactions, between the core RNA polymerase and certain sites or regions on the DNA which lead to the termination of RNA chains in the cell without the involvement of an independent termination factor.

Recently, we have found that ribosomal RNA (rRNA) can be preferentially synthesized *in vitro* on DNA complexes purified from *E. coli*[7,8]. The synthesis occurs as an extension of nascent rRNA chains isolated with the complex and new chains are not initiated[7,8]. The RNA polymerase molecules on the complex have initiated synthesis in the cell, and therefore they should be correctly started on the template. The system would seem to be well suited for studies of rRNA chain termination.

As described below, RNAs of discrete sizes are synthesized in this *in vitro* system, and the major product is homogeneous in polyacrylamide gel electrophoresis. The electrophoretic mobility of the latter RNA is indistinguishable from a labelled RNA obtained from pulse labelled cells. These results suggest that the synthesis of at least

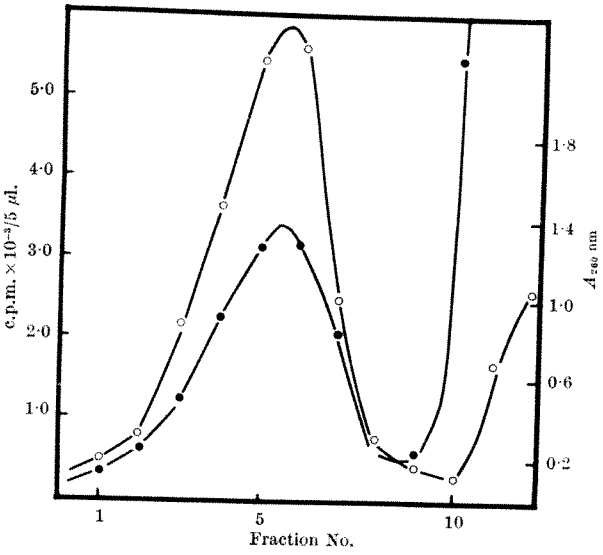


Fig. 1. Isolation of the DNA complex on a sucrose gradient. A culture of D-10 (90 ml.) growing in the log phase was pulse labelled for 10 s with 5-³H-uridine (100 μCi, 8 Ci/mM), quick chilled to 0° C while adding sodium azide to a final concentration of 10 mM, and was rapidly harvested by centrifugation. The bacteria were resuspended at 0° C in 0·2 ml. of a solution containing 20 per cent w/v sucrose, 0·01 M Tris (*p*H = 8·1 at 4° C), 0·1 M NaCl and then mixed with 0·05 ml. of a solution containing 50 mM EDTA, 5 mg/ml. lysozyme, 0·12 M Tris (*p*H = 8·1). After 30 s at 0° C, 0·25 ml. of a solution containing 1 per cent brij 58, 0·4 per cent deoxycholate, 10 mM EDTA and 2·0 M NaCl was added. The mixture was held for about 3 min at 0° C, during which time it cleared considerably but did not become more viscous. The lysate was centrifuged 5 min at 4,000*g* and 4° C to remove debris and the supernatant was layered on a 10–30 per cent w/v sucrose gradient containing 1·0 M NaCl, 0·01 M Tris (*p*H = 8·1 at 25° C), 1 mM EDTA and 1 mM β-mercaptoethanol. Centrifugation was at 17,000 r.p.m. for 10 min at 4° C in an SW50 rotor. The entire procedure was completed within 50 min. ○, Acid precipitable radioactivity; ●, absorbance.

some RNA species is precisely chain terminated *in vitro* at sites or regions on the DNA template similar to those used *in vivo*. It cannot be positively excluded that termination factors bound to the purified complex are responsible for the observed termination, but we can find little evidence of them. Analysis of the proteins of the complex by acrylamide gel electrophoresis reveals that they are predominantly RNA polymerase and from these data we estimate that there can be no more than one molecule of ρ-factor per 40 RNA polymerase molecules (of 13S size).

Preparation and Properties of the DNA Complex

The DNA complex was purified from *E. coli* strain D-10 (ribonuclease I⁻) (ref. 9) by a modification of our previous methods[8] and using some of the lysis procedures developed for the preparation of polyribosomes[10]. The purified complex is about 10 per cent protein by weight and includes nearly all of the DNA and DNA-bound RNA polymerase activity of the cell free lysate[8]. As shown in Fig. 1, nascent RNA chains (identified by pulse labelling the bacteria with ³H-uridine just before lysis) are bound to the complex. Only a small fraction of the pulse labelled RNA sediments free of the complex at the top of the sucrose gradient. This released fraction becomes much larger when the period of labelling is longer. Lysis at a low temperature and minimum preparation times are essential to obtain high yields of undegraded nascent chains on the complex. In general, the precautions are similar to those in preparing undegraded polyribosomes[10]. The DNA of the complex does not have the usual hydrodynamic behaviour of purified DNA, but seems to be folded into a compact structure (sedimentation coefficient is about 4,000S). The physical–chemical properties of this complex will be described in a future communication.

A large fraction of the RNA polymerase on the purified complex is bound to rRNA cistrons; when the complex is incubated in an assay mixture for RNA polymerase, the RNA synthesized is 45 ± 5 per cent rRNA[8]. The rate of synthesis and amount of RNA synthesized is independent of ionic strength[8] in the range 0·20 to 1·2 and is not influenced by agents that inactivate non-initiated RNA polymerase such as heparin[7].

RNA Molecules of Discrete Sizes are Synthesized

RNA was synthesized on the isolated DNA complex, purified and analysed by electrophoresis on acrylamide gels (Fig. 2). The period of synthesis (20 min) was sufficient to complete the reaction[8]. Because rRNA is guanosine rich, approximately 55 per cent of the incorporated ³H-GMP is in rRNA[8]. Two major species of RNA are resolved, both of lesser electrophoretic mobility (larger molecular weight) than the mature 23S rRNA marker. The smaller of these two RNAs appears to be as homogeneous in molecular weight as the mature rRNA markers (see also Fig. 3), while the larger may be somewhat heterogeneous. Only a small fraction of the total RNA product passes through the 2·8 per cent gel to become concentrated in the 7 per cent gel, showing that little of the RNA product is of sizes smaller than 16S rRNA. The mRNA sequences which were synthesized on the complex would be expected to be heterodisperse in size, because there are many different species and the nascent mRNA may be in various stages of degradation[12,13]. These mRNA sequences are probably distributed throughout the gel to account, at least in part, for the high backgrounds and the incomplete resolution of the major peaks.

Another method of labelling the RNA, which consistently gave lower backgrounds and better resolution of the RNA bands, is described in Fig. 3. In this case the nascent RNA on the complex was labelled *in vivo* before isolating the complex and then chains were completed *in vitro* using non-radioactive substrates. As shown in Fig. 3*b*, the same two RNA species as described in Fig. 2 were obtained. In addition, RNA species of mobilities similar to mature 23S rRNA (m23) and the 16S rRNA precursor (p16) (refs. 14–17) are observed. These latter

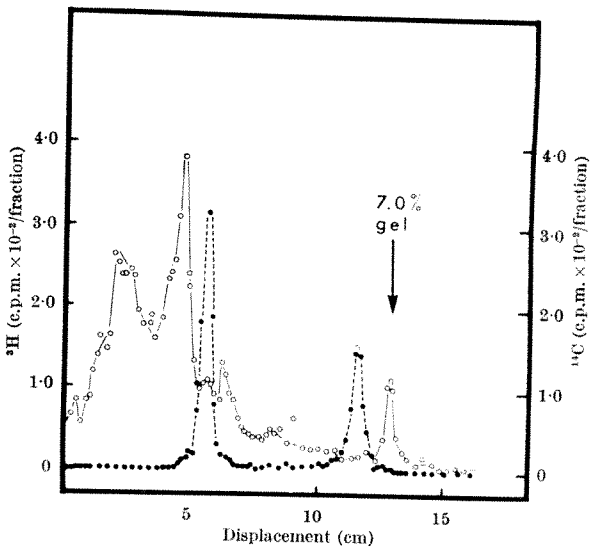


Fig. 2. Polyacrylamide gel electrophoresis of RNA synthesized *in vitro*. DNA complex was isolated from non-radioactive bacteria as described in Fig. 1. A reaction mixture (0·50 ml.) containing 18 μg of the purified complex, 0·02 M Tris (*p*H = 7·8 at 37° C), 12 mM $MgCl_2$, 1 mM EDTA, 1 mM β-mercaptoethanol, approximately 20 per cent w/v sucrose, 1·0 M NaCl, 0·15 mM ³H-GTP (0·6 Ci/mM) and 0·4 mM each of ATP, CTP and UTP was incubated for 20 min at 37° C. The RNA was purified[8], precipitated with ethanol, resuspended in buffer E (containing 0·04 M Tris, 0·02 M sodium acetate, 1 mM EDTA, 0·2 per cent SDS, *p*H = 7·2) and after the addition of sucrose to 20 per cent, it was layered into one slot in a slab of 2·8 per cent polyacrylamide gel overlaid on a slab of 7·0 per cent gel. The gels were made up in buffer E as described by Bishop *et al.*[11], run for 73 V h cm^{-1}, sliced, the slices dissolved in 30 per cent H_2O_2 and counted in an aqueous scintillation reagent. ● - - - ●, 23S and 16S ¹⁴C-rRNA markers (0·25 μg total) run in a separate slot of the same gel; ○—○, ³H-RNA synthesized *in vitro*, 3×10^4 applied (approximately 1 μg) mixed with 8 μg tRNA carrier.

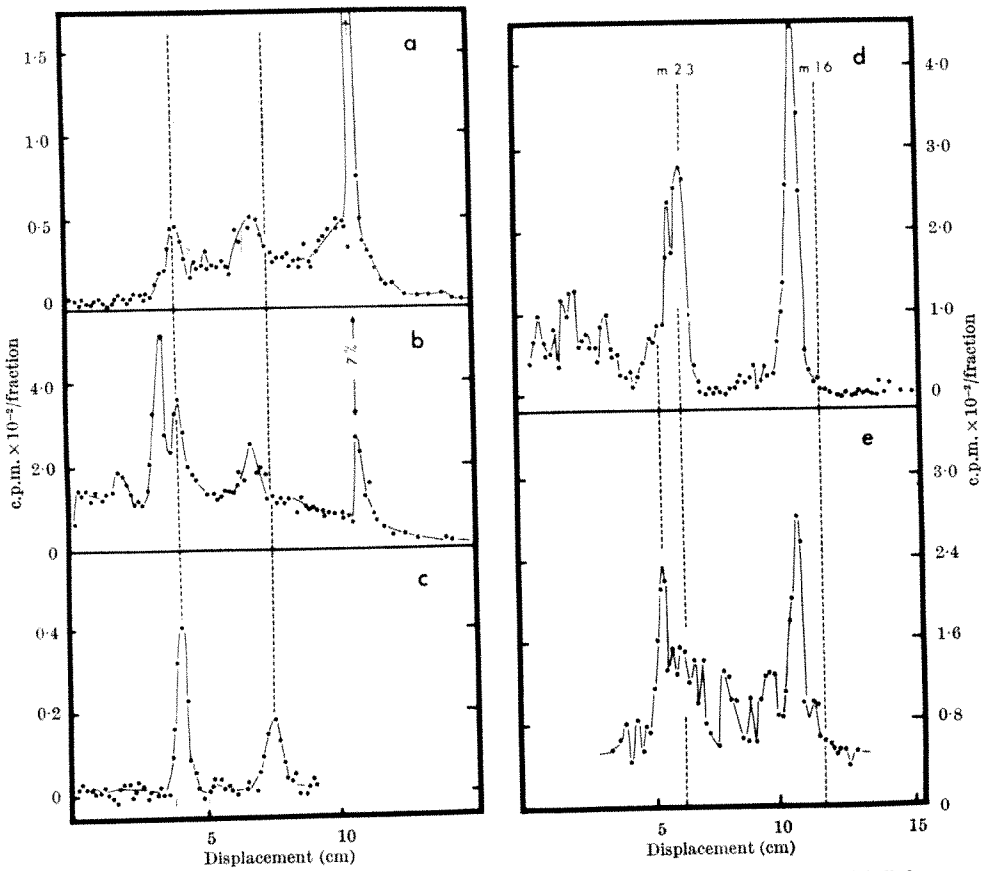


Fig. 3. Electrophoresis on polyacrylamide gel of RNA labelled *in vivo* and of nascent chains labelled *in vivo* and completed *in vitro*. A DNA complex was isolated from bacteria which had been pulse labelled as described in Fig. 1 except that ^{3}H or ^{14}C-guanosine was used. The RNA was purified from the complex and analysed by electrophoresis as described in Fig. 2. *a*, ^{3}H-RNA isolated directly from a portion of the purified complex, duration of pulse label 15 ± 3 s; *b*, ^{3}H-RNA from another portion of the same complex as used in *a* which had been incubated with non-radioactive ATP, CTP, UTP and GTP in a reaction mixture which was otherwise identical to that described in Fig. 2; approximately 3·5 times the amount of ^{3}H-RNA was applied as in *a*; *c*, ^{14}C-rRNA marker (23S and 16S) which were mixed with the RNA of *a*; *d*, released ^{14}C-RNA extracted from fractions 10, 11 and 12 (see Fig. 1) of another preparation which was pulse labelled for 90 s with ^{14}C-guanosine; *e*, nascent ^{14}C-RNA isolated from a complex which had been purified from bacteria which were pulsed for 10 ± 3 s with ^{14}C-guanosine. *a*, *b* and *c* were run on the same gel slab for 53 V h cm^{-1}. *d* and *e* were run on separate gels for 73 V h cm^{-1}; the dotted lines show positions of rRNA markers run together on the same gels. In *a* and *b* the peaks at the discontinuity between the 2·8 per cent and 7 per cent gels are due to concentration at the interface of small molecular weight RNA.

RNAs, however, were neither synthesized nor chain terminated *in vitro*. This is shown by two lines of evidence: (1) we never observe incorporation *in vitro* of a labelled ribonucleotide into RNAs which give well defined electrophoresis bands at the mobilities of p16 or m23 (see Fig. 2, for example); (2) the p16 and m23 molecules are present on the complex before the *in vitro* synthesis (see Figs. 3*a* and 3*e*, for example). Apparently, some newly terminated RNA molecules are retained on the complex; perhaps because there is delay in the normal release after termination or perhaps some newly released RNA is trapped within the folded DNA of the complex.

Relationship to RNA synthesized *in vivo*

The electrophoresis profiles of RNA synthesized *in vivo* which is released from the DNA complex or retained on the complex are shown in Figs. 3*d* and 3*e* respectively. The released RNA labelled during a 90 s pulse has a distribution similar to that previously reported for total pulse labelled RNA[14–17]. Prominent is the m23 rRNA band as well as the rRNA precursor bands p23 and p16 which have mobilities slightly less than m23 and m16, respectively. The RNA retained with the complex following the 10 s pulse label is more heterodisperse, as expected, because this RNA includes nascent chains. The broad distribution in the region of the 23S rRNA marker is strongly weighted toward mobilities smaller than m23 and has a peak at a mobility somewhat less than p23. This peak has only been seen after very short pulse labels; frequently, it is not as well defined as in Fig. 3*e* and appears only as a shoulder on the low mobility side of m23 as in Fig. 3*a*. With longer periods of labelling the m23 band becomes so prominent that it obscures this RNA species. The mobility of this RNA is very close to that of the major RNA species which is chain terminated *in vitro* (see Fig. 4), suggesting that at least one of the RNA species synthesized *in vitro* may be of the same size as a labelled RNA synthesized in the cell. It should be pointed out that pulse labelled RNA such as that described in Fig. 3 is predominantly labelled in rRNA sequences[8,18,19]. It is therefore unlikely that any single mRNA species would give rise to bands of peak heights comparable with those of rRNA.

Estimated Molecular Weights

The relative electrophoretic mobility of RNA on polyacrylamide gel is linearly related to the logarithm of its molecular weight[11]. In Fig. 4 the mobilities of the *in vitro* synthesized RNAs are compared with the known rRNAs m23, m16, p23 and p16. These data indicate that the two major RNA species which are chain terminated *in vitro* on the protein–DNA complex have molecular weights of $1{\cdot}25 \times 10^{6}$ and $1{\cdot}7 \pm 0{\cdot}15 \times 10^{6}$ daltons. The error range of the larger RNA reflects the possible heterogeneity in the electrophoresis band. The molecular weights of the p23 and p16 species from the same data

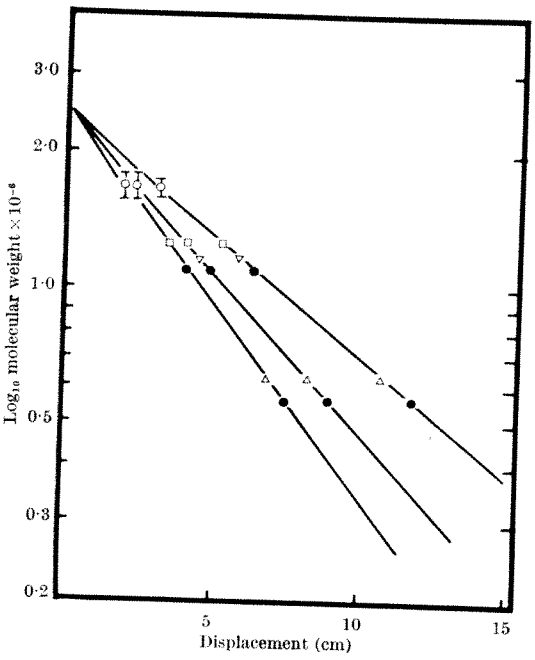


Fig. 4. Estimation of molecular weight of *in vitro* RNA products and *in vivo* rRNA precursors. The relative electrophoretic mobilities of various RNAs compared with mature rRNA markers are plotted. Three independent determinations taken from the data of Fig. 3 as well as two other experiments are shown above. ●—●, 23S rRNA and 16S rRNA taken as $1{\cdot}1 \times 10^6$ and $0{\cdot}56 \times 10^6$ daltons respectively; △, p16 synthesized *in vivo*; ▽, p23 synthesized *in vivo*; □ and ○, the two major RNAs synthesized *in vitro*.

are $1{\cdot}17 \times 10^6$ and $0{\cdot}62 \times 10^6$ daltons, respectively. The above figures are relative to m23 and m16 markers which may have slight anomalies in their mobilities[11].

Proteins of the Purified Complex

The finding that RNAs of discrete sizes are synthesized on the purified DNA complex suggests that precise chain termination events occur during the synthesis. We have tried to bring evidence to bear on the possibility that factors other than the RNA polymerase and DNA of the complex participate in this termination. The conditions of isolation of the complex are quite demanding in that only molecules sedimenting at $4{,}000 \pm 500$S are selected. Molecules bound to the complex must remain tightly associated in the presence of 1·0 M NaCl. The known proteins of *E. coli* which bind to DNA are dissociated at these high ionic strengths[20]; only RNA polymerase which has initiated synthesis is known to be an exception[21,22]. In addition, the conditions of synthesis are such that any chain termination factor must be active at ionic strengths greater than 1·0 in order to influence the size of the products. The ρ-factor is known to be inactive at ionic strengths greater than 0·15 (ref. 23).

The proteins of the purified complex have been isolated and analysed on polyacrylamide gels in the presence of SDS (Fig. 5). The major bands correspond to the α, β and β′ subunits of RNA polymerase[26,27]. Little or no σ-factor protein is observed, which is consistent with previous findings *in vitro*[28,29] that the σ-factor is released from the RNA polymerase core after initiation. Our results suggest that the σ-factor also recycles *in vivo* on the *E. coli* DNA template. The fractionation described in Fig. 5 reveals only one minor protein component other than those of RNA polymerase; but when the gels are overloaded with protein (approximately ten-fold greater in amount than in Fig. 5*b*) several additional minor bands do appear. The largest of these in the region of the gel between α and σ (the region expected for the ρ-factor[3]) had a scanning area less than 0·06 of that of α. If we assume that the ratio of stain to protein is the same for all proteins in the region of α, the published values for molecular weights and subunit compositions of rho[3] and RNA polymerase[26,27], then we estimate that less than one ρ-factor per 40 RNA polymerase (13S size) molecules is on the complex.

These results are in agreement with those of Millette[30], who has shown that the major transcription product of purified RNA polymerase on T7 DNA template is a single homogeneous RNA. In this work it was estimated that the amount of ρ-factor contaminating the RNA polymerase preparation was less than one ρ-factor per 50 polymerase molecules. Also, Roberts[3] has observed RNA chain termination at specific regions of λ DNA templates in the absence of added ρ-factor. It therefore seems likely that there is one kind of chain termination in which RNA polymerase recognizes certain sites on its DNA template and, independent of other components, specifically terminates its synthesis. There are probably then, at least two different processes in the cell for RNA chain termination.

Ribosomal RNA Synthesis

As presented in this communication, the relationship of the RNA species synthesized *in vitro* to rRNA synthesis is circumstantial. That is, we depend on previous findings that the primary product of the *in vitro* system is rRNA[8].

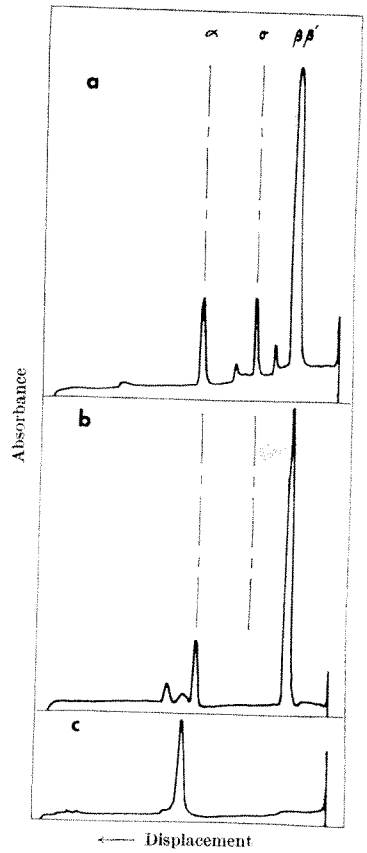


Fig. 5. Polyacrylamide gel electrophoresis of the proteins from the purified DNA complex. The DNA complex, isolated by a scaled-up modification of the procedure described in Fig. 1 (to obtain larger amounts of complex), was degraded with pancreatic deoxyribonuclease and applied to a 'Biogel P-4' column. Material alluting with the excluded volume was lyophilized, redissolved in a buffer containing 0·01 M sodium phosphate (*p*H 7·2), 0·1 per cent SDS, 0·14 M β-mercaptoethanol, heated for 10 min at 65° C, and run for 20 V h cm^{-1} on 5 per cent polyacrylamide gels as described by Shapiro *et al.*[24]. The gels were stained with coomassie blue, destained with trichloroacetic acid, and scanned with a Densicord recording electrophoresis densitometer which had been calibrated with protein standards in gels to give band areas linearly related to protein concentration. Direct tracings of the actual scans are shown above. The β and β′ bands which were visually distinct were not resolved by the densitometer. *a*, RNA polymerase (5 μg) purified by the double glycerol gradient method of Burgess[25]; the minor band between β and σ is the commonly observed contaminant τ[26,27]; the minor band between σ and α is another contaminant. *b*, Proteins isolated from the purified complex (about 5 μg); the two minor bands to the left of α are, respectively, deoxyribonuclease added to degrade the complex and a non-RNA polymerase protein present with the complex. *c*, Pancreatic deoxyribonuclease run in a separate gel.

Recently, however, we have isolated the RNA of the two major electrophoresis bands (as in Fig. 2) and observed that this RNA can compete with rRNA during DNA–RNA hybridization. These experiments will be described in a future publication.

The overall significance of the observation that rRNA synthesized *in vitro* is larger than the known rRNA precursors is not yet clear. The smaller of the *in vitro* rRNAs is similar in electrophoretic mobility to a labelled RNA obtained from pulse labelled bacteria, suggesting that it may correspond to an rRNA precursor synthesized in the cell. This ostensible precursor is about 7 per cent larger than the p23 rRNA precursor previously reported[15–17] and observed here. 23S rRNA may be the product of at least two steps of maturation similar to the 16S rRNA for which two precursor steps are indicated[16]. The *in vitro* rRNA of $1{\cdot}7 \pm 0{\cdot}15 \times 10^6$ daltons cannot yet be correlated with known rRNA precursors. Two possible explanations for this RNA have occurred to us: (1) It may result from polymerase molecules which pass a termination signal and respond thereafter to an adjacent termination site. This might happen, for example, if the missed site is one requiring an independent termination factor such as the ρ-factor. (2) In the cell the "unit of transcription" for rRNA may include both the sequences of 16S and 23S rRNA and the primary transcription product may be cleaved before the completion of its 3′ end by a specific nuclease into sequences containing only p23 or p16. Thus polymerase molecules which had not passed the "cleavage region" or which missed the termination site on the DNA complex would synthesize large precursors *in vitro*, while those which had passed the region would synthesize the smaller rRNA molecules of molecular weight $1{\cdot}25 \times 10^6$ daltons. These considerations are consistent with the relative sizes of the observed rRNA molecules, that is, the sum of the molecular weights of p16 and the smaller *in vitro* rRNA are about equal to that of the larger *in vitro* rRNA. Either of these two considerations could also explain the observation that while the sequences of 16S rRNA are synthesized *in vitro*[8], molecules of the size of p16 or m16 are not. It would then be required that the 16S rRNA region of the primary transcription product always occurs on the 5′ side of the cleavage region or missed termination site. It is pertinent to this discussion that the 23S and 16S rRNA genes are very closely linked and probably alternating in *B. subtilis*[31] (which is similar to *E. coli* in many aspects of rRNA synthesis and maturation[15]). Furthermore, either of the above explanations predict that the fraction of an incorporated radioactive label which appears in the larger rRNA synthesized *in vitro* will be less when labelling occurs *in vivo* (as in Fig. 3*b*) than when the label is incorporated *in vitro* (as in Fig. 2). We have consistently observed this predicted difference (for example, the experiments of Figs. 2 and 3*b*).

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Selective Binding of Ribosomes to Initiation Sites on Single-stranded DNA from Bacterial Viruses

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Bacterial ribosomes can bind to initiation sites on single-stranded DNA acting as messenger. Factors are synthesized during phage development which are required for binding to late gene sites but not to early gene sites. They may be involved in the control of translation.

Ribosomes are known to bind to specific sites on mRNA. For example, in the presence of protein initiation factors, f-Met-tRNA$_f$, and the proper Mg^{2+} concentration, ribosomes bind only to the three sites on R17 phage RNA which are used as initiation sites *in vivo* for polypeptide synthesis[1]. Each binding site contains the triplet AUG which has been shown to code for the first amino-acid incorporated into protein, N-formyl-methionine[2]. The triplet AUG is not sufficient by itself to create an initiation site because many internal AUG triplets exist which are not used as initiation sites. Some other feature, possibly the nucleotide sequence and secondary structure around the initiation triplet, must also be involved in the recognition of the true initiation region by the ribosome and initiation factors. Single-stranded DNA can function as a template for the incorporation of amino-acids into

protein in the presence of the antibiotic neomycin. Neomycin, however, appears to be necessary only for the extensive incorporation of amino-acids. The binding reaction between factors, ribosomes, tRNA and single-stranded DNA to form the initiation complex does not require neomycin[4,5] and short oligopeptides can be synthesized in the absence of neomycin[6].

Do ribosomes bind to the specific sites on single-stranded DNA that correspond to initiation sites on mRNA? Because mRNA is a complementary copy of one of the two strands of DNA, one strand of DNA contains the base sequences corresponding to those in mRNA which constitute the binding sites, except that thymine is substituted for uracil and therefore mRNA and single-stranded DNA may have in common whatever features constitute the binding site. If so, the use of DNA as an analogue for mRNA would facilitate study of the initiation complex because DNA can be readily isolated in a pure, undamaged form from phage particles whereas mRNA must either be isolated from the cell as a heterogeneous and partly degraded mixture of phage and bacterial mRNA species or synthesized *in vitro* using RNA polymerase. Neither of these two procedures is entirely satisfactory at present.

A long standing problem of intracellular phage development is the time-dependent changes in protein synthesis that occur after infection. Some phage-induced proteins are synthesized early after infection (early proteins) while the synthesis of others (late proteins) is delayed. Recent studies have demonstrated that there are several categories of phage-specific mRNA formed after infection. The host sigma factor and host RNA polymerase are used for the synthesis of the first mRNA species[7,8]. RNA species synthesized later depend on the synthesis of a new phage-specific sigma factor which, with the host "core" RNA polymerase, initiates transcription of these RNA species[9,10].

While transcriptional control by sigma factors is sufficient to explain the early–late phenomenon, recent work has suggested that there may be some form of translational control as well. It was found that ribosomes from T4-infected cells are unable to translate messenger obtained from RNA phages, although they translate T4 mRNA, and that this effect is connected with the synthesis of one or more new T4-induced proteins[11,12]. Translational control might plausibly be expected to occur at the initiation step. For example, *E. coli* ribosomes might be unable to bind to initiation sites for late T4 messengers unless modified, possibly by the synthesis of new binding factors, during the course of phage development.

We show here that *E. coli* ribosomes bind selectively to only one of the two separated strands of T7 DNA, that strand which contains within it sequences corresponding to initiation sites in T7 mRNA. We cannot discriminate in these experiments between early and late T7 genes. With phage T4, however, we can show that ribosomes from uninfected cells will bind to DNA containing sequences corresponding to initiation sites on early messenger but not to such sequences for late messenger. Preliminary results indicate that ribosomes from T4-infected cells do bind to DNA containing initiation sequences for late messenger.

Purification of Single-stranded DNA

The individual strands of DNA obtained from T7, lambda and T4 bacteriophage particles were purified by complexing the denatured DNA with poly rU,G. One of the two strands has been shown to bind more poly rU,G and to have, therefore, a greater buoyant density in CsCl than its complement[13] (Fig. 1*a*). We will use the term "heavy" to refer to the DNA strand which bands at the greater density and "light" to refer to the complementary strand without regard to the species of DNA used or whether it contains early genes, late genes or both. Poly rU,G contains GUG sequences which function

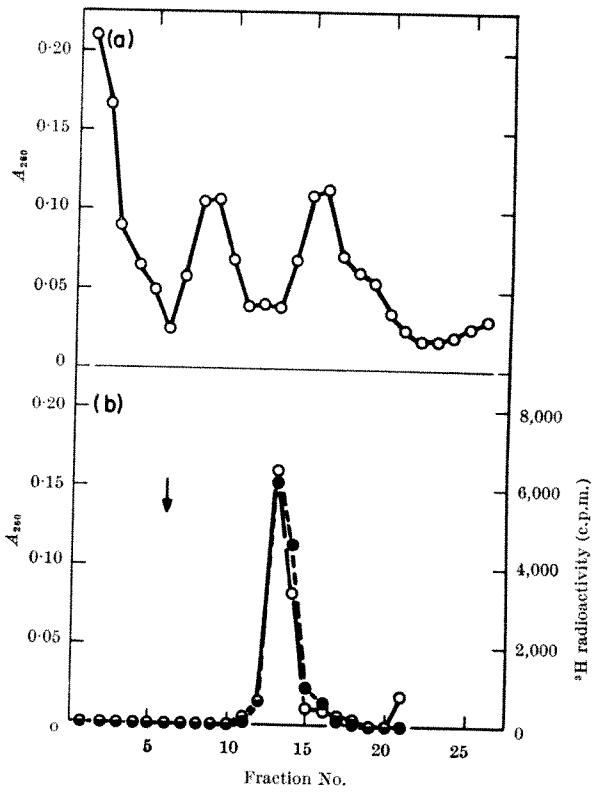


Fig. 1. Equilibrium CsCl density gradient centrifugation of T7 DNA. *a*, Separation of heavy and light strands of T7 DNA. 10^{13} T7 phage particles were dialysed against 0·02 M Tris buffer (*p*H 7·4) and 0·01 M NaCl in a volume of 0·5 ml. EDTA to 0·01 M and Sarkosyl to 0·1 per cent were added and the phage particles were lysed by heating at 60° C for 30 min. NaOH to 0·2 M was added to denature the DNA. The solution was diluted to 4·5 ml. with 0·05 M Tris (*p*H 7·4) and enough 1 M HCl was added to neutralize 90 per cent of the NaOH. One mg of poly rU,G (Miles) was added, followed by solid CsCl (Harshaw) to a refractive index at 1·4052. The solution was centrifuged for 60 h in the Spinco fixed angle 65 rotor at 18° C, 35,000 r.p.m. One-tenth of each fraction (20 μl.) was used to determine the A_{260}. *b*, Rebanding of heavy strands of T7 DNA after alkaline hydrolysis. Heavy strands of T7 DNA were dialysed in 0·01 M Tris (*p*H 7·9), 0·001 M EDTA and 0·01 M NaCl. NaOH (1M) was added to a final concentration of 0·2 M and the DNA was incubated at room temperature for 24 h. The NaOH was neutralized with Tris-HCl buffer and dialysed in 0·005 M Tris (*p*H 7·9) and 0·0005 M EDTA. The DNA was then mixed with denatured ^{3}H-T7 DNA, and CsCl was added to a refractive index at 1·4033. Both heavy and light strands banded at the same density after alkaline hydrolysis relative to denatured ^{3}H-T7 DNA, centrifuged either in the same gradient or in separate gradients. (*a* and *b* are not directly comparable because of the different initial densities.) The arrow indicates the position of the heavy DNA after denaturation and prompt neutralization which was ineffective in removing bound poly rU, G. ○—○, A_{260} of heavy strands of T7 DNA; ●– – –●, radioactivity of denatured ^{3}H-T7 DNA.

as well as AUG sequences for the formation of complexes with ribosomes and f-Met-tRNA$_f$ (ref. 14), and therefore it was necessary to remove the residual poly rU,G present both bound and unbound to the DNA. We first attempted to do this by exposing the DNA to denaturing conditions (0·2 N NaOH for 5 min) followed by banding in CsCl. The DNA, however, banded at a higher density than denatured T7 DNA, indicating that the treatment did not remove bound poly rU,G. Alkaline hydrolysis (0·2 N NaOH for 24 h at room temperature) was sufficient to hydrolyse the poly rU,G. After hydrolysis, both strands now banded at the same density in CsCl as denatured T7 DNA which had not been exposed to poly rU,G (Fig. 1*b*). Using poly rU,G (1 : 3) obtained from Schwartz Bioresearch, it was necessary to treat the DNA solutions further after alkaline hydrolysis with ribonuclease (0·1 μg/ml.) for 15 min at room temperature. The ribonuclease activity was then destroyed by a brief re-exposure to 0·2 N NaOH. We found that ribonuclease alone, without alkaline hydrolysis, was insufficient to destroy all the poly rU,G.

Table 1. SINGLE-STRANDED PHAGE DNA-SPECIFIC BINDING OF ^{14}C-f-Met-tRNA$_f$ TO RIBOSOMES

Nucleic acid added	^{14}C-f-Met-tRNA$_f$ bound pmoles	Δ pmoles	Binding sites per single-stranded nucleic acid
—	0·95	—	—
MS2 RNA, 7 μg	3·82	2·87	0·41
T7 DNA, light strand 4·7 μg	2·98	2·03	5·18
T7 DNA, heavy strand 4·7 μg	0·93	—	—
T7 DNA, native double strands 9·5 μg	1·01	0·06	—
λ DNA, light strand 4·7 μg	2·00	1·05	3·69
λ DNA, heavy strand 4·7 μg	1·87	0·92	3·23

The reaction mixture in the presence of 5 mM Mg^{2+} was incubated at 35° C for 15 min and radioactivity retained by a 'Millipore' filter was counted[16]. The reaction mixture, in a total volume of 0·4 ml., contained: 100 μg NH_4Cl-washed ribosomes from *E. coli* 238 cells (endonuclease$^-$); 10 μg crude initiation factors; 6,000 c.p.m. ^{14}C-f-Met-tRNA$_f$ (150 c.p.m. = 1 pmole); 20 μmoles of Tris (pH 7·2) and 2 μmoles of magnesium acetate; 20 μmoles of NH_4Cl; 0·1 μmole of GTP. Nucleic acid was added as indicated. All single-stranded DNAs used in this and following experiments were treated with alkaline hydrolysis and ribonuclease.

Binding of Ribosomes to Single-stranded DNA

We have used single-stranded DNA from phage T7 to demonstrate specific binding of ribosomes. All the mRNA transcribed from T7 DNA *in vivo* has been shown to be complementary to only the heavy strand of the DNA[15]. Consequently we would expect the light strand to contain all the sequences corresponding to T7 mRNA and therefore to contain all the ribosome binding sites. The experiments described in Table 1 show that the light strand but not the heavy strand of T7 DNA on a 'Millipore' filter retains significant amounts of radioactive f-Met-tRNA$_f$ (ref. 16). The amount of f-Met-tRNA$_f$ retained is roughly proportional to the amount of nucleic acid added. Single-stranded DNA was specifically required, for no binding was detected using native double-stranded T7 DNA (Table 1). Retention was not affected by ribonuclease treatment, but was completely abolished by deoxyribonuclease, indicating that the ribosome was bound to DNA (Table 2*a*). Retention was dependent on the addition of ribosomes and on the presence of protein initiation factors known to be required for the formation of specific initiation complex with mRNA (Table 2*b*). No binding was detected using three other radioactive aminoacyl tRNAs, Lys-tRNA, N-acetyl-Phe-tRNA and Phe-tRNA, indicating that the complex forms specifically with f-Met-tRNA$_f$ (Table 2*c*).

We believe that the heavy strand of T7 DNA does not bind f-Met-tRNA$_f$ in a complex with ribosomes because it lacks the specific sites needed for ribosome binding. One alternative explanation might be that the heavy strand does not contain any ATG triplets (which are needed for complementary interaction with the anti-codon site of f-Met-tRNA$_f$). This possibility is unlikely because we found that both strands of T7 DNA bind met-tRNA equally when the Mg^{2+} concentration is raised (Table 2*d*). It is known that at high Mg^{2+} concentrations, ribosomes can bind non-specifically to many sites on mRNA, although the codon specificity for the tRNA is retained[17]. We conclude, therefore, that the heavy strand of T7 DNA must indeed contain ATG sequences which can be recognized in the abnormal conditions of high Mg^{2+} concentrations.

Phage lambda, unlike phage T7, used both strands of DNA for the synthesis of mRNA, some mRNA molecules being derived from the heavy strand and some from the light strand. Early mRNA in particular is known to be derived from both strands[18]. We would therefore predict that both heavy and light strands of lambda DNA should bind ribosomes and f-Met-tRNA$_f$. This prediction was confirmed by the experiment shown in Table 1 where it can be seen that both strands cause the retention of equal amounts of f-Met-tRNA$_f$. Thus, in agreement with the expectation, with one phage, T7, only one strand participated in complex formation while with another, lambda, both strands participated.

Early messengers of phage T4 are transcribed from the heavy strand and late messengers from the light strand[19]. Consequently, the ribosome binding sites for late genes are contained primarily in the heavy DNA strand. Using ribosomes, factors and f-Met-tRNA$_f$ from uninfected cells, we found that the heavy strand (late genes) fails to bind f-Met-tRNA$_f$ while the light strand (early genes) does bind f-Met-tRNA (Table 3*a*). This implies that some new component must be synthesized during phage development to allow ribosomes to bind to initiation sites for late genes. We have conducted some preliminary experiments to test this possibility. Using unwashed ribosomes isolated 22 min after infection with T4 phage, we found that these ribosomes can bind to the heavy strand (late genes) but cannot bind to the light strand (early genes). These ribosomes also do not bind well to MS2 RNA (Table 3*b*).

From the known specific activity of the radioactive f-Met-tRNA$_f$ and the amount and molecular weight of the added DNA, we can estimate the number of binding sites per single strand. We find about 0·5 binding sites per MS2 RNA molecule, in general agreement with previous results showing that ribosomes can bind to only one initiation site on unfragmented MS2 RNA, that site being the coat protein gene[16]. We find about five sites on the light strand of T7 DNA (Table 1). This number is considerably smaller than the twenty-five to thirty known proteins[20] and also smaller than the twelve *in vivo* RNA species found[21]. It is, however, about equal to the number of early T7 genes transcribed in the absence of the T7-specific sigma factor[20]. We find three to four sites on the heavy strand of λ DNA and an equal number of sites on the light strand, again in rough agreement with the distribution of early genes between the two strands[18].

We found about twelve sites on the light strand (early genes) of T4 DNA using ribosomes and factors from

Table 2. SPECIFICITY OF PHAGE DNA-DIRECTED BINDING OF ^{14}C-f-Met-tRNA$_f$ TO RIBOSOMES

(*a*) Effect of deoxyribonuclease treatment

Nucleic acid added	^{14}C-f-Met-tRNA$_f$ bound, c.p.m. No deoxyribonuclease	Deoxyribonuclease treated
—	88	82
MS2 RNA, 7μg	426	340
T7 DNA, light strand 3·3 μg	187	97
T7 DNA, heavy strand 3·3 μg	91	93
λ DNA, light strand 3·3 μg	167	90
λ DNA, heavy strand 3·3 μg	169	86

(*b*) Requirement for initiation factors

Nucleic acid added	^{14}C-f-Met-tRNA$_f$ bound, c.p.m. −Ribosomes −Factors	+Ribosomes −Factors	+Ribosomes +Factors
—	26	51	83
MS2 RNA, 7 μg	25	131	476
T7 DNA, light strand 4·7 μg	32	110	344

(*c*) Specificity for f-Met-tRNA$_f$

Nucleic acid added	Aminoacyl-tRNA bound, c.p.m. f-Met-tRNA$_f$ (^{14}C)	Lys-tRNA (^{14}C)	Acetyl-Phe tRNA (^{3}H)	Phe-tRNA (^{3}H)
—	46	78	39	65
MS2 RNA, 5 μg	227	98	39	67
T7 DNA, light strand 2·3 μg	151	94	45	58
T7 DNA, heavy strand 2·3 μg	48	59	51	59
λ DNA, light strand 2·3 μg	122	89	45	66
λ DNA, heavy strand 2·3 μg	110	83	30	83

(*d*) Effect of Mg^{2+} concentrations

Nucleic acid added	^{14}C-f-Met-tRNA$_f$ bound, c.p.m. 5 mM Mg^{2+}	^{14}C-Met-tRNA bound, c.p.m. 20 mM Mg^{2+}
—	60	113
MS2 RNA, 5 μg	191	244
T7 DNA, light strand 2·3 μg	164	246
T7 DNA, heavy strand 2·3 μg	94	224

The reaction mixture in a total volume of 0·4 ml. in the presence of 5 mM Mg^{2+} was the same as described in Table 1 and incubated at 35° C for 15 min.

(*a*) Nucleic acid was treated with 0·1 μg deoxyribonuclease at 35° C for 10 min just before the reaction.

(*c*) 5 μl. each of ^{14}C-Lys-tRNA (1,400 c.p.m./μl.), ^{3}H-acetyl-Phe-tRNA (800 c.p.m./μl.) and ^{3}H-Phe-tRNA (1,100 c.p.m./μl.) was added where indicated instead of ^{14}C-f-Met-tRNA$_f$.

(*d*) At 20 mM Mg^{2+}, ^{14}C-Met-tRNA (5,000 c.p.m.; 90 c.p.m. = 1 pmole) was added instead of ^{14}C-f-Met-tRNA$_f$.

Table 3. T4 PHAGE DNA-SPECIFIC BINDING OF ^{14}C-f-MET-tRNA$_f$ TO RIBOSOMES

Source of ribosomes	Nucleic acid added	^{14}C-f-Met-tRNA$_f$ bound, pmoles	Δ pmoles	Binding site per single-stranded nucleic acid
(*a*) Uninfected cells	—	0·70	—	—
	MS2 RNA, 14 μg	7·16	6·46	0·46
	T4 DNA, light strand 4·7 μg	1·62	0·92	11·74
	T4 DNA, heavy strand 4·7 μg	0·72	0·02	—
(*b*) T4-infected cells	—	0·68	—	—
	MS2 RNA, 14 μg	1·31	0·63	0·045
	T4 DNA, light strand 4·7 μg	0·67	—	—
	T4 DNA, heavy strand 4·7 μg	1·26	0·58	7·40

Conditions for the reaction were the same as described in Table 1, except 5,000 c.p.m. ^{14}C-f-Met-tRNA$_f$ (100 c.p.m. = 1 pmole) was used. Single-stranded T4 DNAs used in this experiment were treated with alkaline hydrolysis and ribonuclease. Ribosomes (100 μg) washed with NH_4Cl and initiation factors (10 μg) were present in (*a*), and ribosomes (100 μg) prepared from T4-infected cells (22 min after the infection) and not treated with NH_4Cl were present in (*b*).

uninfected cells (Table 3*a*). There are at least twenty to twenty-five early genes in T4, which fall into several subdivisions of early proteins. It is not known whether all of these early proteins can be synthesized *in vitro* using factors from uninfected cells. Several T4 proteins which are, however, transcribed from the strand containing early genes have been synthesized *in vitro* using initiation factors from uninfected cells[22–24]. Most early proteins may use host initiation factors. Using ribosomes from T4-infected cells, we find less than ten sites on the heavy strand (late genes) of T4 DNA (Table 3*b*). This number is somewhat smaller than the number of late genes, which is about fifty.

We conclude that ribosomes can bind to specific sites in single-stranded DNA, presumably corresponding to initiation sites in mRNA, for ribosomes bind to the light strand of T7 DNA but not to the heavy strand. The experiments with T4 DNA suggest that ribosomes from uninfected cells bind only to initiation sites for early genes and that some new component is synthesized during phage development, allowing ribosomes to bind to initiation sites for late genes. Our results with ribosomes and factors obtained from T4-infected cells indicate that these ribosomes can bind to sites for late genes but not to sites for early genes.

We feel that the use of DNA rather than mRNA offers a simplified approach to several problems in addition to the question of translation control. Mutants of the *lac* operon have been isolated[25] which contain polypeptide reinitiation sites. By using transducing phages containing these sites, it should be possible to determine if these mutations create new ribosome binding sites. It may also be possible to sequence the binding sites and to investigate the mechanism of initiation of protein synthesis in mammalian systems using mammalian virus DNA.

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Translation of a Purified DNA-RNA Complex

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A eukaryotic DNA–RNA complex, which seems to be a transcription complex, can be translated *in vitro* by a cell-free system from *E. coli*.

THE occurrence of a DNA–RNA complex in several strains of *Euglena gracilis* and in a related organism, *Astasia longa*, has been demonstrated in this laboratory, and methods for the isolation and purification of this complex have been reported[1–3]. Spiegelman, Hall and Storch[4] were the first to identify a DNA–RNA complex in viral infected *Escherichia coli*. On the basis of the experimental data then available, they postulated that the "complex" represented a stage of transcription during which specific "informational" RNA molecules were formed on the viral DNA template. Since then, a number of complexes containing RNA have been reported in many types of cell[5–10]. In most cases, the investigators either assumed or gave indirect evidence to show that the RNA contained in the complex was messenger RNA. Recently, Jachymczyk and Cherry[11] reported that a fraction of RNA from peanut seedlings containing free DNA and DNA–RNA stimulated the incorporation of amino-acids into trichloroacetic acid (TCA) insoluble material when added to a cell-free protein-synthesizing system. It is not clear from this study[11], however, whether the stimulation was due to the DNA–RNA (presumably a complex) or to the free DNA. The latter is possible because there are reports of DNA acting as a direct template for protein synthesis in some systems[12,13]. Here we demonstrate that a purified DNA–RNA complex from

E. gracilis and from *A. longa* is translated very efficiently in a cell-free protein-synthesizing system from *E. coli*.

Cells used were two photosynthetic forms (strain Z and variety bacillaris) of *E. gracilis*, a streptomycin-bleached, chloroplast-free strain (SM–L1) of *E. gracilis*, and the closely related, naturally colourless, chloroplast-free flagellate, *A. longa* (strain J). All cells were grown in the dark on a defined medium[14] as described[15]. In some experiments, the photosynthetic *Euglena* were also grown under constant light at room temperature on defined medium[14] without the carbon source (acetate).

The total RNA from all cell types was isolated by the phenol technique[15]. The isolated total RNA was extracted with 1·0 M NaCl to give "saline-soluble RNA", and the DNA–RNA complex was isolated from the latter by precipitation with isopropanol[1–3]. The composition of the complex from strain SM–L1 *Euglena* is 52 per cent RNA, 31 per cent DNA and 17 per cent protein[1,2].

The S-30 fraction from *E. coli* A19 was extracted and pre-incubated according to Clark *et al.*[16] except that the pre-incubation time was increased from 40 min to 60 min. The final preparation was stored as small aliquots in liquid nitrogen until used for amino-acid incorporation studies. Assays were run at 37° C in 0·05 ml. final volumes. Turnip yellow mosaic virus (TYMV) RNA was used as a standard and was prepared as described by J. M. Clark, jun. (personal communication).

Protein-synthesizing Activity

During the initial stages of standardization of the cell-free system, various parameters such as amounts of template RNA, of the S-30 fraction, and of the amino-acids were adjusted to arrive at optimal conditions. For TYMV RNA, the conditions used by Clark *et al.*[16] were found to be optimal. The TYMV RNA in these conditions[16], therefore, was used as a standard reference in subsequent experiments. For *Euglena*, 20–25 μg of total RNA and other RNA fractions (see tables) or 10 μg of DNA–RNA complex gave maximum activity when 0·02 ml. of the S-30 fraction was used. The activity of the system with either TYMV or various *Euglena* templates was dependent on the addition of ATP, GTP and twenty amino-acids. For example, omission of five L-^{12}C-amino-acids lowered the activity by about 75–80 per cent.

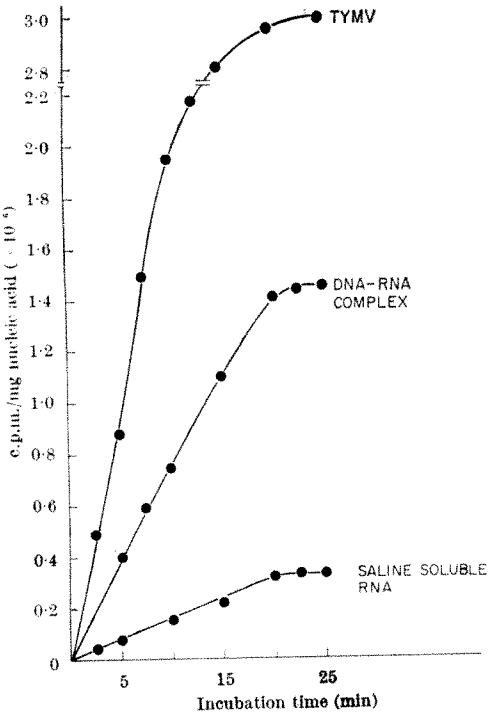


Fig. 1. Template activity and time of incubation of TYMV RNA and two fractions from strain SM–L1 *Euglena*. Details are given in Table 1.

Table 1. PROTEIN-SYNTHESIZING ACTIVITY OF RNA FRACTIONS FROM STRAIN SM–L1 *Euglena*

Fraction	c.p.m./mg nucleic acid
Total RNA	$6·0–8·0 \times 10^4$
Saline-insoluble RNA	$2·0–3·5 \times 10^3$
Saline-soluble RNA	$3·9–4·0 \times 10^5$
DNA–RNA complex	$1·3–1·5 \times 10^6$
TYMV RNA	$2·5–3·1 \times 10^6$

Assays were run at 37° C for 25 min in final volumes of 0·05 ml. The reaction mixture was modified from Clark *et al.*[16] and included the following: 0·04 M Tris-HCl (*p*H 7·8), 0·0125 M magnesium acetate · 4 H_2O, 0·04 M KCl, 0·01 M β-mercaptoethanol, 0·01 M NH_4Cl, 0·006 M phosphoenolpyruvate (Sigma), 0·0015 M ATP (Sigma), 0·003 M GTP (Sigma), 0·1 mM polyethylene glycol 4,000 (Union Carbide Chemical Co.), 0·5 μCi algal hydrolysate containing fifteen ^{14}C-amino-acids (New England Nuclear, 1·8 μCi/mg), 4×10^{-5} M each of L-^{12}C-amino-acids (asparagine, glutamine, tryptophan, methionine and cysteine), 2·5 μg of crystalline pyruvate kinase (Sigma), 0·01 ml. or 0·02 ml. of the S-30 fraction as desired and 0–40 μg of TYMV RNA or various fractions of *Euglena* RNA. The S-30 fraction was extracted from *E. coli* A19 and pre-incubated according to Clark *et al.*[16] except that the pre-incubation time was increased from 40 min to 60 min. The amount of radioactivity incorporated into the TCA-insoluble fraction was measured as described[16]. The blank—complete system minus added template—was usually between 600–900 c.p.m. for the 0·05 ml. system and was subtracted from the above values. The amounts of nucleic acid were determined by A_{260} readings (1·0 *A* unit = 40 μg).

Amino-acid incorporation by the TYMV RNA and by the DNA–RNA complex and the saline-soluble RNA from strain SM–L1 *Euglena* was maximal between 20 and 25 min (Fig. 1). In subsequent experiments, the time of reaction was therefore kept at 25 min. Total RNA isolated by our usual procedure[15] from strain SM–L1 *Euglena* had considerable activity and most of this activity was extractable in 1·0 M saline (Table 1). Saline-insoluble RNA had a low activity whereas the DNA–RNA complex exhibited about twenty times higher activity than the total RNA. Electrophoretic patterns of the total RNA, the saline-soluble fraction, and the DNA–RNA complex are shown in Fig. 2.

To locate the species of RNA molecules which incorporated labelled amino-acids into the TCA-insoluble fraction, the individual regions designated as 1, 2, and so on in Fig. 2*a*, *b* were separated at 0°–4° C and then assayed for template activity. The activity (Table 2) was localized in regions 1 and 4 of the total RNA and in regions 1 and 2 in the saline-soluble RNA (that is, hRNA and 13S RNA, Fig. 2*a*,*b*). The other fractions—regions 2, 3 and 5 of total RNA and region 3 from saline-soluble RNA (22S, 17S and 4S RNA, Fig. 2*a*,*b*)—did not show activity. The DNA–RNA complex (Fig. 2*c*), which is from the 13S region of total RNA[1–3], had the highest template activity (Table 2). These results on template activity of *Euglena* RNA agree with the $^{32}P_i$-labelling experiments reported previously[17,18]. Experiments on the template activity of hRNA, which does not contain any DNA or protein[1], will be considered in detail elsewhere.

Other workers using a cell-free system detected template activity in preparations of ribosomal RNA from *Euglena*[19]. The RNAs of these preparations[19], however, were not completely resolved because, for example, only a single rRNA was reported from the cytoplasm of *Euglena*. It is therefore difficult to compare these earlier results[19] with the present findings. Regions 2 and 3 in Fig. 2*a* are ribosomal RNA[15,17] and these regions do not have any template activity (Table 2).

Effects of Drugs and Nucleases

DNA in the presence of certain drugs, for example streptomycin, can initiate amino-acid incorporation in some systems[12,13]. The effect of drugs on the translation of the complex was therefore checked. Actinomycin D did not affect the activity as expected (Table 3). Cycloheximide, which is inhibitory to eukaryotic cell-free systems[20], also did not affect the activity. By contrast, both puromycin and streptomycin strongly inhibited the activity. Further, none of the drugs used (Table 3) either initiated or enhanced the activity of the complex,

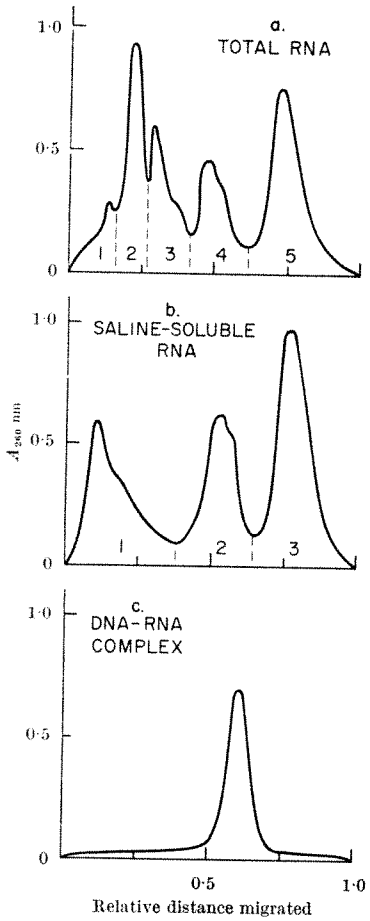


Fig. 2. Electrophoretic pattern of the total RNA (*a*), the saline-soluble RNA (*b*) and the DNA–RNA complex (*c*) from strain SM–L1 *Euglena*. About 4·0 mg of total RNA was electrophoresed on forty polyacrylamide gels[17] (100 μg per gel); each gel was carefully marked and cut as shown by the dotted lines into five regions (*a*); the corresponding pieces from all forty gels were pooled and the RNA was eluted in 100 ml. of 5 mM Tris–HCl (*p*H 7·4) and precipitated with chilled ethanol; the RNA precipitate was dried with nitrogen gas and dissolved in 5 mM Tris-HCl (*p*H 7·4) at a final concentration of 2 mg/ml. Regions 1 to 5 represent the hRNA, 22S, 17S, 13S and 4S RNAs, respectively, of *Euglena* total RNA[1,17]. The same technique was used to separate the saline-soluble RNA into three fractions (*b*). Regions 1 to 3 represent the hRNA, 13S and 4S RNAs respectively[1].

a finding which excluded the possibility of this activity being the type obtained with DNA[12,13].

As judged by electrophoretic patterns on polyacrylamide gels, the DNA–RNA complex is resistant to the action of ribonuclease and its absorbance increases on treatment with deoxyribonuclease[1]. Also, ribonuclease was without effect on the translation of the complex (Table 4). This resistance to ribonuclease is probably due to the structural assembly of the complex, but the complex lost more than 60 per cent of its activity on treatment with deoxyribonuclease (Table 4). This result indicates that a structural alteration in the complex, as reflected in hyperchromicity, leads to biological inactivation. When ribonuclease was added directly to the cell-free system, the complex showed very low activity as expected[22].

Table 2. TEMPLATE ACTIVITIES OF THE INDIVIDUAL RNA REGIONS OF FIG. 2

RNA	Region	c.p.m./mg nucleic acid
Total RNA (Fig. 2*a*)	1	4×10^4
	2	Negligible
	3	Negligible
	4	8×10^4
	5	Negligible
Saline-soluble RNA (Fig. 2*b*)	1	4×10^4
	2	$1{\cdot}2 \times 10^5$
	3	Negligible
DNA–RNA complex (Fig. 2*c*)		$1{\cdot}3 \times 10^6$

Individual regions from total RNA (1 to 5) and from saline-soluble RNA (1 to 3) were obtained as described in Fig. 2. These RNAs and also the DNA–RNA complex which has only one peak (Fig. 2*c*) were assayed for template activity as described in Table 1. The term "negligible" means that the c.p.m. were less than twice the c.p.m. of the 0·05 ml. blank.

Table 3. EFFECTS OF DRUGS ON PROTEIN SYNTHESIZING ACTIVITY OF THE DNA–RNA COMPLEX

Drug added	c.p.m./mg nucleic acid
None	$1{\cdot}30 \times 10^6$
Actinomycin D	$1{\cdot}25 \times 10^6$
Cycloheximide	$1{\cdot}31 \times 10^6$
Streptomycin	$5{\cdot}20 \times 10^5$
Puromycin	$3{\cdot}10 \times 10^4$

Drugs were added (20 μg/0·05 ml. reaction mixture) at zero time. Correction has been made for the quenching effect of actinomycin D. Other details were as in Table 1.

Table 4. EFFECT OF NUCLEASES ON PROTEIN-SYNTHESIZING ACTIVITY OF THE DNA–RNA COMPLEX

Treatment	c.p.m./mg nucleic acid
None	$1{\cdot}40 \times 10^6$
Ribonuclease	$1{\cdot}35 \times 10^6$
Deoxyribonuclease	$0{\cdot}52 \times 10^6$

The complex was treated with Worthington bovine–pancreatic deoxyribonuclease (ribonuclease-free) and Worthington bovine–pancreatic ribonuclease (deoxyribonuclease-free) as described[1]. After 20 min incubation at 2°–4° C, the reaction was stopped by adding 0·01 ml. of diethyl pyrocarbonate[21] (K & K laboratories) to each 0·2 ml. of reaction mixture. The mixture was treated with chilled ethanol. The precipitate thus obtained was dried with nitrogen gas, dissolved in 5 mM Tris buffer[1], and assayed as in Table 1.

DNA–RNA Complex from *Astasia* and Various *Euglena*

Assays for protein-synthesizing activity (Table 5) were carried out using the DNA–RNA complex isolated[1] from two wild-type strains of *Euglena* (both grown in the dark as well as in the light) and also from another flagellate, *A. longa*, which is naturally chloroplast-free. The activity of the complex was about the same in all cases ($0{\cdot}9 \times 10^6$–$1{\cdot}2 \times 10^6$ c.p.m./mg nucleic acid), indicating that the activity is not strain specific or dependent on the presence of chloroplasts.

Table 5. TEMPLATE ACTIVITY OF THE DNA–RNA COMPLEX ISOLATED FROM WILD-TYPE *Euglena* AND FROM *A. longa*

Strain	c.p.m./mg nucleic acid
Euglena bacillaris*	$1{\cdot}0 \times 10^6$
Euglena Z*	$1{\cdot}2 \times 10^6$
Euglena bacillaris†	$1{\cdot}1 \times 10^6$
Euglena Z†	$1{\cdot}2 \times 10^6$
*Astasia longa**	$0{\cdot}9 \times 10^6$

* Grown in the dark with acetate as carbon source.
† Grown in the light without acetate. Details were as given in Table 1.

Saline extraction offers a good method for separating tRNA and those species of RNA (both hRNA and the DNA–RNA complex) which have template activity from the rest of the RNAs (Fig. 2, Tables 1, 2). DNA–RNA complexes can then be isolated from the saline-soluble RNA, and such complexes have comparable template activity in all cases (Tables 1, 5). This method is applicable to *Euglena* cells, whether or not they contain chloroplasts, as well as to another flagellate, *A. longa*, which lacks chloroplasts (Tables 1, 2, 5). Similar results on the preferential extraction of template RNA with saline were reported in the case of rabbit globin messenger RNA[23]. Our results clearly show that the DNA–RNA complex isolated from various *Euglena* strains and from *Astasia* was translated very efficiently in a cell-free protein-synthesizing system. The activity of the complex was about 50 per cent of that of the highly active TYMV RNA and, further, was maximal in salt concentrations which were optimal for the TYMV RNA. The results with deoxyribonuclease indicated that translation of the complex is affected by structural alterations. In recent experiments, the bacterial message for β-galactosidase was transcribed and translated *in vitro*[24]. In the present work, it seems that we have isolated eukaryotic DNA in the

process of transcription *in vivo* and have translated the resultant RNA *in vitro*. Experiments are under way to elucidate the exact mechanism of translation of the complex on the ribosomes.

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Mechanism of Inhibitory Action of Histones on DNA Template Activity *in vitro*

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If RNA is synthesized on the chromatin deoxyribonucleoprotein (DNP) template, the chains formed are four to five times shorter than in RNA synthesized on a DNA template. This correlates with the decrease of the period of elongation. After the removal of histone FI from DNP the RNA chains synthesized are as long as or longer than those formed on free DNA. Histone FI seems to prevent the movement of RNA polymerase along the DNA.

It is well known that complete chromatin deoxyribonucleoprotein obtained at low ionic strength (DNP_0) is a poor template compared with free DNA, usually making about ten times less RNA[1-4]. What is the mechanism of this decrease and which steps of the RNA polymerase reaction are inhibited by proteins combined with DNA? The RNA polymerase reaction has several stages: binding of the enzyme with the template, initiation of RNA synthesis, chain elongation and termination[5-6].

To find out whether histones interfere in this process we have studied the synthesis of DNA on calf thymus DNA and DNP templates, using RNA polymerase isolated from *E. coli*, UTP randomly labelled with ^{14}C, and ATP or GTP labelled with ^{32}P in the γ-position. The incorporation of ^{14}C gave information about the total RNA synthesis, and that of ^{32}P told us the number of chains initiated[7]. In some experiments rifampicin was used to inhibit the reinitiation of RNA chains[8,9], for even at low ionic strength there is some reinitiation with GTP[10].

In this article S is taken as full RNA synthesis in moles of nucleotides related to 1 mole of DNA nucleotides. It was calculated by multiplying the amount of ^{14}C-UMP incorporated by 3·4 as calf DNA contains about 29 per cent of dAMP. n is the total number of initiated chains (in moles) also related to 1 mole of DNA nucleotides. It is the sum of ^{32}P-ATP and ^{32}P-GTP incorporation. The value of n gives information about enzyme binding and initiation. l is average chain length of nucleotides, so that $l = S/n$. It depends on the rate of elongation (V) and time of elongation (t)—$V = \Delta l/\Delta t$. It can be determined more precisely using rifampicin. V usually decreases with the elongation of the RNA chain[10].

As well as these parameters one can also determine t', the time of initiation, and V', the rate of initiation ($V' = \Delta n/\Delta t$).

DNA Synthesis on DNA and DNP Templates

Table 1 shows the principal parameters of RNA synthesis on different templates. As Maitra and Hurwitz[7] have shown, RNA chains synthesized *in vitro* are initiated with pppA or pppG. But a difference between the initiation kinetics of ATP and GTP was found: with ATP, initiation is completed during the first 10 min, whereas initiation with GTP continues for a long time. It depends on the reinitiation of the chains, beginning with GTP. Additional factors (similar to σ) may be responsible for the difference between initiation with ATP and GTP.

Table 1. PRINCIPAL PARAMETERS OF RNA SYNTHESIS ON DIFFERENT TEMPLATES

Template	S after 33 (34) min: Without rifampicin	S after 33 (34) min: With rifampicin	$n \times 10^6$: After 3 (4) min	$n \times 10^6$: After 33 (34) min	l* After 33–34 min	V For 0–8 (9) min interval	t (min)
DNA	0·50	0·79	300	494	1,700	130	>30
DNP_0	0·023	0·072	58	173	400	140	≦3
$DNP_{0.6}$	0·32	0·52	137	450	2,300	144	>30
DNPur	0·040	0·14	86	164	470	—	~5–6

* Values of l are taken from experiments when rifampicin was added. In general they are very close to values obtained without rifampicin.

The value for V, determined using rifampicin, is about 130 nucleotides/min (for the first 8 min). It should be noted that samples were incubated at 25° C which would decrease the rate of RNA synthesis (Fig. 1).

The value of n is 300 in experimental conditions, which means that one RNA polymerase molecule combines with a DNA piece of about 1×10^6 daltons. This figure is not far from those obtained by Pettijohn and Kamiya[11] using DNA saturated with enzyme.

RNA synthesis on the DNP_0 template (Fig. 2) is about ten times slower than on free DNA. This depends first on the sharp decrease (four to five times) of l. This may be a result of the decrease of t or V. The use of rifampicin confirmed the first suggestion. After the addition of rifampicin no further increase of l was observed. The value of t is not more than 3 min. It is impossible to determine V precisely from such experiments, but if we assume t to be 3 min and l to be 400 (Table 1), then V is at least 140 nucleotides/min. Thus the rate of elongation with the DNP_0 template is of the same order of that with DNA. Thus RNA is synthesized on DNP_0 and DNA at a similar rate, but it is stopped earlier on DNP_0 because of obstacles to the movement of RNA polymerase.

The value of n is also decreased on DNP_0 (two to three times after 30 min of incubation; after a short incubation this difference is much greater). This is probably a result of decrease of V', whereas t' increases. Such initiation kinetics may simply depend on the lower availability of the template for the enzyme, for the DNP is precipitated in the conditions of the RNA polymerase reaction.

In general the decrease in the rate of binding and initiation influences the total RNA synthesis less than the early stopping of chain elongation.

RNA Synthesis on DNP extracted by Sodium Chloride

The removal of histone F1 (according to Johns's classification of histones[17]) by 0·6 M NaCl extraction leads to a sharp increase of DNP template activity. On the other hand, RNA synthesized on such DNP ($DNP_{0\cdot6}$) could not be distinguished from RNA synthesized on DNA by its hybridization properties[4,12]. Although some non-histone proteins are also extracted from chromatin by 0·6 M NaCl, the reconstitution experiments indicated that of the extracted proteins only histone F1 inhibits RNA synthesis[14].

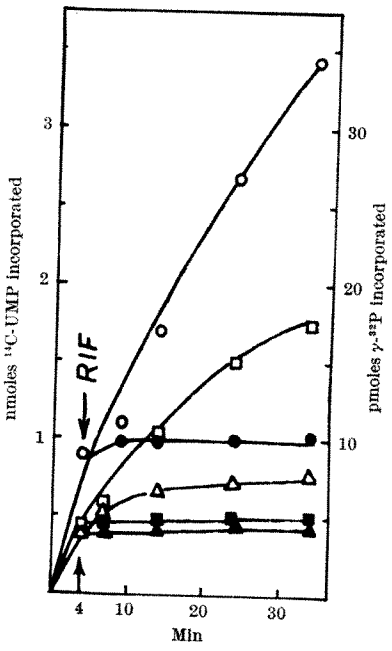


Fig. 2. RNA synthesis on the DNP_0 template with and without rifampicin. Conditions were the same as for Fig. 1 except that DNP_0 (containing 50 μg of DNA) was added instead of DNA, and the time of preincubation lasted 4 min before the addition of rifampicin. Symbols are as in Fig. 1.

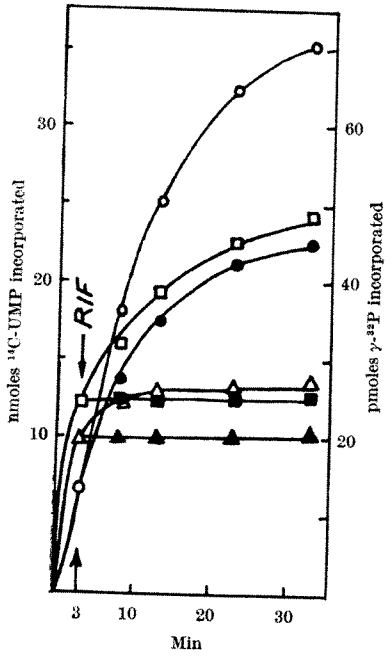


Fig. 1. RNA synthesis on the DNA template with or without rifampicin. Each tube contained in 0·5 ml.: 20 μmoles of Tris-HCl (pH 8·1); 2 μmoles of $MgCl_2$; 0·5 μmoles of $MnCl_2$; 0·2 μmoles each of ^{14}C-UTP, specific activity 200 counts/nmole, γ-^{32}P-ATP and γ-^{32}P-GTP-specific activity 100–200 counts/pmole; 5·4 μmoles of mercaptoethanol; 50 μg of DNA and 22 U of RNA polymerase (1 U gives rise to the incorporation of 1 nmole of UMP in 10 min at 25° C). RNA polymerase was isolated from *E. coli* by the method of Burgess[15] with slight modifications—it contained σ factor. γ-^{32}P-ATP and GTP were synthesized according to Penefsky *et al.*[16]. Samples were incubated at 25° C. Rifampicin was added to half of the sample 3 min after the start of incubation. The reaction was stopped by the addition of 1·5 ml. of 0·5 per cent sodium dodecyl sulphate in 0·01 M pyrophosphate (pH 8). RNA was deproteinized by phenol and chloroform, purified by gel filtration through 'Sephadex G-25', washed with 5 per cent trichloroacetic acid on a 'Millipore' filter and counted in a Geiger counter. ^{32}P and ^{14}C were separated by using a corresponding filter. Samples 1 (○), 3 (△) and 5 (□) were incubated without rifampicin; rifampicin was added after 3 min of incubation (5 μg/0·5 ml.) to samples 2 (●), 4 (▲) and 6 (■). 1 and 2 represent ^{14}C-UMP incorporation; 3 and 4 represent ^{32}P-ATP incorporation, and 5 and 6 represent γ-^{32}P-GTP incorporation.

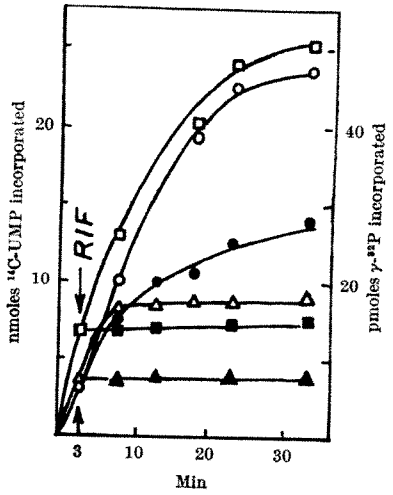


Fig. 3. RNA synthesis on the $DNP_{0\cdot6}$ template with and without rifampicin. Conditions were the same as for Fig. 1, except that $DNP_{0\cdot6}$ containing 50 μg of DNA was added instead of DNA. Symbols are as in Fig. 1.

Fig. 3 shows that the limitation of RNA elongation typical of DNP_0 is absent in the case of $DNP_{0\cdot6}$. The RNA polymerase moves along the $DNP_{0\cdot6}$ strand at the same rate and for the same time as in the case of free DNA. $DNP_{0\cdot6}$ is insoluble in the reaction medium, so that aggregation cannot explain the difference of transcription from DNP_0 and $DNP_{0\cdot6}$.

The removal of histone F1 must also remove the obstacles to the movement of RNA polymerase along DNA. Other histones (F2a1, F2a2, F2b and F3) do not influence this process.

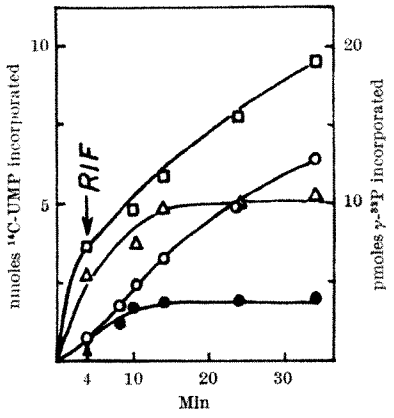


Fig. 4. RNA synthesis on the DNP_{ur} template with and without rifampicin. Conditions were the same as in Fig. 1 except that the DNP_{ur} (contains 50 μg of DNA) was added instead of DNA and the time of preincubation lasted 4 min before addition of rifampicin. Symbols are as in Fig. 1.

Other findings include a smaller value of n compared with those typical of DNA, especially after a short incubation. After 30 min of incubation this difference is negligible, perhaps because of the lower availability of aggregated template for the RNA polymerase, with a resulting decrease in V'.

RNA Synthesis on DNP treated by Urea

Does the early stopping of RNA polymerase progression depend on interference with histone F1 itself or on the DNP superstructure for which histone F1 is responsible? To resolve this question we have investigated the template properties of DNP_0 treated by 4 M urea (DNP_{ur}). It had been found that DNP_{ur} contained all the proteins of the original DNP_0, including histone F1, but the superstructure was destroyed[12]. After the removal of urea by dialysis against 0·001 M Tris this superstructure was not reconstituted.

Fig. 4 and Table 1 show that in the case of DNP_{ur}, as well as DNP_0, RNA elongation stops very soon. Values of t and l are only slightly higher than for DNP_0. Thus the presence of histone F1 on the DNP strand seems to be responsible for the early stopping of enzyme movement.

Our findings indicate that the poor template activity of chromatin or DNP_0 depends first on the formation of stop-points for the movement of RNA polymerase along the template. For this reason the time of chain growth and the average length of the chains decrease four to five times. Histone F1 is probably responsible for this effect, for its removal leads to the removal of obstacles to RNA polymerase progression.

Although the relation of this phenomenon to the *in vivo* situation is not clear, *in vivo* histone F1 could also prevent the movement of the RNA polymerase.

It is interesting that bacterial repressors, in particular the lac repressor, act in a similar way. The lac repressor combines with the operator, localized between the promotor and the structural genes, and prevents the RNA polymerase from moving to the structural genes[13].

Perhaps F1 histones are localized in the proximal parts of operons near promotors, and so inhibit RNA synthesis in corresponding operons[14].

Another important conclusion from our results is that the presence of four principal histone fractions in a complex with DNA does not influence the template properties of DNA—long RNA chains are synthesized. Thus RNA polymerase does not need a "naked" DNA for its action. DNP with a high protein content (total protein to DNA ratio in $DNP_{0.6}$ is equal to 1·1) may serve as a very effective template.

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A Ligand Exclusion Theory of Allosteric Effects

by

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Cooperative and allosteric effects are explained by a theory involving not conformational changes but steric exclusion through interactions between multifunctional ligands which bind to common and topologically distinct subsites.

An increasing amount of attention has recently been focused on a group of enzymatic phenomena generally categorized as "allosteric" or "cooperative" effects. These phenomena may include: a sigmoid dependence of reaction velocity on substrate or cofactor concentration; activation by compounds structurally unrelated to reactants or products; effects of ligands on binding at sites topologically distinct from their own; and the obligatory sequential binding of substrates. Elegant and ingenious theories[1–3] have been developed to provide a comprehensive explanation for this diverse group of effects. These theories and the evidence supporting them

have been reviewed by Koshland[4], who has devised a nomenclature that is most useful for discussions of such enzyme-regulatory effects. In spite of important differences in these theories, their proponents are in general agreement with Stadtman[5], who has concluded that "one is led to the inescapable conclusion that the binding of the inhibitor to the allosteric site results in a change in the conformational state of the protein to a form with lowered affinity for the substrate at the catalytic site". We present here an alternative class of mechanisms which demonstrate that the conclusion just cited is by no means inescapable.

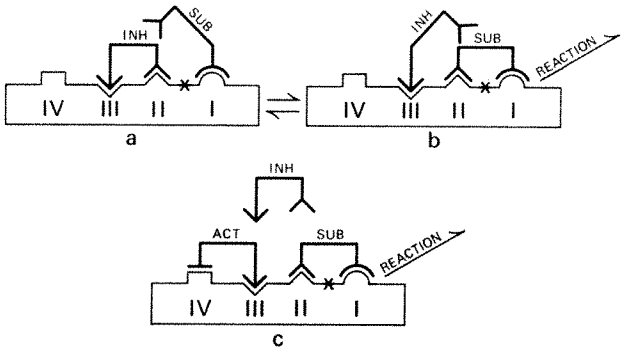


Fig. 1.

Fig. 1 is a schematic representation of an enzyme surface possessing four subsites, each capable of binding a functional group on a ligand. Also shown in Fig. 1 are three different ligand species, each possessing two functional groups which are capable of binding independently to a subsite. Assume a general case in which at any given moment each bidentate ligand may be bound to the enzyme at either one or two subsites; further assume that catalysis can occur only when the substrate ligand (Sub.) is bound to both subsites I and II. In the absence of activator (Act.), both inhibitor (Inh.) and substrate may bind to the enzyme simultaneously, as shown in Fig. 1*a* and *b*, but at a given moment only one ligand can be bound in a bidentate manner, for subsite II is required for the bidentate binding of either ligand. If, as assumed in Fig. 1, both ligands bind much less tightly to subsite II than to the other two subsites (I and III) a non-competitive inhibition will be observed because the competition for subsite II by the substrate and inhibitor ligands is through an isomerization step which is not a concentration dependent one[6]. The binding of an activator ligand at subsites III and IV relieves the inhibition and results in the complex depicted in Fig. 1c. The binding of an activator ligand in a monodentate manner to subsite III would also decrease the inhibition. It is obvious that an enzyme system exhibiting such behaviour would certainly be described as allosteric. It should be noted that it is by no means necessary that the ligands involved have recognizably similar structures; they need only possess single functional groups capable of binding in some manner to the same subsite. Some simple variations of the scheme just described in Fig. 1 exhibit other aspects of cooperative or allosteric interaction.

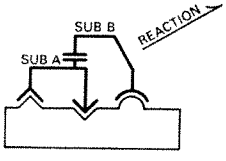


Fig. 2.

Fig. 2 portrays the active complex of a two-substrate reaction in which substrate A contains one of the two subsites required for the productive binding of the second substrate (B). An obligatory sequential ordered reaction must result and the two substrates will now each enhance the binding of the other in a typical cooperative interaction. Such behaviour frequently has been taken as evidence of a substrate-induced conformational change (ref. 4, pp. 381 and 395). If the two substrates are identical, the rate equation is

$$v/V = \frac{\alpha s^2}{1+\beta s+\alpha s^2} \qquad (1)$$

This equation is a special case of a more general one discussed by Reiner[7] and permits a sigmoidal dependence of velocity on substrate concentration.

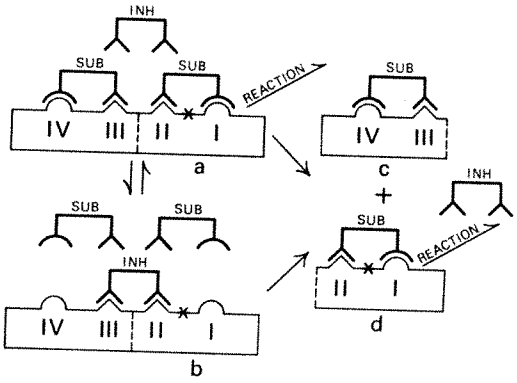


Fig. 3.

Now consider the system shown in Fig. 3*a* and *b*, representing an enzyme which can bind an identical substrate in two different and non-interacting modes (I–II and III–IV), only the former of which is productive, as indicated by the asterisk. The enzyme can also bind an inhibitor to a combination of subsites II and III, each of which also forms part of the two separate substrate binding sites. Assuming the simplest case in which all ligands are either bound in a bidentate manner to two subsites or not bound at all, the enzyme can form two different complexes: (*a*) in which the inhibitor is not bound and both substrates may be bound, and (*b*) in which the inhibitor is bound and neither substrate can be bound. The kinetic equation which results is

$$v/V = \frac{\alpha s+s^2}{(\alpha+\beta)s+s^2+\alpha\beta(1+\gamma I)} \qquad (2)$$

In the presence of a sufficient concentration of inhibitor, this system will display a sigmoid dependence of velocity on substrate concentration. In the absence of inhibitor a normal hyperbolic dependence will be seen. The maximal velocity is independent of inhibitor concentration. If the enzyme consists of two dissociable subunits as indicated by the dotted line in Fig. 3*a* and *b*, dissociation will lead to a mixture containing subunit c which is catalytically inactive but still capable of binding a substrate molecule, and subunit d which is catalytically active. The ability of either subunit to bind inhibitor will be greatly diminished. The system will now show normal hyperbolic kinetics even in the presence of inhibitor at any concentration. If the dissociated subunits differ in physical properties sufficiently to be separable, then subunit d would be found to have full catalytic activity and would exhibit normal hyperbolic kinetics even in the presence of inhibitor; and subunit c would be found to be catalytically inactive. Subunit c would then most reasonably be described as a "regulatory subunit" and d the "catalytic" subunit. It is also apparent that the location of specific subsites on dissociable subunits in cases similar to those we have

described could lead to a "normalization" of sigmoid kinetics by subunit dissociation. If the dissociation into subunits represents a reversible system, then the scheme of Fig. 3 predicts that the presence of inhibitor could favour formation of the associated form by forming a strap across the interface, and thus cause the formation of more of its own ligand binding sites. The behaviour of this scheme presents an interesting analogy to certain features of a number of multisubunit enzymes.

A significant distinction between the concepts proposed here and conformation-change theories is that we specifically assume that the occupancy of a given subsite by a ligand functional group has no effect on the micro-constants of binding of functional groups to any other subsite. On the other hand, such occupancy of a subsite can effect an overall ligand binding constant (generally the product of two or more micro-constants). Thus the ability of multifunctional ligands to connect a group of topologically distinct subsites can lead to a variety of effects commonly described as allosteric.

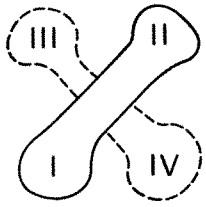


Fig. 4.

A somewhat more general model, freed even of the simple assumption of ligand sharing of common subsites, is quite capable of explaining the same range of allosteric and cooperative effects. Fig. 4 represents an enzyme with four subsites, no two of which are capable of binding the same functional group, which are arranged on an enzyme surface so as to constitute the corners of a rough square. If one bidentate ligand, possessing the proper pair of functional groups and the required inter-group distance, can bind to the I–II pair of subsites, and a totally different ligand can bind to the III–IV pair; then the binding of either one of the two ligands may exclude the binding of the other by simple steric hindrance even though the two do not have a single binding subsite in common and have absolutely no structural resemblance to each other. Such simple steric exclusion can result in cooperative and allosteric effects similar to those produced by common subsite occupancy. The combination of these two mechanisms can result in even more complex behaviour.

It is interesting to note that the definition of allosterism[8] arose from the apparent inadequacy of an overlapping site theory proposed by Changeux[9] to explain the effects of L-isoleucine on L-threonine deaminase activity and abandoned by him in favour of a separate but interacting site model. The kinetic effects described by Changeux are easily explained by the concepts proposed here. The difference in viewpoint involves primarily the distinction between subsites and ligand binding sites; the ligand binding site consists of the sum of its specific subsites and all of the surrounding space occupied by the bound ligand.

Conformational change theory explains the effect of the binding of one ligand on the binding of another as a result of ligand–enzyme–ligand connectivity whereas ligand exclusion theory proposes that these same effects are caused by direct ligand–ligand interactions; in this case the connectivity is afforded either by common binding subsite utilization or by simple steric exclusion. Both theories predict identical kinetic consequences and therefore only direct physical evidence of protein conformational changes or ligand–ligand interactions causally connected with ligand binding can distinguish between the two mechanisms.

Recent spectroscopic studies in our laboratory have permitted the resolution of multiple binding of several ligands to glutamate dehydrogenase[10]. The results provide direct evidence of a number of situations quite similar to combinations of the interactions postulated above, and have led us to consider the applicability of such ligand exclusion as a possible explanation of similar effects in other enzyme systems. Our purpose here is not to attempt to assess the relative weights of evidence supporting the ligand exclusion theory and those theories which explain identical effects by assuming changes in tertiary conformation, but rather to suggest an alternative general mechanism which should be given careful consideration in any specific case in which conformational changes have not been actually observed and shown to be causally connected to the kinetic behaviour.

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Palaeohistory of the Spruce in Switzerland

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Pollen and carbon dating suggest that the Post-Glacial spread of the spruce (*Picea*) in Switzerland has been influenced by human activity. This is different from the situation in the eastern Alps.

BASED on a preliminary comparison of dated sections of sediments from different localities (Fig. 1), I should like to suggest that human activity was responsible for the spread of the spruce (*Picea abies* H. Karst) in the Swiss forests during Post-Glacial times. The comparison was made possible by numerous carbon-14 dating and pollen analyses of soil profiles (Fig. 2), on the assumption that changes in vegetation are reflected in the relative pollen composition of sediment horizons[1].

In Switzerland the oldest traces of *Picea* pollen, which mark the immigration of this tree, generally reach back to Atlantic times[2–5]—7,000 to 6,000 BP*, as in central Europe[6]. Only east of the upper Inn River did *Picea* arrive and spread as early as Pre-Boreal and Boreal times, about 9,000 BP (unpublished results of M. Welten)—as in the

* Dates are given in conventional carbon-14 years before AD 1950 called BP, before present) as calculated from the measured activities and use of adopted standards without use of calibration curves[6,7].

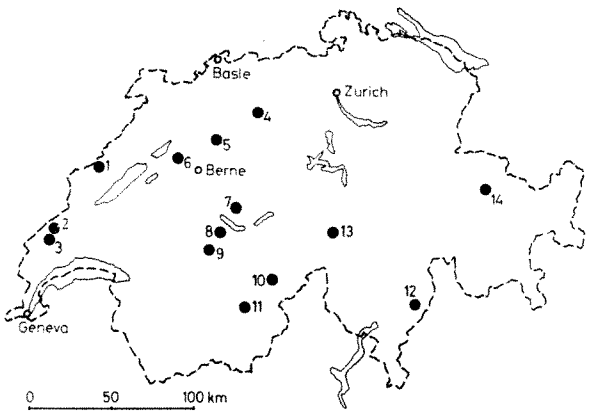


Fig. 1. Map of Switzerland showing localities where pollen was investigated (see profiles in Fig. 2). (1) Le Cachot, altitude 1,050 m, near La Brévine, in central Jura (canton Neuchâtel) (F. Matthey, in preparation); (2) Les Cruilles, 1,035 m, near Lac de Joux, in South-West Jura (canton Vaud)[3]; (3) Le Marais des Amburnex, 1,300 m, near Col du Malchairuz, in South-West Jura (canton Vaud)[3]; (4) Fätzholz, 476 m, near Murgenthal, Mittelland (canton Aargau)[21]; (5) Burgmoos, 460 m, near Seeberg, Mittelland (canton Berne)[2]; (6) Lobsigensee, 514 m, near Aarberg, Mittelland (canton Berne)[22]; (7) Hängstli, 1,260 m, valley of Eriz, near Thun, N limestone Alps (canton Berne) (K. Heeb, in preparation); (8) Egelsee, 1,000 m, Simmenthal, N High Calcareous Alps (canton Berne)[2]; (9) Sewlenboden, 2,120 m, near Lenk, Simmenthal, N High Calcareous Alps (canton Berne)[2]; (10) Aletschwald, 2,010 m, near Brig, central Alps (canton Valais) (M. Welten, in preparation); (11) Bonigersee (or Böhnigsee), 2,095 m, near Visp, central Alps (canton Valais)[4]; (12) Pian di Signano, 1,540 m, Misox valley, S Alps (canton Ticino)[5]; (13) Göscheneralp, profile Berg, 1,940 m, central Alps (canton Uri)[11]; (14) Faninpass, 2,220 m, near Chur, Swiss E Alps (canton Graubünden) (P. Wegmüller, in preparation).

Eastern Alps[9–11] (Fig. 2, locality 15), where *Picea* became established immediately when climate improved at the beginning of the Post-Glacial. West of the upper Inn River, however, spruce seems to have spread no farther for several thousand years after its arrival (unpublished results of P. Wegmüller), a pattern which has also been reported for Germany[6].

It was previously thought that the amount of spruce pollen deposited increased in the Sub-Boreal period[2–5], but radiocarbon dates[12] suggest that expansion was not simultaneous in different places. Even pairs of localities less than 20 km apart at comparable altitudes differ in the date of expansion by about 2,000 yr: for example, Les Cruilles–Le Marais des Amburnex (Fig. 2, profiles 2 and 3) and Aletschwald–Bonigersee (Fig. 2, profiles 10 and 11). Dates of expansion for *Picea* vary from 7,000 BP (Fig. 2, profile 14) to 1,000 BP (Fig. 2, profiles 5 and 6) with a mode at about 5,000 BP (Fig. 2, profiles 2, 7, 8, 9, 11 and 13).

To interpret the problem of *Picea* it is necessary to explain the interruption of its development west of the upper Inn River and the different dates of expansion at different localities. The cause of these effects must have been local and strong enough to have induced the changes in vegetation which made spread possible. Climatic changes alone cannot have been responsible, particularly in view of Frenzel's observations[13] that a "climax" forest has its own climate, as a result of centuries of undisturbed development. Such a forest is not easily altered, even by minor environmental changes. In any case, recent investigations of the Atlantic and Sub-Boreal climate have shown fluctuations to have been of minor importance[13]: for example, a short cold spell (*Piora Kaltphase* of Zoller[5]) occurred about 5,000 BP and has previously only been detected in sections near the alpine timber line[14,15]. The change to a continental climate during the Sub-Boreal period was too slow and weak to explain the sudden expansion of *Picea*.

Signs of Human Influence

Another solution was suggested by the changes in vegetation indicated in the profiles contemporaneous with the increase in *Picea* pollen. These changes can be summarized as follows. First, there was a general decrease of pollen of *Abies alba* (fir), *Pinus cembra* ("Arve") and the so-called mixed oak forest (*Ulmus*, *Tilia* and *Quercus* spp.) with a simultaneous increase of herb pollen, chiefly Gramineae. Soon after this the dominant species were shrubs and heliophilous trees (*Corylus*, *Alnus*—also *Alnus viridis*—or *Betula*), and then trees which required shade. Iversen thought that this succession indicated re-afforestation[16]. In two places (Fig. 2, profiles 11 and 12) associated charcoal horizons were found, indicating that forest had

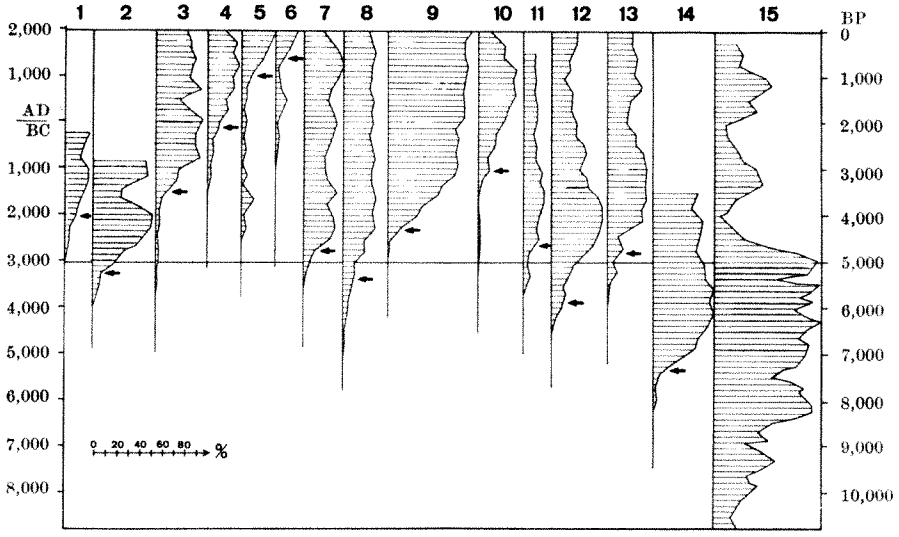


Fig. 2. Pollen profiles at localities numbered in Fig. 1. No. 15, not marked on Fig. 1, is in Austria: Lengholz I, 570 m, in Drau valley, Carinthia, South-East Alps[10]. The abscissae show the amount of *Picea abies* pollen relative to total pollen in each horizon. The ordinates give time in ^{14}C years: to the right as BP (before present), to the left their equivalent in conventional ^{14}C years AD/BC. Profiles 4 and 9 were not directly radiocarbon dated, but pollen analysis data were compared with ^{14}C dated profiles. The arrows mark the onset of the spread of *Picea* at the point where *Picea* pollen exceeds 10 per cent of all pollen.

been cleared[2,5]. Second, there was a decline of *Ulmus* (elm) in the mixed oak forest pollen, indicating a final change in the composition of that forest type. Third, there were the first traces of cereal pollen, which showed that agriculture was being practised, and there was the invasion of weeds such as *Plantago lanceolata*.

These three changes are all related to human activity[16–18], although the importance of this influence has been questioned[13,19]. The charcoal horizons at the upper limit of the forest confirm the great impact of man on nature at the onset of the Neolithic. Because these changes were contemporaneous with the dates of expansion of *Picea* I think that the spread of this species was also due to human influence.

Local Activity

Human activity is likely to have been local for two reasons. First, Neolithic people almost certainly immigrated from the Mediterranean region, bringing agriculture with them. It is unlikely that their clearing activity would have been simultaneous throughout Switzerland (that is, one should find their traces earlier in southern than in northern localities). Second, the different suitability of the localities for cultivation could be accounted for by the different times of expansion; the places estimated to have been of low agricultural productivity were cleared distinctly later than the more productive places (Fig. 2, localities 10 and 11).

Human activity is also likely to have been a strong influence. Not only the more or less continuous clearing of the forests by fire, but especially subsequent grazing by cattle, destroyed the previous vegetation and made natural re-afforestation impossible (private communication from H. Oberli). Thus species which were less adaptable to continuous disturbances would not have survived, and *Picea*, which is less sensitive, came to be dominant.

I therefore consider that the conditions for the spread of the spruce in the forests of Switzerland were different from those described for the eastern Alps, where *Picea* arrived earlier than any other Post-Glacial tree. The interruption of expansion west of the upper Inn River was due to competition from *Abies alba*[21], *Pinus cembra* and *Larix decidua*, which had already become established after immigrating from the south into Switzerland across the Alpine passes.

In summary, large scale expansion of *Picea* was only possible in Switzerland after man had destroyed the previous natural composition of the forests. Such great changes in vegetation were not necessary in pre-agricultural times—until about 5,000 BP[20]. The subsequent rapid development of *Picea* was also supported by climate, even if the climatic fluctuations were minor. Other factors such as soil development and plant diseases certainly contributed in some degree to the spread of *Picea*, but none were effective enough to have started the process by themselves.

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Characterization of Prehistoric Flint Mine Products

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Trace element analyses of flint show statistically valid differences between products of major British and European Neolithic flint mines.

STONE axe-heads for tree felling and clearing the land were part of the staple equipment of the early farmers. In the Neolithic and Early Bronze ages outcrops of suitable hard rocks and easily accessible flint bands in the chalk regions were exploited on a large scale for the manufacture of axe-heads. Such major axe factories are relatively rare; there are perhaps fifteen–thirty sites in the British Isles and it seems likely that many of the axe-heads found elsewhere are their products.

Petrological analysis by thin section has enabled the locality of origin of axes made from hard rock to be identified and, incidentally, has demonstrated their trans-

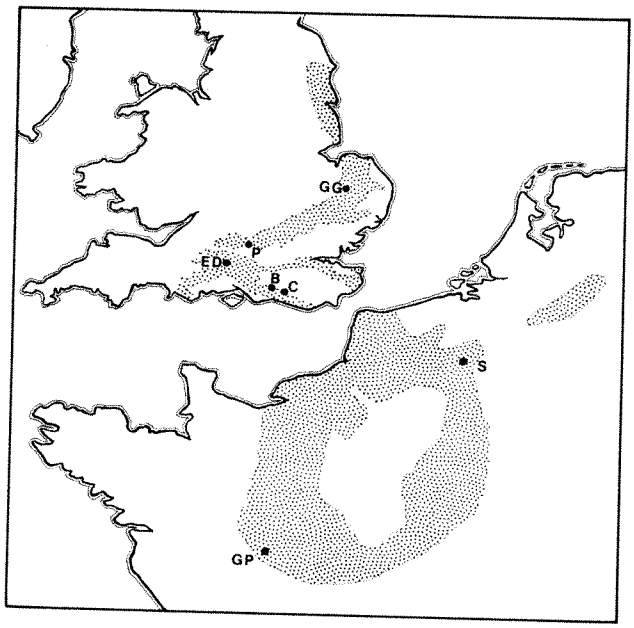


Fig. 1. Distribution of the flint mines and axe factories whose products have been analysed. Shading indicates outcrop of the Cretaceous Chalk. The sites are ED, Easton Down; GG, Grimes Graves; GP, Grand Pressigny; B, Black Patch; C, Cissbury; P, Peppard; S, Spiennes.

port over long distances[1]. Lake District axes are found in datable prehistoric contexts as far afield as Wiltshire, and specimens of Cornish origin occur in East Anglia. Such are isolated instances. The majority of the tools were used in areas with easier access to the factory sites although large scale distribution of the Langdale factory extends as far as the West Riding of Yorkshire.

In the Chalk regions it has not yet been possible to demonstrate such trade or transport of axe-factory products because the majority of axe-heads are made of flint whose place of origin cannot be identified petrologically. Certain geographically restricted varieties of flint are said to have a characteristic appearance, but no satisfactory objective method has yet been found of demonstrating these differences[2,3].

Because flint is largely composed of silica and water it was thought that analysis for trace elements might prove a satisfactory method of distinguishing flint from different localities. To check this hypothesis it was decided to analyse a restricted sample of flint from the flint seams of some of the major axe factory/flint mines in southern Britain and Continental Europe. It was recognized that flint might be similar in its trace element constitution and therefore we selected sites which were well separated. Two British sites were chosen specifically, however, because they were close together and might be expected to be indistinguishable by their trace element content. These are Cissbury and Black Patch, here taken as representative of the South Downs group of flint mines. In the analysis that follows, the results from these sites are tabulated and their localities are shown in Fig. 1.

It will be seen that on the restricted sample of twenty specimens analysed from each flint mine/axe factory, all the sites whose products are analysed can be clearly distinguished from one another, except in the instance of the two sites in the South Downs group, whose trace element composition is very similar.

Petrology of the Flints

The flint discussed here is a form of chalcedony that occurs as bands and nodules in the soft white limestone of Upper Cretaceous age called the Chalk (Fig. 1). Chalcedony is composed of micro-crystalline alpha quartz with crypto-pores filled with an aqueous solution[4]. This means that pure chalcedony consists of silica, water and the dissolved constituents of the water. If the flints found in the chalk were pure chalcedony they would probably not have a very variable trace element content.

The origin of nodular and tabular flints and cherts of the type found in chalk has long been a source of interest. There are two principal theories for their origin: (1) syn-depositional precipitation of silica gel which matures into chalcedony, and (2) post-depositional segregation of silica. With Chalk flints the second theory seems best to fit the evidence provided by mode of occurrence. The nodule or vein of flint would form, at least in part, by the replacement of calcium carbonate by silica. Growth of the flint would be outwards from a nucleus or nuclei and the replacement would be piecemeal. No cavity would be formed and most of the non-carbonate material in the chalk would be incorporated in the flint. The most common non-carbonate materials are quartz, clay minerals and heavy minerals; these occur in various proportions and they are the most likely source of the trace elements. The distribution of non-carbonate material in Chalk varies both laterally and vertically[5–8]. The lateral changes in composition are normally not rapid except where the margins of Chalk deposition are approached. Vertical variations may, however, be quite sudden and considerable which means that the flints from one horizon in one locality will not normally vary widely.

Chalk flints have a noticeable outer skin, cortex or patina, which is present both on flints freshly removed from the calcareous matrix and on well weathered flint fragments or implements. The origin of the layer is, however, different under these two circumstances. The cortex on the unweathered flint is the result of the growth of the nodule or vein, while the patina on the fragments or implements is the result of weathering and partial solution. From an analytical viewpoint both layers are undesirable and are removed before analysis.

Analytical Methods

Samples were obtained by drilling the flint specimen with a high speed, diamond impregnated, hollow core drill which gives a cylinder of material. The top of this core, comprising the weathered outer layer and the first few millimetres of unaltered flint, was removed and discarded. The cylinder of flint was cleaned in boiling aqua regia for approximately two minutes to remove any surface contamination arising from the process of sampling. The cylinders are extremely hard and dense and there is no danger of leaching out the trace elements by this procedure. One sample was boiled for several hours with no apparent change in either its appearance or chemical composition on analysis.

In preparing a finely powdered sample for analysis, difficulties arise because the flint is extremely hard. Initial experiments using a combination of steel percussion and boron carbide mortars resulted in heavy sample contamination and severe damage to the surface of the boron carbide mortar. It was found, however, that the sample could be broken up and its hardness reduced by rapid heating and quenching in water. This was done by dropping the cleaned sample into a red hot pure nickel crucible and immediately replacing the lid. Because of the expansion of small amounts of structural water the flint shattered violently. The fragments were then tipped into cold distilled water, which rendered them more friable. They were then dried at 120° C and were ground in an agate mortar to a fine powder. The sample was then ready for weighing and analytical treatment by the following methods.

The initial quantitative survey was carried out by emission spectroscopy using a technique in which the finely ground powdered flint, mixed with twice its weight of 'Specpure' graphite powder, was packed into cupped, necked, graphite electrodes and arced for 30 s at 8 A with

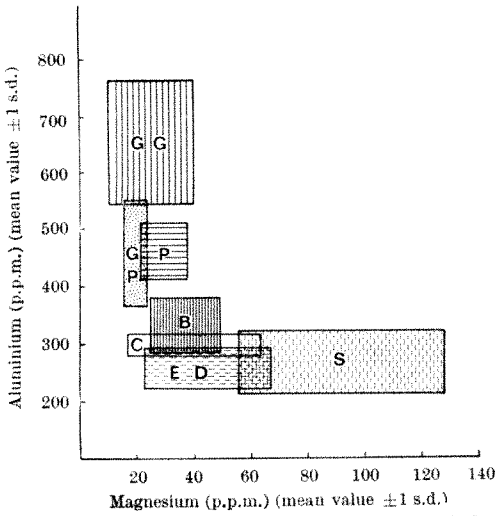


Fig. 2. Trace element analysis of flint mine products. Al/Mg. Site abbreviations as in Fig. 1.

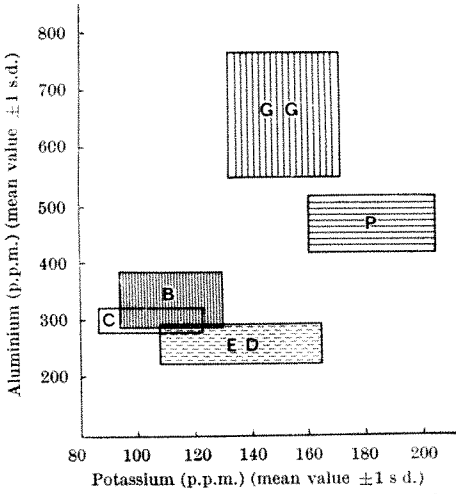


Fig. 3. Trace element analysis of flint mine products. Al/K. Site abbreviations as in Fig. 1.

a graphite counter electrode in a d.c. arc. The spectra were recorded photographically using a rotating stepped sector and a Hilger and Watts large quartz spectrograph. Silicon was used as an internal standard and suitable line pairs were measured using a Joyce Loebl 'Mark III C' microdensitometer. The method is relatively insensitive because of the suppressive effect of silica on the spectral lines of the other elements present, and suffers from the poor reproducibility which is inherent in d.c. arc methods. Consequently it was possible to measure only the major impurities and the results showed a relatively high coefficient of variation of 20 per cent on replicates of the same sample. Nevertheless, in spite of these limitations, the results indicated that differences in the levels of major impurities in flint from flint mines might be used as an indication of place of origin. Aluminium, calcium, magnesium, iron and manganese (and phosphorus from one site) were detected by this technique, but in view of the general lack of sensitivity and suspected systematic errors, particularly in the case of aluminium, other methods were investigated.

We then turned our attention to atomic absorption spectroscopy, which requires the samples to be in solution. The flint powder, produced as before, was digested on a water bath with a mixture of hydrofluoric and perchloric acids in a 'Teflon' beaker. There was a small black residue in each of the resulting perchlorate solutions; some of these residues were analysed on the spectrograph and it was inferred from the general absence of spectral lines that they were probably composed of elemental carbon and certainly would not contribute to the analytical results. After centrifugation to remove these residues, the solutions were analysed for aluminium, iron and magnesium with a Hilger and Watts 'Atomspek' atomic absorption spectrophotometer, using a nitrous oxide acetylene flame for aluminium, and an added excess of lanthanum chloride for the determination of magnesium in the presence of aluminium. The alkali metals were also found to be present, and consequently sodium and potassium were determined using an EEL flame photometer. The analyses by both these methods presented no difficulties, and results could be estimated with a much higher accuracy than that obtainable by emission spectroscopy. The atomic absorption method can clearly be extended to other elements because qualitative spectrographic examination of residues from the hydrofluoric acid treatment of 0·5 g quantities of prepared flint has shown that up to twenty elements (including Al, Sb, Be, Ca, Cu, Ge, Fe, Pb, Li, Mg, Mn, P, K, Ag, Na, Sr, Sn and Ti) can occur in these samples.

The results for aluminium by atomic absorption spectroscopy were shown to be much more reliable than those obtained by emission spectroscopy and all the aluminium results quoted and discussed later relate to the atomic absorption technique. No systematic differences were, however, found between the two methods in the case of magnesium and iron, and the results by the two methods have therefore been combined in the figures. This is regarded as being valid because the spread of results from flint to flint at any one site is much greater (a coefficient of variation of 50 per cent) than the variation inherent in the two methods of analysis.

Thus in the discussion which follows, the results previously obtained by emission spectroscopy are combined with the results since obtained by atomic absorption on fresh flints. The sodium and potassium results were all obtained by flame photometry. Complete results are not yet available for Le Grand Pressigny and Spiennes, but the initial spectrographic examination has already revealed that Spiennes samples can be uniquely identified by their high levels of phosphorus, which was not detected by this method in samples from the other sites, and, similarly, flints from Le Grand Pressigny are uniquely characterized by very high levels of iron content.

The results shown in Figs. 2, 3 and 4 are the mean values for each element determined in the twenty samples taken from each site. Work is currently in progress

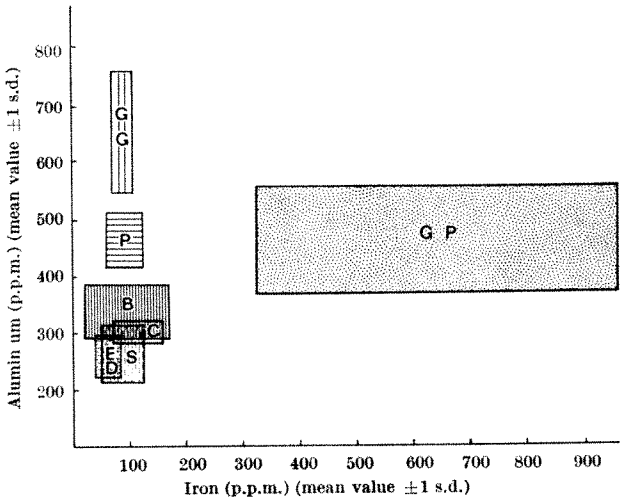


Fig. 4. Trace element analysis of flint mine products. Al/Fe. Site abbreviations as in Fig. 1.

on the estimation of Li, P, Ba, Ti, in order to provide further criteria for the differentiation of flint mine sites.

Statistical Analysis of Chemical Data

Complete data for the concentrations of Al, Mg, K and Fe in samples from six localities were available for this preliminary study. The results were first studied graphically, by plotting the concentration (in p.p.m.) of one element against another (Figs. 2–4). These plots showed that some localities occupied distinct fields; for example, in Fig. 2, Grimes Graves is distinct from the others, showing only a slight overlap. Other combinations of elements in other localities are equally or more clearly distinct (for example, Fig. 3). As a result of this preliminary study it was decided that some more sophisticated analysis of the data should be carried out and we used factor and discriminant analysis: we used a standard method and the data were processed on the University of London CDC 6600 computer, using modified versions of the programs given in the IBM 'System 360' manual[9].

Discriminant functions were calculated on the raw data and the *a priori* classification was subsequently tested by determining the group to which an individual flint was best assigned using the calculated coefficients. By this means it was found that 85 per cent of the individual flints were correctly classified. The majority of those that were incorrect belonged to the Cissbury/Black Patch sites which make up the South Downs group. Geologically this would be expected because the two sites are close together and the flint used could have been extracted from similar levels in the Chalk. A second set of functions was calculated after combining the Cissbury/Black Patch data; the new set of discriminant functions, when applied to the data, showed that the *a priori* classification was now 95 per cent correct.

The factor analysis was performed using the method of principal components, and, because the resulting matrix was easily interpreted, no rotation was needed. The results are summarized in Table 1; the first two factors only are reported and these account for 68 per cent of the variation in the data.

Table 1. SUMMARY OF FACTOR ANALYSIS

Factor 1	Factor 2
Al +ive correlation with K Al −ive correlation with Mg K −ive correlation with Mg	Fe not correlated with either Mg, Al or K

The results of both techniques are provisional until more data from the five sites (and further new sites) are available. The discriminant function analysis indicates, however, that our method, using trace element data to identify flint implement source areas, may be valid. The non-ferrous trace elements which we used are probably accounted for by the presence of clay minerals incorporated in the flint during its formation, which would explain the correlations found in the factor matrix.

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Glassy Particles in Lunar Fines

by

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Most of the Apollo 11 glassy particles differ from those generated by terrestrial volcanism, by meteoritic impacts, or by the condensation of the parent bodies of meteorites. Some of the particles may have been formed by the action of solar flares on the lunar surface.

To begin with, we describe the morphology and optical properties of the Apollo 11, sample 86 of lunar fines and samples 91·8 and 19·9 of the conglomerates as observed with Wild petrographic and Leitz phase contrast microscopes and a Cambridge 'Stereoscan'. Chemical composition was studied with a microspectrophotometer[1] and the Philips electron microprobe. Scanning microscopy was done on gold-coated particles attached to rivets.

Most of the glass particles are of either regular or pumiceous habit. Each gram contains approximately 5×10^6 regular and 7×10^6 pumiceous particles with sizes of 1 to 1,000 μm (Table 1). The regular particles include globules, prolate and oblate spheroids, ellipsoids, dumbbells, dumbbells with central bulge, rings, crescents, tops, spindles, filaments and tear drops (Figs. 1–5, 6*A*). The pumiceous particles are asymmetrical and rugged (Fig. 6*B*). Aggregates of two or more particles are rare (Figs. 3, 7) and resemble some of the twin and grapebunch chondrules[2] from carbonaceous meteorites. A globule adhering to a glassy fragment by a slender protrusion has been observed (Fig. 1).

The prolate spheroids and dumbbells often have zonation patterns (Fig. 8) and flow lines of crystallites (Fig. 8*A*). Gas inclusions occur in the central portions of light, intermediate, or dark shaded glass particles (Fig. 8*B*). Towards the extremities of some of the particles, a zone

Table 1. RELATIONSHIP BETWEEN SIZE AND MORPHOLOGY OF THE PARTICLES OF THE FINES

Diameter (μm)	Spheroids with eccentricity 0–10 per cent	10–50 per cent	Over 50 per cent	Dumb-bells	Rings	Tear-drops	Overall percentage of particles
1–2	83	15	1	0	1	0	70
2–5	66	19	8	2	3	2	4·1
5–10	58	22	7	5	4	5	0·8
10–20	46	25	16	3	6	4	1·3
20–50	54	21	11	6	3	5	1·4
50–100	70	13	6	7	2	2	1·4
100–200	68	17	9	3	0	3	1·7
200–500	64	18	11	4	1	2	4·0

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of orange occurs next to a zone of dark brown (Fig. 8*B*). The orange coloured glass is not seen in any unzoned particles. Numerous particles are tipped with minute biconvex grains of troilite and, more frequently, nickel–iron (Figs. 4, 8*A*). Zonation may be restricted to only one extremity, which is usually narrower than the opposite one (Fig. 8*C*). Elongate particles in which one extremity is occupied with a gas inclusion tend to taper towards the opposite end (Fig. 8*D*). Occasionally, crystalline silicates occupy the extremities of the elongate particles. These may be rather symmetrically displaced in alternate sides of the long axis of the particles (Fig. 9). Some of the oblate spheroids have a more or less regular zone of darker glass or inclusions of nickel–iron along the equatorial regions (Fig. 11*B*).

Several rare regular particles (less than 1 p.p.m.) from the most globular 0·1 per cent fraction of specific gravity > 3·4 fraction yielded a few magnetic particles with an octagonal outline (Figs. 10*A*, *B*, *C*). These resemble the microchondrules of magnetite in type I carbonaceous chondrites[2]. The more angular and flattened fraction of the same density range contained some hexagonal plates of troilite (Fig. 10*D*).

Particles may be covered by semitransparent films of nickel–iron. Surface dimples and inclusions of nickel–iron, troilite, glasses of high refractive index, or crystalline silicates are common (Figs. 11, 12). Most particles with crater-like pits contain a high proportion of gas inclusions (Fig. 8*D*). Rare globules of colourless to pale blue glass contain octahedra of an opaque or a slightly translucent spinel (Fig. 13*A*).

Many globules have stalk-like appendages (Figs. 6*A*, 13*A*). The elongate forms with appendages are markedly asymmetrical (Fig. 13*B*). A count of 561 transparent particles in unsorted fines showed 196 particles with no appendages, 318 particles with one, 40 particles with two, and 7 particles with three or more. Inspection in polarized light indicates that approximately 3 per cent of the glassy particles are partly or wholly recrystallized. The conglomerates contain about 15 per cent recrystallized particles.

The glasses may be colourless, light yellow, blue or green, or dark brown. Opaque glasses of sky blue, brown and black shades are also present. They have densities of 2·6 to 3·2 and many of the relatively denser ones are magnetic. Table 2 shows the relations between morphology, shade, density and magnetism of the glasses.

Preliminary electron microprobe analysis[3] has revealed glassy regular particles of salic, mafic or basaltic composition. The pumice particles are of relatively more uniform basaltic composition. The crinkled surface of a fragmented spherule was found to be rich in Mg, Al, Si and some Zr.

Table 2. RELATIONS BETWEEN COLOUR, DENSITY, MAGNETISM AND MORPHOLOGY OF PARTICLES OF THE FINES

Sieving fraction (μm)		Percentage spheroids with eccentricity of 0–10 per cent	10–50 per cent	Over 50 per cent	Percentage dumbbells	Percentage rings	Percentage tear drops
	Shade						
44–53	Light	79	12	4	1	4	0
	Dark	56	28	9	1	3	3
	Opaque	51	24	13	6	1	5
	Spec. gravity						
10–105	Below 2·85	68	15	9	4	2	2
	Above 2·85	59	22	9	4	4	2
	Magnetism						
5–53	Less magnetic 5 per cent	60	25	6	5	1	3
	More magnetic 95 per cent	51	23	12	7	3	4

Microspectrophotometric analyses of the salic particles in the visible and near ultraviolet show a continuous absorption spectrum increasing towards the ultraviolet. The darker particles have the same type of spectrum with a higher percentage absorption. The spectra of basaltic pumices from Hawaii and the Orgueil meteorite are similar to that of the average lunar pumice. A blob of orange glass from the terminal portion of a zoned prolate ellipsoid showed a maximum absorption feature at 340 μm which was absent from the spectra of the fourteen unzoned particles.

There are deep cavities in some particles filled with foreign grains. A single microshattercone of brown glass was observed (Fig. 14). Most of the particles show little or no internal fragmentation.

The lunar pumice is characterized by irregular vesicles containing quantities of loosely anchored particles (Fig. 15*A*). Tubules of glass protruding from the main mass have been also reported[4]. By contrast, vesicles of terrestrial pumice tend to be more regular and free of attached particles (Fig. 15*B*).

Interpretation of the Structures

The gross morphology of either the regular or the pumice particles clearly indicates the solidification of freely suspended liquid or viscous liquid droplets. These

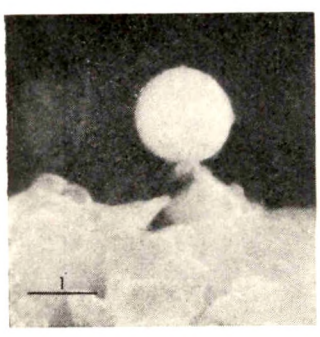


Fig. 1.



Fig. 2.

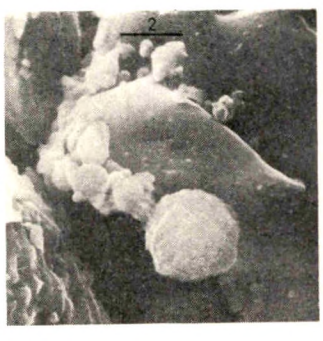


Fig. 3.

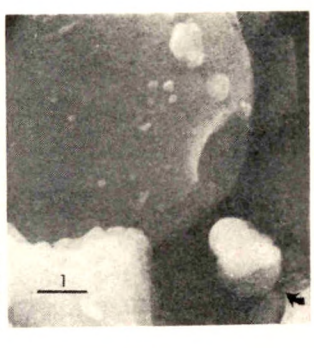


Fig. 4.

Fig. 1*. Glass globule sintered to a slender stalk and hemispheres welded to fragment of glass (left). Scanning electron micrograph (SEM).

Fig. 2. Irregular dumbbell with a large opening, and markings indicative of impacting the lunar surface in a viscous state. Small filament of glass (bottom centre). (SEM.)

Fig. 3. Tear drop with groups of small globules, spheroids and ellipsoids in different degrees of sintering and coalescence. A small spinel and a small top beneath scale marker. Part of large crinkled globule (left). (SEM.)

Fig. 4. Tip of prolate spheroid with end plug of nickel-iron (arrow) which fell out during specimen preparation. Note dimples and bud-like protrusions on surface of spheroid. (SEM.)

* All the figures are photographs of sample 86 of Apollo 11 fines, unless otherwise stated. Magnifications are indicated on the photographs in μm.

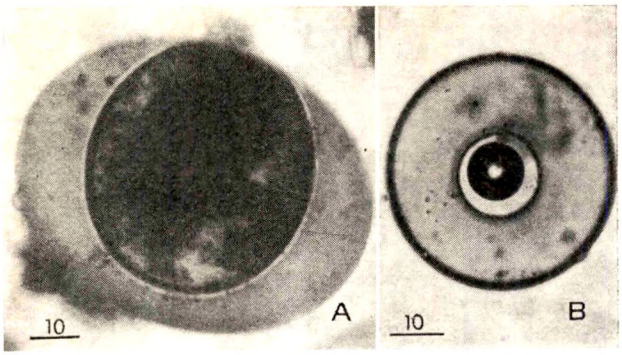


Fig. 5. Flattened rotational forms. (*A*) Crescent of brown glass with central dust covered film. (*B*) Ring of yellow glass; the bubble in the centre is bridged with a thin film of glass.

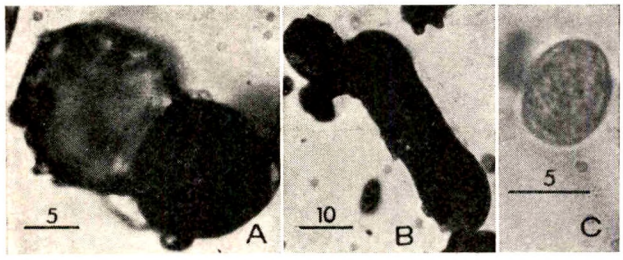


Fig. 7. Types of aggregates. (*A*) Loosely sintered, "grape bunch type" aggregate of large globule of salic glass (left) and nickel-iron (right) with several smaller glass globules. The convex surface of the nickel-iron indicates that it was already solid when the droplet of glass settled. (*B*) Dumbbell with a slight central bulge fused to an ellipsoid. (*C*) Coalesced, "globular" type aggregate of spheres of uniform diameters. The individual spheres are close to the upper limits of resolution with the optical microscope.

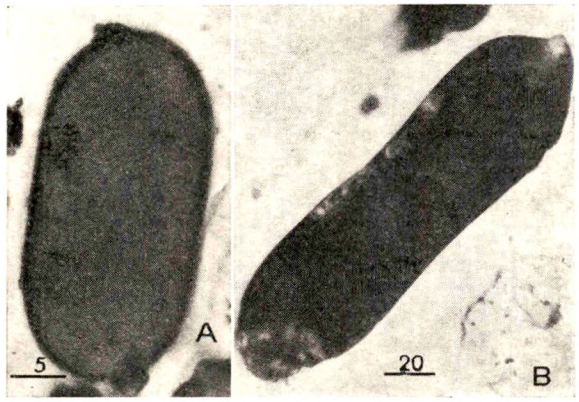


Fig. 9. Indications for rotation of elongated particles in some resisting medium. (*A*) Prolate spheroid of brown glass with two pyroxen crystals displaced in an anticlockwise direction from the long axis. (*B*) Dumbbell of turbid glass with recrystallized terminations similarly displaced.

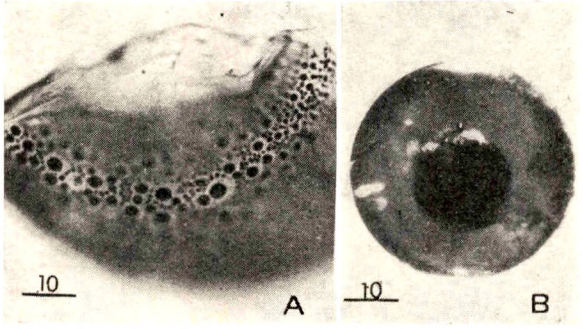


Fig. 11. Association of nickel-iron and glass. (*A*) Surface condensation pattern of nickel-iron on fragmented globule of pale blue glass. (*B*) A particle simulating the internal structure of the Earth; an oblate spheroid of basic glass with a nickel-iron core. Note irregular patches of darker glass concentrated towards the equatorial zone.

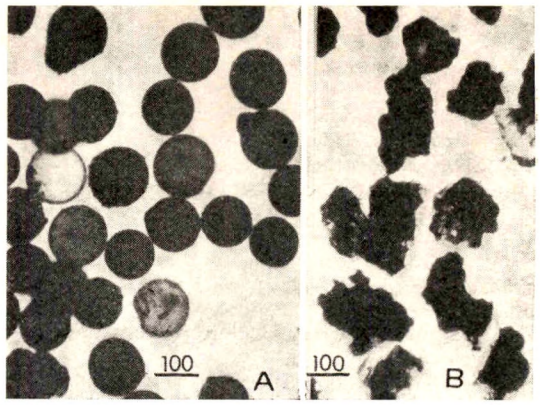


Fig. 6. Illustration of the degrees of morphological separation possible after four re-rollings on inclined sheets of paper of the globular (fast rolling) and angular (slow rolling) fractions of sieved lunar fines. (*A*) Smooth globules, some with appendages and fractures. (*B*) Pumice and flat fragments.

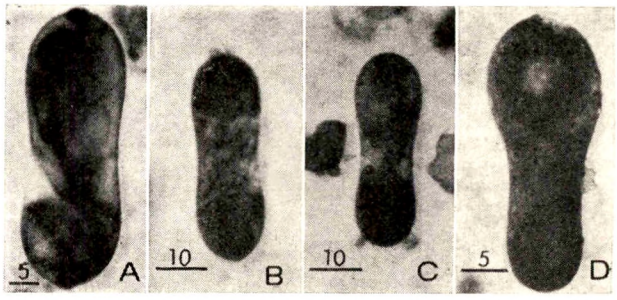


Fig. 8. Zoned dumbbells. (*A*) Terminal knobs of nickel-iron and flow structures of brown crystallites in clear glass. (*B*) Central zone of yellow glass with some bubbles, followed by zones of orange and black glass towards both terminations. (*C*) Dark brown glass occupying narrower lobe. (*D*) Glass gradually darkening towards narrower lobe, and broader lobe occupied by bubble. Note crater-like pit over bubble.

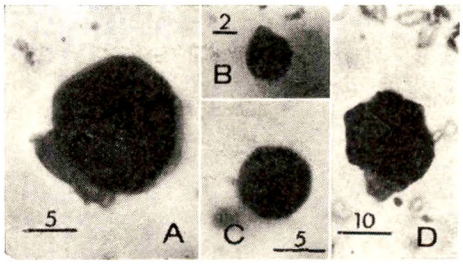


Fig. 10. Some rare magnetic particles separated from lunar fines, which resemble particles from type I carbonaceous chondrites. (*A–C*) Grains of magnetite of rounded, octagonal outline. (*D*) Hexagonal plate of troilite.

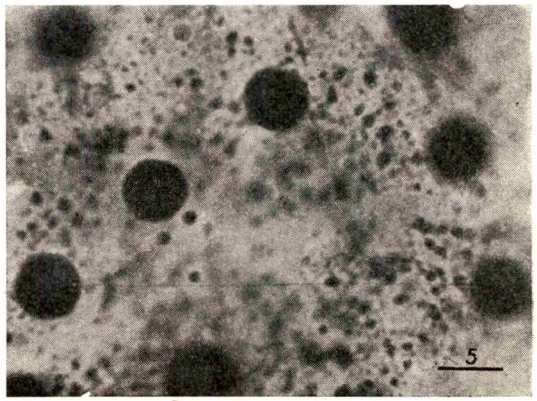


Fig. 12. Part of spheroid of yellow glass with a surface pattern of two generations of more or less regularly spaced troilite crystals of rounded hexagonal habits.

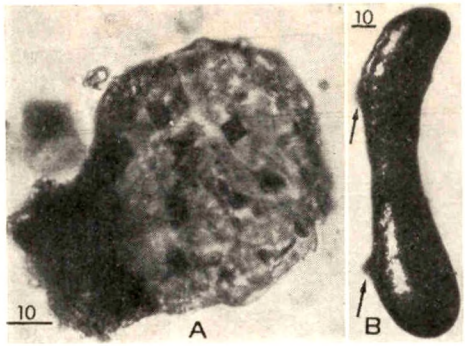


Fig. 13.



Fig. 14.

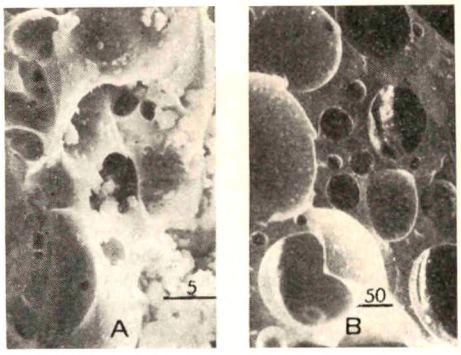


Fig. 15.

Fig. 13. Appendages of spray particles. (*A*) Chipped globule of pale blue glass with porphyroblasts of octahedra of an opaque spinel, from the powder of sample No. 91. 8 of the conglomerates. The globule has a tapering appendage of sintered dust. (*B*) Distorted dumbbell of black glass with two fused appendages (arrows) partly out of focus.

Fig. 14. Microshattercone of brown glass.

Fig. 15. Comparison of (*A*) large lunar pumice particle, (*B*) Hawaiian pumice, as explained in text. (SEM.)

may be termed spray particles Someø of the particles may have been partially or wholly recrystallized during the course of relatively slow cooling or metamorphosed subsequent to cooling.

The small proportion of partially sintered particles of the grape-bunch type suggests condensation of the spray from tenuous incandescent clouds in which the probability of collision–coalescence was small.

The morphologies of the regular glass particles suggest their solidification at various stages of centrifugal deformation from a molten state. Elongated forms may be produced when a rotating globule develops two diagonal centres of gravity such as spheroids, ellipsoids, dumbbells, and finally filaments and tear drops as the dumbbells split. The splitting of the dumbbells would convert the rotational momentum to linear momentum with the reversion of the tear drops so formed to a globule. As long as the force which causes rotation prevails, the elongation–division process could continue until a size is reached in which capillary forces on the surface would prevent further elongation. The constancy of ratios between globular and elongate forms throughout a range of diameters (see Table 1) supports this hypothesis of the rotational origin of the forms through the action of a sustained and uniform force. The momentum required for the elongation increases with decreasing dimensions of the particle, because of the increase of capillary forces. Thus, if rotation of the sprays occurred as a single event, such as a meteorite impact or a volcanic explosion, it would be anticipated that, statistically, the larger spray particles would have received the more intensive centrifugal deformation effects and, accordingly, the elongation ratios would increase with diameter. But the increase with elongation of magnetism and the density and darkening colour (Table 2) cannot be accounted for by any theory of random impact or explosive volcanism, because such processes would not have a selective effect. Rotational momentum caused by radiation or an electron flux might account for the higher elongation ratios of the more magnetic particles, or the darker and denser particles which are usually optically less homogeneous.

Free rotation of markedly homogeneous glasses may not produce two diagonal centres and, therefore, might lead to the formation of oblate spheroids, crescents and rings. Top or spindle shaped particles may form through their rotation in a viscous state on the lunar surface.

The zoning of spheroids and dumbbells suggests that the constituents of the particles separated through centrifugation. Some of the constituents may have been present as immiscible phases in the original particle. The orange coloured glass, however, which is only present in the centrifuged structures, may be a product of centrifugation of an originally homogeneous glass. The clockwise or anticlockwise displacement of extremities from the long axis of elongate particles may indicate the occasional presence of a slightly resistant medium, possibly a tenuous gas, within which the particle rotated.

The grains of magnetite and troilite seem to be products of desublimation of ultimate lunar or meteoritic origin. The surface patterns of nickel–iron and troilite seem to indicate a lunar condensation process of the vapours of the respective substances.

The prevalence of pits on particles which are also rich in gas inclusions indicates that most of these pits were produced through bursting of some of the gas inclusions before the final solidification of the globule.

The marked prevalence of a single sintered or fused appendage of each regular spray particle seems to indicate that the hot particles settled on the lunar surface at low velocities, without much bouncing or other high velocity impact effects. The results of preliminary electron probe work suggest that the relatively volatile elements evaporated from the surface of some of the spray particles after their solidification. The relatively higher proportion of recrystallized spray particles in the conglomerates indicates that these may be older or more metamorphosed than the fines.

The lunar pumice sprays may have been formed by the heating of relatively larger masses of silicates. The more irregular character of the vesicles, when compared with the average terrestrial pumice, may possibly result from the lower gravitational field of the Moon. Loose protrusions and particles within the vesicles would not settle and fuse to the walls before the cooling of the melt.

Sprays from the Moon and from Other Sources

Particles transitional between the regular, the pumiceous and the rare types are uncommon, and it is conceivable that each type was produced in different conditions. The modes of formation of spray particles of terrestrial or extraterrestrial origin so far suggested include primary condensation of celestial bodies, volcanism and meteoritic impact. Terrestrial volcanic sprays differ from most lunar particles by a marked degree of chemical homogeneity and by the lack or rarity of symmetrical rotational forms[2]. The absence of flattened rotational forms is particularly significant because the development of such forms could not be efficiently damped by the terrestrial atmosphere.

The glassy particles from Apollo 11 show relatively few structures which can be recognized as due to impact.

The terrestrial meteoritic impact particles contain symmetrical, chemically rather homogeneous globules of glass, many with opaque cores[5]. There are no rotational forms. The meteoritic microchondrules and chondrules have been interpreted recently as condensates from the solar nebula[6], or products of incandescent clouds which constituted the mantles of condensing asteroid-type parent bodies[2]. The chondrules are characterized by the absence of rotational forms and a high incidence of sintered and coalesced aggregates[7]. Only some of the rarer structures from the Apollo 11 material show any detailed resemblance to chondrules. Such structures include some partially crystallized glassy particles[8], magnetite grains and grape-bunch type aggregates. The cosmic spherules from the ocean bottoms and other locations show a high degree of recrystallization with no pronounced rotational structures[9].

Tektites and microtektites[10] are believed to be products of impacts on the Earth[11] or the Moon[12]. Although the rotational forms of tektites more closely resemble the rotational forms of lunar particles than the other forms, they differ from the bulk of the Apollo 11 particles in having a less basic mean chemical composition and a greater degree of homogeneity.

Theoretical Considerations

Might solar radiation explain the origin of the lunar glassy particles? It was predicted before Apollo 11 (ref. 13) that because of the increased solar radiation in vacuum, the mainly basaltic lunar surface would become depleted in Na, K and organic molecules. Gold[14] has attributed the glassy sheets seen in craters to fusion by solar flares of unusual intensity. Loose particles on the lunar surface would need relatively less radiation to melt because heat would be conducted away only through narrow contacts with the rest of the lunar material. If the exceptionally intensive solar flare is powerful enough to fuse the concave portions of the lunar surface, it would therefore also be adequate to melt loose particles. Some of the fused particles would remain on the lunar surface. The heat escaping through their narrow contacts would sinter the dust particles beneath and produce the characteristic appendages. Thermal decrepitation before melting would throw up other particles from the surface. Such particles would eventually melt in a suspended state, and the high electron flux and radiation would cause the rotational forms throughout a broad range of particle diameters.

This hypothesis is also supported by the existence of particles of both salic and mafic composition. Our terrestrial sprays clearly indicate that both volcanism and meteorite impact involve the production of homogeneous or quasi-homogeneous glasses. A hypothesis of glass formation through hypervelocity impacts of minute cosmic particles may possibly account for the chemical diversity of the spray, but according to this theory one may expect a higher than observed degree of other impact structures. The extreme composition range of the lunar sprays can be most readily explained by solar flares melting minute, essentially monomineralic particles on the lunar surface.

The conditions of vacuum and high electron flux of the lunar surface have been simulated with the electron microscope. Fox *et al.*[3] reported that the focusing of the electron beam of lunar fragments results in sintering and the formation of globules with dimples and bud-like protrusions on their surfaces.

The solar flare origin implies the possibility that the reported depletion of volatiles in the lunar basalts from Apollos 11 and 12 would be at least partially caused by solar evaporation. A decrease of spray particles and an increase of volatiles, including organic molecules, may therefore be anticipated with increasing depth beneath the lunar surface, and also at the surface of those areas which were not close to the equator at any time in the history of the Moon.

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Comments on the Big-bang

by

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Is the big-bang hot, warm or cold? And are galactic masses determined by the interplay of gravitational and strong interactions in the very early universe?

MANY of the most intriguing problems encountered in big-bang cosmology are concerned with the nature of the very early universe. Elsewhere[1-3] it is suggested that cosmology and high energy particle physics are intimately related during the earliest moments of the expanding universe, and that large structures such as galaxies already exist in primitive form. In this discussion comments are made on the problem of whether the big-bang is hot,

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warm or cold, and on the possibility that galactic masses are determined by the interplay of gravitational and strong interactions.

From the present average values of the baryon, lepton and photon densities it is possible[4-6] to follow roughly the changing composition of the universe back to the onset of strong interactions at a density and temperature of

$$\rho_\pi \sim m_\pi/\lambda_\pi^3 \sim 10^{14} \text{ g cm}^{-3} \quad (1)$$

$$T_\pi \sim m_\pi c^2/k \sim 10^{12} \text{ K} \quad (2)$$

where $\lambda_\pi = \hbar/m_\pi c$ is the pion wavelength, and symbols have their usual meaning. If time is measured from a singularity of infinite density the "hadron era" terminates at

$$t_\pi \sim (\lambda_\pi^3/Gm_\pi)^{1/2} \sim 10^{-4} \text{ s} \quad (3)$$

One might, with perhaps pardonable audacity, refer to the universe in the period $t > t_\pi$ up to the present epoch as the "known" universe. But before t_π the universe is "unknown", and our theories of its composition and nature are highly uncertain and speculative. The hadron spectrum is known only at relatively low energy and there is no generally accepted theory of the properties of matter at extreme densities and temperatures. Moreover, the universe may be far from uniform, and attains ultimately its homogeneous and isotropic state through interconnexions opened up by "mixmaster" convulsions, as proposed by Misner[7]. (Daroshkevich and Novikov, however, in an unpublished paper, find that non-uniformities do not entirely disappear by this method.) Alternatively, the global homogeneity and isotropy of the universe may derive from an initial underlying uniformity of a primordial chaos at the threshold epoch of classical cosmology[1,2]

$$t^* \sim (G\hbar/c^5)^{1/2} \sim 10^{-43} \text{ s} \quad (4)$$

when the density is

$$\rho^* \sim c^5/G^2\hbar \sim 10^{93} \text{ g cm}^{-3} \quad (5)$$

and the hadron gravitational and strong interactions are of comparable strength. Before t^* there exists the physically indescribable chaos of quantum cosmology[1]. According to this hypothesis all astronomical structure on the galactic scale evolves from the fluctuations of the primordial chaos[3].

One might adopt the attitude that $t < 10^{-4}$ s is only a fleeting moment and ask: why therefore bother with it? But from the point of view of logarithmic time, which possibly is more meaningful, the known universe of $40 \lesssim \log t/t^* \lesssim 60$ is of shorter duration than the hadron era of $0 \lesssim \log t/t^* \lesssim 40$. Or one might argue that in the hadron era the universe may be totally unlike anything hitherto imagined, and ask whether it is sensible to discuss so speculative a subject. From time immemorial man has wondered about the origin of the universe, and no doubt he always will. Apart from man's usual motives for speculating on such an obscure subject, the big-bang is itself a fascinating subject for study. And at the present moment rival theories of the early universe offer us an interesting choice between hot, warm or cold big-bangs.

An equation of state frequently used for extreme densities is

$$p = (\gamma - 1)c^2\rho \quad (6)$$

where p is an isotropic pressure, and γ is a constant in the range $1 \leq \gamma \leq 2$. There are reasons for thinking[8] that in fact $1 \leq \gamma \leq 4/3$, despite arguments[9] favouring an upper limit of 2. If thermodynamic equilibrium is assumed, the temperature in the early universe is[6]

$$T \propto \rho^{(\gamma-1)/\gamma} \propto t^{2(1-\gamma)/\gamma} \quad (7)$$

and therefore rises with density when $\gamma > 1$. (If the density of hadronic states increases as some power of particle mass, then[10] $1 \leq \gamma \leq 5/4$.) This is the hot big-bang theory, and at the threshold epoch the temperature for $\gamma \leq 4/3$ is $T \leq T^*$, where

$$T^* \sim (\hbar c^5/Gk^2)^{1/2} \sim 10^{32} \text{ K} \quad (8)$$

Layzer[11] suggests that on the contrary the universe has initially a zero temperature. This theory postulates a complete absence of antimatter in the initial stage[12], and assumes that the 3 K microwave radiation is not a remnant of the primordial fireball. It can be argued that a cold big-bang implies perhaps a universe which must forever expand. Consider the case of a universe that first expands and then eventually collapses. Because of the 3 K radiation the collapse phase terminates in a hot big-bang[13] and this occurs independently of whether the previous big-bang is hot or cold. If now collapse is followed by renewed expansion, as in the oscillating or bouncing cosmological models, then all succeeding big-bangs are hot. The probability of being at the beginning of such a sequence is presumably very small, and therefore the cold big-bang theory limits the available cosmological models to those which continually expand.

Hagedorn[14,15] has proposed a theory of dense matter—in thermodynamic equilibrium—of great importance in cosmology. He presents a strong case in support of an exponentially increasing mass spectrum, and from this has developed the "hadronic boiling-point" theory. The exponential smallness of the number of particles in the high mass states is offset by the exponentially large number of such states, and as a result the temperature approaches asymptotically a limiting value T_π for densities large compared with ρ_π, where ρ_π and T_π are given by relations (1) and (2). Hence, according to this theory the big-bang has a temperature of the order 10^{12} K, and is "warm" in comparison with the hot and cold models.

An integration over all hadronic states in Hagedorn's theory gives a total number of particles in volume V of

$$N \sim \ln Z(V,T) \sim -\frac{V}{V_0}\ln\left(\frac{T}{T_0} - 1\right) \quad (9)$$

for $\rho \gg \rho_\pi$, where Z is the partition function, $V_0 \simeq \lambda_\pi^3$, and $T_0 \simeq T_\pi$. In place of equation (6) the equation of state is now

$$p = \frac{T}{V}k \ln Z \sim \frac{T_0}{V_0}k \ln\left(\frac{V_0\rho c^2}{kT_0}\right) \quad (10)$$

and therefore $p/\rho c^2 \to 0$ as $\rho \to \infty$. Thus a uniform universe in the hadron era is similar to the Einstein–de Sitter model. It is possible, however, that Hagedorn's theory applies only for a limited period of time in the very early universe. The integration over all hadronic states of $m_\pi \leq m \leq \infty$ is allowable as long as the Hubble distance, of order ct, is large compared with the pion wavelength. When $t < \lambda_\pi/c$, the observable universe is smaller than the pion wavelength and points separated by a distance λ_π move apart at a speed greater than c. To avoid this anomalous situation and preserve the elementarity of particles it is necessary to eliminate the particles of mass $m < \hbar/tc^2$. In other words, particles of mass $m < \hbar/tc^2$ cannot exist, and the lower mass hadron states are depopulated when $t < \lambda_\pi/c \sim 10^{-23}$ s. The hadronic boiling-point is therefore the

Hagedorn value $T_0 = T_\pi \sim 10^{12}$ K in the range

$$10^{-23} \sim \lambda_\pi/c \lesssim t \lesssim (\lambda_\pi^3/Gm_\pi)^{1/2} \sim 10^{-4} \text{ s} \quad (11)$$

$$10^{56} \sim c^2/G\lambda_\pi^2 \gtrsim \rho \gtrsim m_\pi/\lambda_\pi^3 \sim 10^{14} \text{ g cm}^{-3} \quad (12)$$

and the universe has an age of at least one chronon, or one "jiffy". (I am indebted to W. A. Fowler for the term "jiffy", who recalls that it was used by R. C. Tolman for the time light takes to travel a distance of 10^{-13} cm.) When the age of the universe is less than one jiffy, Hagedorn's theory still applies, but now $V_0 \sim (ct)^3$ and the hadronic boiling-point is $T_0 \sim \hbar/kt$. Hence, as we go back in time from 10^{-4} s to 10^{-23} s the big-bang is warm and has roughly a constant temperature of 10^{12} K. And then, at earlier epochs, the temperature rises as $1/t$ and ultimately attains the original value given by relation (8) of 10^{32} K at the threshold epoch. To the question whether the big-bang is hot, warm or cold, the answer is that perhaps at first it is hot, and is then warm for most of the time.

Density fluctuations in the Hagedorn theory are of the form

$$\frac{\delta\rho}{\rho} = \alpha\left(\frac{V_0}{V}\right)^{1/2} \sim \left\{\frac{1}{N}\ln\left(\frac{T_0}{T}-1\right)^{-1}\right\}^{1/2} \quad (13)$$

(where $\alpha \sim 1$) and are of the order unity in volume V_0. In the warm era of the big-bang the nucleon volume V_0 is small compared with the observable universe, and equation (13) may be used for $V_0 \lesssim V \lesssim (ct)^3$. But in the earlier hot era, $V_0 \sim (ct)^3$, equation (13) tells us that fluctuations are of order unity within the observable universe, and is unable to specify the fluctuation spectrum for wavelengths λ greater than ct. The fluctuation spectrum for $\lambda > ct$ is in a frozen state, and is essentially a relic of the fluctuations prevailing at the threshold epoch. Let $\delta\rho/\rho = \beta(N)N^{-n}$ be the fluctuation spectrum at the threshold epoch, where $N = V/V^*$ is the number of particles in volume V, and $V^* \sim (ct^*)^3$. In order that these fluctuations develop into galaxies it is shown[2] by arguments of a quite general nature that $n = 2/3$, and $10^{-2} < \beta_g < 1$, where β_g is for galactic masses. It is also shown semi-quantitatively that metric fluctuations at the threshold epoch have the remarkable property of obeying the $n = 2/3$ rule. A weakness of the theory, however, is its inability to determine $\beta(N)$ and therefore, in principle at least, galaxies of all masses are possible. But if, as a result of the initial fluctuations or even possibly convulsive oscillations, it is possible for weak correlation to exist (weak in the sense $\alpha \ll 1$) over distances larger than a pion wavelength, then β will tend to maximize at wavelengths of the order λ_π owing to pion interactions, as in Hagedorn's theory. This leads to the surprising conclusion that the mass contained in a nucleon volume of λ_π^3 at the threshold epoch is ultimately the typical mass of a galaxy. A mass M in the primordial fireball becomes ultimately a material mass of[6] ηM, where $\eta \sim b(\hbar c/kT)^3$, b is the present average density of nucleons, and $T \simeq 3$ K is the background radiation temperature. Hence the ultimate material mass in an initial nucleon volume is

$$M_g = \eta\rho^*\lambda_\pi^3 \sim 10^{44} \text{ g} \sim 10^{11}\, M_\odot \quad (14)$$

for $\eta \sim 10^{-10}$. Because η is of the same order as the dimensionless number $(Gm_\pi^2/\hbar c)^{1/4}$, equation (14) can be expressed as

$$M_g \sim m_\pi(\hbar c/Gm_\pi^2)^{7/4} \quad (15)$$

and forms an interesting contrast to a solar mass of $M_\odot \sim m_n(\hbar c/Gm_n^2)^3$ [2] where m_n is the nucleon mass. What determines the baryon number constant η is, of course, unknown. But it is interesting to notice, nonetheless, that stellar masses are governed by gravitational and Coulomb interactions, whereas galactic masses may be determined primarily by the interplay of gravitational and strong interactions in the very early stages of the big-bang.

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Generation of Cherenkov Light Flashes by Cosmic Radiation within the Eyes of the Apollo Astronauts

by

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It is proposed that the primary cosmic radiation is responsible for the light flashes observed by astronauts in translunar flight. Cherenkov radiation may be an important or even the dominant mechanism. An alternative mechanism is the direct excitation of the retina by cosmic ray particles.

CREW members of Apollos 11, 12 and 13 have reported the observation of flashes of light within the command module during the long periods of translunar flight (private communication from D. E. Hagge). In this article we suggest that because nearly all the observations were reported to have occurred when the eyes were closed, the flashes are probably caused by primary cosmic ray particles which penetrate the cabin walls and the astronauts' eyes. Two mechanisms are proposed here, the first that the flashes arise from Cherenkov radiation generated

Table 1. ASTRONAUTS' OBSERVATIONS

Flight	Astronaut	Observations
Apollo 11*	Edwin Aldrin	All incoming light blocked by shades, spacecraft revolving; pinpoint-like flash that dissipated rapidly; single flash, white; frequently streak, occasional double flash; random location; saw them at will; static electricity not the cause; never recalled seeing them with eyes closed
	Neil Armstrong	Saw approximately 100 flashes; rate about one per minute
Apollo 12*	Charles Conrad	Observed flashes with eyes closed; approximately 10 per cent of flashes were streaks and double points; streaks appeared horizontal; all streaks appeared in the upper field of view
Apollo 13†	General comments	Each crewman saw light flashes, but only while in the dark and with the eyes closed. All observations were made in the command module, both in and under the couches. None of the flashes were obtrusive. The crewman had to be relaxed before he noticed them. They observed many more dots than streaks, and the colour was always white
	James Lovell	Frequency of flashes about once every two minutes
	Jack Swigert	Counted only two flashes in one half hour
	Fred Haise	First evening counted 10 flashes in a 5-min period; time intervals varied from 7 s to 1·5 min; all but one flash were dots that looked like "roman candles"; in this set there was one streak which passed from left to right; the second night 12 to 15 flashes observed before falling asleep (time interval not defined)

* Comments by Astronaut Edwin Aldrin, recorded by G. G. Fazio; also private communications with D. E. Hagge.

† From memorandum by Astronaut Anthony England on debriefing of crew of Apollo 13.

in the ocular media or the retina of the eye or, alternatively, that the receptors or their accompanying cells in the retina are excited directly by ionization. We shall discuss chiefly the former mechanism.

There is already evidence[1], at a reasonable statistical significance, that even sea level cosmic radiation, chiefly μmesons, can be detected by the Cherenkov radiation which they produce in the vitreous humour of the eye. At the same time, however, it was pointed out that the observed effects could possibly be attributed to fluorescent light in the vitreous humour or even direct excitation, either of the optic nerves or the areas of the brain associated with vision.

All the observations so far on the Apollo flights have been in translunar flight, and never in Earth orbit or lunar orbit. On Apollos 11 and 12 the observations were reported without the astronauts receiving special briefing before the flights, but on Apollo 13 the astronauts had received special instructions before the flight, so that their reports may be more reliable and detailed than was the case for the two earlier flights.

The observations, taken straight from the astronauts' notes and amplified in debriefing interviews, are listed in Table 1.

Cherenkov Radiation in the Ocular Media and Retina

A single primary cosmic ray charged particle of atomic number Z and velocity βc will produce Cherenkov radiation in the ocular media of the eye at the rate

$$dN/dl = 2\pi\alpha Z^2 \sin^2\theta \int(1\lambda^{-2})d\lambda \text{ photons cm}^{-1} \qquad (1)$$

where θ, the Cherenkov angle, is $\theta = \cos^{-1}(1/\beta n)$ (ref. 2). For the vitreous humour of the eye, $n = 1{\cdot}34$, so that the threshold kinetic energy for protons is 470 MeV, $\theta(\max) = 42°$, and the asymptotic value of $dN/dl = 210$ photons cm^{-1}, over the band $\Delta\lambda = 3800$ to 6200 Å. The duration of the flash will depend on geometrical factors, but is always $\leq 10^{-10}$ s in this case, that is, short compared with the integration time of the eye, ~ 50 ms.

The response of the eye to this internally generated light will depend on a variety of factors, physical, physiological and psychological. As well as the wide fluctuations which always arise in numerical data in this field, there are additional factors because of the unusual environment in translunar or orbital flight.

First consider a relativistic proton passing through the centre of the eye and striking the retina normal to its surface. Because the light is at best of very low intensity, we shall be concerned exclusively with scotopic (rod) vision, and because the cosmic ray particles will usually arrive with a random orientation, we can neglect the foveal region of the eye and consider mainly the peripheral regions. Near threshold the retina exhibits an areal integration, but this is sufficiently restrictive to limit the effective path length of the particle and hence the maximum available light. Various values appear in the literature for the effective area of integration in scotopic vision. We will here adopt 20′, quoted[3] for the diameter of the basic unit, the area believed to be served by a single ganglion cell. For the Cherenkov radiation considered here, the light flux falls off as $(1/r)$ from the impact point of the particle on the retina. In these circumstances both physiological summation and probability summation[4] will play their part in extending the effective area. This suggests that 2° may be a reasonable value to take for the effective diameter of the area of integration in this particular problem. With a posterior–nodal distance of 17 mm, this corresponds to an absolute radius of 0·29 mm on the retina (Fig. 1) so that the effective track length Δl is only 0·32 mm. We note that this is comparable with the figure for the thickness of the retinal layer itself, typically 0·36 mm (ref. 5), so that the effective portion of the particle track occurs within the retinal layer itself rather than in the vitreous humour in front; the effective part of the Cherenkov cone is thus formed in and among the various layers of cells and their synapses. The larger portion of the cone which develops throughout the vitreous humour and the other ocular media is therefore relatively ineffective.

For the absolute sensitivity for the dark adapted eye, we take the figures obtained by Hecht *et al.*[6], namely, that 5–14 quanta are required at the retina to produce a signal from a 1 ms flash at $\lambda = 5100$ Å on a 10′ diameter area, with a 60 per cent probability of detection. At the present level of approximation we will adopt a figure of 10 photons for the threshold sensitivity, somewhat higher than the limit of 4 photons quoted in some later work[7].

With these values of (dN/dl), $\Delta\lambda$ and Δl, 6·7 photons on average are thus available, per proton ($\beta = 1$), on the elemental area. The effective number of photons is then given by $6{\cdot}7\ (\int\lambda^{-2}L(\lambda)d\lambda/\int\lambda^{-2}d\lambda)$ where L is the scotopic luminosity factor[8] normalized to unity at $\lambda = 5100$ Å and corrected for transmission of the ocular media[9]. Using the data from refs. 8 and 9, the ratio of these integrals is 0·43, so that the average effective photon number is reduced to 2·9. This falls short of the accepted

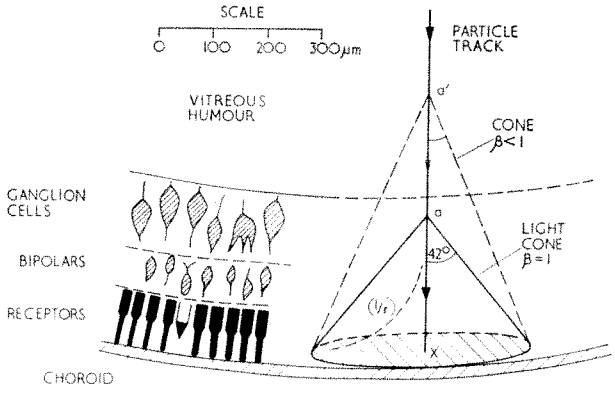


Fig. 1. Left, the essential features of the layers of cells in the human retina. Right, the formation of a Cherenkov light cone formed from a primary cosmic ray particle traversing the retina.

threshold of 10 by a factor of ~ 3, so a singly charged relativistic proton (or electron) will produce a detectable flash only once in $\sim 10^3$ impacts, bearing in mind that Hecht's threshold itself only represented a 60 per cent probability of detection.

But because the Cherenkov light yield varies as Z^2, α particles should be just detectable at the 60 per cent confidence level, while for the Li, Be, B group ($Z^2 = 9$ to 25) and the C, N, O group ($Z^2 = 36$ to 64), the available light exceeds the threshold by factors of 2·2 to 6·2, and 9 to 16, respectively. In these cases therefore the detection probability may approach unity, even with some relaxation on the requirements of no stray light and full dark adaptation.

If the particles enter the retinal layer obliquely, as the majority will, the pool of light will be ellipsoidal, with the impact point at one focus of the ellipse. With the $(1/r)$ flux distribution, the photons will cluster round the impact point, and for particles of sufficiently high Z we might expect an observer to report that the image had shape, and he might then record an "ellipse" or a "comet head". If we assume the same elemental area of integration, a disk of diameter $\sim 20'$, we find that for an iron nucleus ($Z = 26$) the area of sensitive detection may extend over an area having a diameter 7°, and that even for an oxygen nucleus ($Z = 8$) this disk will still have a finite size, a diameter of $\sim 0{\cdot}66°$.

Much of the Cherenkov light impinges on the receptors at larger angles than those which occur in normal vision, so the directional sensitivity of the rods could also be quite important. There is evidence[10] for such directivity, and it may be quite marked in this case. For particles entering the eyeball at random orientations and impinging at random locations on the retina, averaging will, however, occur, permitting much of the light to fall on the receptors at favourable angles.

On the Cherenkov interpretation of the Apollo experiments, we would not expect colour to be reported. The threshold for photopic or cone vision is ~ 800 times higher than for rod vision[5] so that if α particles are just detectable in the latter case, colour would appear only when $Z \geq 56$.

These estimates apply for particles having $\beta \to 1$. We note that for a constant area of integration, and particles with $\beta < 1$, as θ decreases Δl increases ($a'X$ in Fig. 1), so that the number of effective photons varies as $\sin^2\theta \cot\theta$. Because this function (Fig. 2) rises more rapidly with kinetic energy, above the threshold, than the normal Cherenkov yield, equation (1), it is sufficiently accurate to assume that any cosmic rays within the spacecraft cabin which arrive at the retina above the threshold will generate light at the asymptotic value of 6·7 photons per nucleon.

As already mentioned, a number of unknown factors will influence the absolute threshold in the Apollo observations. Many of these factors will combine to raise the threshold of vision, and we mention, for example, that it is known[5] that oxygen deficiency lowers the sensitivity. We do not know, however, what effects the highly enriched oxygen atmosphere of the spacecraft might have on the sensitivity.

Estimates of Flash Rates due to Primary Cosmic Ray Nuclei

The integral number of nuclei with charge Z and with energy greater than E_0 passing through the retina per unit time is given by

$$\frac{dN(>E_0)}{dt} = I_z(>E_0)A\Omega \qquad (2)$$

where I_z is the integral intensity of nuclei of charge Z in units of (cm² s sr)⁻¹, A is the effective cross-sectional area of the eye, and Ω is the effective solid angle subtended by the retina. The value of A was taken to be 3 cm². The value of Ω was determined from the density distribution of rods along a horizontal meridian of the retina. We have taken the sensitive region to be $\pm 70°$ from the fovea[4] which gives a value of $\Omega = 4{\cdot}13$ sr.

Table 2. COSMIC RAYS INCIDENT ON THE ASTRONAUTS' EYES

Primary particle	Z	Primary flux above 1·5 GeV nucleon⁻¹ (k.e.) at solar min. (m² s sr)⁻¹	Interaction m.f.p. (water) (g cm⁻²)	Flash rate (min)⁻¹	Integral flash rate for nuclei $\geq Z$ (min)⁻¹
He	2	89	39	5·1	5·6
Li	3	0·71	30	0·038	0·49
Be	4	0·31		0·017	0·45
B	5	0·99		0·053	0·43
C	6	2·8	24	0·14	0·38
N	7	0·94		0·046	0·24
O	8	1·8		0·088	0·19
F	9	0·22		0·011	0·10
Ne	10	0·49	19	0·022	0·090
Na	11	0·23		0·010	0·068
Mg	12	0·54		0·024	0·058
Others	≥13	0·83	17	0·034	0·034

Taking the effective threshold energy for the Cherenkov radiation process to be 1·5 GeV per nucleon (Fig. 2) we have computed the number of cosmic ray nuclei above this energy at the eye of the astronaut, assuming that they have penetrated a thickness equivalent to 10 g cm⁻² of water (unpublished work of S. B. Curtis). Attenuation was considered but not secondary production. The primary cosmic ray fluxes used were based on values outside the Earth's magnetosphere at solar minimum[11]. The results are presented in Table 2 and Fig. 3.

The actual counting rate depends on the sensitivity of the retina. In ideal conditions one could detect helium nuclei with a count rate of $\sim 5{\cdot}6$ min⁻¹. Unless ideal conditions were attained it would seem that only nuclei with $Z \geq 6$ could have been detected; the calculated rate in this case is 0·38 flashes min⁻¹.

Curtis (unpublished) has computed the intensity of heavy galactic cosmic rays ($Z \geq 6$) expected at the eye of an astronaut inside the Apollo command module outside the Earth's magnetosphere. He estimated the total flux of particles with $Z \geq 6$ that could penetrate the cabin to reach the eye to be between 0·5 and 1·0 (cm² min)⁻¹ at solar minimum. These rates, considering the lower energy, are essentially consistent with our own estimates.

In these calculations the spallation products (most probably alpha particles) resulting from cosmic ray nuclei interacting in the spacecraft have been neglected. It is possible, however, that two or more nuclei resulting from spallation may pass through the retina simultaneously, producing two or more flashes.

We have also considered several other cosmic ray processes that could produce a sufficient intensity of Cherenkov light to be detected by the eye: (*a*) production of a shower of particles in the vitreous humour of the eye or in the spacecraft walls, (*b*) simultaneous passage of several protons through the eye during the integration time, and (*c*) production of Cherenkov light in the atmosphere of the spacecraft. The generation of fluorescent radiation in the spacecraft atmosphere or in the eye has also been considered. All these processes produce rates that are negligible compared with the rate caused by primary nuclei.

Direct Excitation of the Retina

It has long been known that the sensation of light (phosphene) can be produced by the effect of ionizing radiation on the retina. In most of this work (ref. 12, for example), the emphasis has been on irradiation by X-rays, in which the sensation of light is produced over the retina as a whole, under conditions in which the dosage is relatively high and the quantum energy low. The situation in space is quite different. Astronauts are exposed to

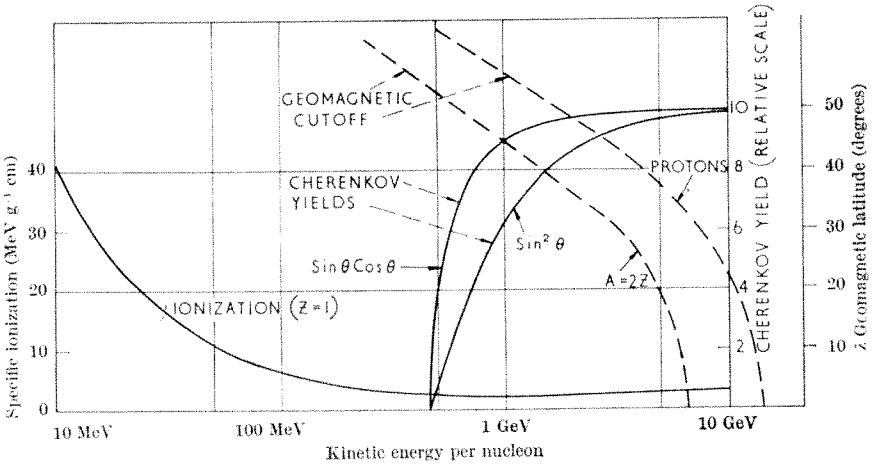


Fig. 2. The Cherenkov light yield, and the specific ionization, for particles traversing the ocular media and retina of the eye. Also shown are lines representing the rigidity cutoff imposed by the Earth's magnetic field.

high energy particles and γ-rays at relatively low fluxes. As the observers were reporting individual flashes, of limited duration and angular size, the stimuli have temporal and positional characteristics.

Fremlin[13] and Tobias *et al.* (unpublished) recently reported that faint flashes of light may be seen by dark-adapted subjects exposed to fast neutrons, in the energy regions 0–8 MeV and 20–640 MeV respectively. We ourselves have recently observed similar effects with 14 MeV neutrons, although we failed to observe such effects with 3 MeV neutrons. It is presumed that these flashes arise from ionization or excitation produced in the retina, either from recoil protons (ref. 13 and Tobias *et al.*) in the hydrogenous material of the retina (or vitreous humour) or by recoil nuclei (Tobias *et al.*) of, say, carbon, nitrogen or oxygen. The experiments are necessarily limited in scope and there is no indication as to whether the sensation is located in the receptors, the various layers of cells or their synapses, or even in the optic nerve. But they do seem to indicate that the excitation was not in the visual cortex. Although at least some of the neutrons in the work of Tobias *et al.* produced protons with energy exceeding the Cherenkov threshold ($E_p = 470$ MeV), this was not the case in the work reported by Fremlin[13]. The latter work therefore provides definite evidence that light flashes can be produced by effects other than the Cherenkov mechanism. In this case the mean neutron energy was 3 MeV, producing recoil protons with a range of about 100 μm, comparable with the length of a rod (60 μm). The observed counting rate was about 25 per cent of that computed. The specific ionization of the recoil proton is about seventy times the minimum value. The minimum value of the specific ionization necessary to generate the light flashes is still unknown, though the experiments described by Tobias *et al.*, where the mean neutron energy was 300 MeV, indicate that this may be as low as twice the minimum value. Thus in the Apollo spacecraft it is possible that cosmic ray nuclei with $Z \geq 2$ generated the light flashes by direct excitation in the retina. Crude extrapolation of X-ray data to the case of single high-energy particles, on the assumption that ionization in the retina is the only mechanism causing the phosphene, also leads to the expectation that relativistic cosmic ray nuclei with $Z \geq 2$ should produce observable effects.

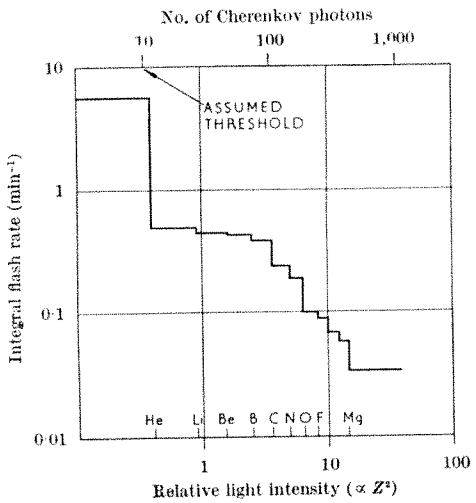


Fig. 3. The integral flash rate calculated for Cherenkov radiation produced by the primary cosmic radiation in the human eye, outside the Earth's atmosphere.

Choice of Interpretations

Of the two interpretations considered, the Cherenkov mechanism has the merit that it is possible to predict the flash rates with reasonable accuracy. The yield of light, spectrum and angular characteristics of Cherenkov radiation are all accurately known, and because the stimulus is optical and hence operates only through the light receptors, whose sensitivity is also well known, there is relatively little uncertainty in the calculations of the flash rates. The theoretical yields (Fig. 3) and the rates observed by the astronauts (Table 1) agree well, within the uncertainties imposed by their environment and the unknown degrees of dark adaptation achieved. We conclude that this mechanism may alone be responsible for the phenomenon.

The alternative mechanism of direct excitation may, however, also occur; it certainly cannot as yet be excluded, although present uncertainties prevent all but very tentative estimates from being made.

We note from Table 1 that some of the astronauts report "streaks" and "double points". Ionization and the formation of elementary Cherenkov cones occur all along the particle tracks, so "streaks" can be expected on both models for those particles which traverse the eye tangential to the retina. The streaks will, however, have a maximum length, represented by a cord tangential to the inner surface of the retina. If the eye is taken to be spherical, of diameter ~20 mm, and the retina has an effective depth of ~0·30 mm, the maximum length of cord is 4·0 mm. With a retinal scale of $1' = 4{\cdot}85$ μm, the streak of maximum length will thus subtend an angle equivalent to 17°. "Double points" can likewise be explained on both models, and may arise when a particle either intercepts the retina

of one eye at two points, or alternatively traverses both eyes in succession.

The "roman candles" referred to on Apollo 13 may also be interpreted on both models. On the Cherenkov model the effect may arise from the oblique interception of the light cones, as discussed earlier, with the clustering of photons around the focus of the ellipse. On the direct excitation model, if the phosphene is related to the specific ionization, we might expect a track to brighten towards the end of its path. We note that impressions drawn freehand by observers in the unpublished work of Tobias *et al.* could easily be identified with "roman candles".

The impressions of motion and/or directions of the flashes, pose problems, however, and we suggest that these effects are illusory.

It is tempting to suggest that the absence of observations on Earth orbital flights may be associated with the effect of the Earth's magnetic field on the primary radiation. But as we see from Fig. 2, the lines which represent the rigidity cutoff fall well to the right of the diagram, at energies above those which already give a Cherenkov yield near to the asymptotic value. We therefore feel that a more likely explanation is that on these earlier Earth orbital flights the astronauts were either too occupied, were not dark-adapted, perhaps were not wearing eye-shields, and in any case were not given rest periods long enough for the appropriate conditions to be realized.

Observations in lunar orbit would be of particular value. The astronauts could then face the lunar surface or away from the surface and, as the Cherenkov radiation is intrinsically directional, it may be possible to distinguish between this mechanism and the alternative one of direct excitation. Even for Cherenkov radiation, however, it is unlikely that a very large ratio in the counting rates would be observed for the two orientations of the astronauts' eyes, for a variety of reasons. First, the Moon obstructs only 2π of the full 4π geometry. Second, the Cherenkov cone has a very large opening angle, 42°. Third, the retinal layer extends over a large fraction, at least 2π, of the inner surface of the eye.

For nuclei of high Z, it should be possible in principle to distinguish between Cherenkov and direct excitation mechanisms by careful observations of the shape and size of the observed images. Because the lateral extent of the ionization will at most be determined by the range of the δ-rays (~ 10 μm) the size of the "points of light", or the thickness of the "streaks", will be independent of Z, so that on the direct excitation model the points and streaks will remain sharp and fine respectively, even at high Z. On the Cherenkov model, as we have seen, as Z increases the images will enlarge into circular or elliptical disks. Two factors are, however, present which are likely in practice to mask this discrimintion. In the first place the frequency of occurrence of nuclei of high Z is so low (Table 2) that the astronaut's concentration is likely to wane during the long periods of observation required, and second, even if the Cherenkov mechanism is dominant, the steep $(1/r)$ dependence of the photon flux might lead to the astronaut reporting a point rather than a disk, even for a particle of really high Z.

We thank Dr C. A. Lundquist for bringing these Apollo observations to our attention.

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Polarization of Pulsar Radiation

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The polarization of radiation from four pulsars has been measured and interpretations of the results are suggested.

OBSERVATIONS of the polarization of both radio and optical pulses from pulsars have been of considerable value in providing information on radiation mechanisms. These observations have generally been of the mean pulse profile as it has been shown[1] that this has a stable form, and so represents structure intrinsic to the pulsar, whereas the polarization of individual pulses varies greatly from pulse to pulse[2]. Also, it is of course possible to decrease random noise fluctuations by averaging, and so weaker pulsars can be studied.

For the Vela pulsar (PSR 0833–45) Radhakrishnan *et al.*[3] showed that the mean pulse profile was essentially completely linearly polarized and that the position angle changed continuously through the pulse. Ekers and Moffet[1], using observations at a frequency of 2295 MHz, found that most of the pulsars observed (except PSR 0833–45) were weakly polarized and that the position angle varied through the pulse, but not in a continuous way as for PSR 0833–45. Instead the position angle was found to change discontinuously between different components of the pulse. More recent observations by Komesaroff *et al.*[4] and Morris *et al.*[5] at similar frequencies have shown that for several pulsars, in addition to PSR 0833–45, the position angle varies through the pulse with no large discontinuities. For the Crab Nebula pulsar, however, Campbell *et al.*[6] observe no variation in position angle of the polarized component. It is apparent that further observations are required to resolve the important question of polarization variation in radio pulses from pulsars.

Observing System

During April 1970 polarization observations at frequencies near 400 MHz were made using the 300-feet transit telescope of the National Radio Astronomy Observatory. Observations at this lower frequency allow profiles with good signal/noise ratios to be obtained in relatively short times because of the increased pulse intensity. Comparison of these observations with those made at higher frequencies is also important.

The telescope was equipped with a travelling feed system which allowed a given source to be tracked for

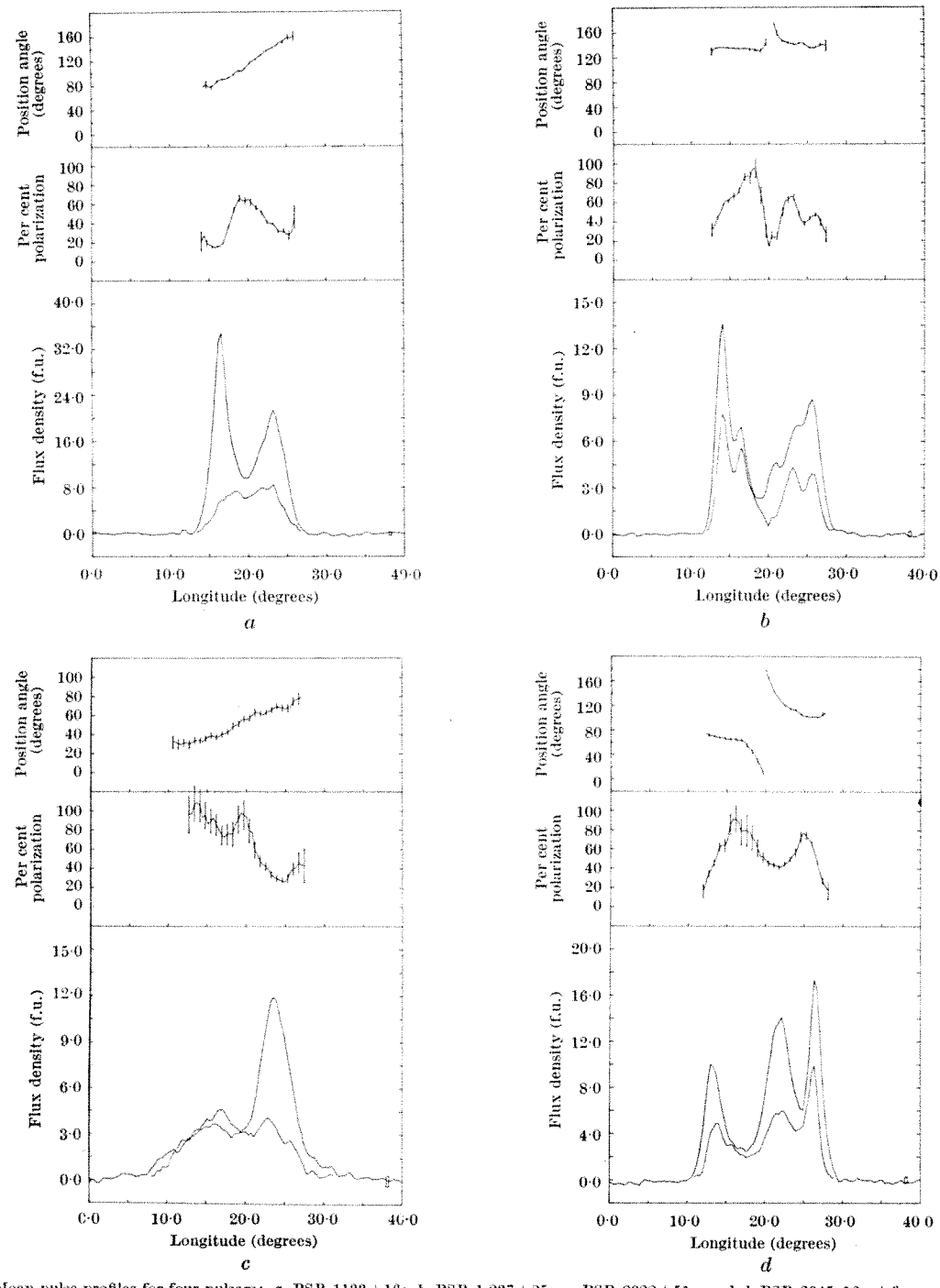


Fig. 1. Mean pulse profiles for four pulsars: *a*, PSR 1133+16; *b*, PSR 1 237+25; *c*, PSR 2022+51; and *d*, PSR 2045–16, at frequencies close to 400 MHz. On the bottom graph in each case the total intensity and intensity of the linearly polarized component are shown. Because the position angle has a 180° cycle, the variation for PSR 2045–16 is continuous through the pulse. Error bars are ±2 standard deviations.

about 40 min. Crossed dipole feeds at position angle 0° and 90° fed phase and gain matched RF amplifiers, the outputs of which were combined in a hybrid network to give four linearly polarized components (P.A. = 0°, 45°, 90° and 135°). These were amplified, band-limited and detected by four nominally identical IF systems, and the outputs recorded using a 4 × 1024 channel signal averager, giving a sampling interval of 1/1024 of the pulsar period. For each pulsar the IF bandwidth was chosen so that dispersion broadening was less than the sampling interval, and the post-detection time constant was similarly chosen approximately equal to the sampling interval. Faraday rotation within the bandwidth would not be excessive unless rotation measures were unexpectedly high. Particular attention was paid to receiver gain calibrations. A pulsed solid state noise source at the front of the receiver was recorded for a short time at intervals of about 10 min and several continuum sources were observed each day to fix the flux density scale. Recording of the redundant linear component gave a check on the gain calibrations during subsequent processing. A rotatable dipole, radiating broad band noise toward the feed from the centre of the reflector, enabled the phase response of the system to be measured.

During observations the mean pulse profile was recorded on magnetic tape at approximately 2 min intervals. In subsequent off-line analysis these profiles were calibrated and then averaged together as desired with weighting

proportional to the square of the signal/noise ratio. These averaged profiles were then combined to give the linear Stokes parameters and other polarization parameters.

Polarization Parameters

In Figs. 1*a*, *b*, *c* and *d*, polarization parameters are presented for the pulsars PSR 1133+16, PSR 1237+25, PSR 2022+51, and PSR 2045−16, respectively. The observing frequency was 410 MHz, and 384 pulses were added to form the mean profile for PSR 1133+16, PSR 1237+25 and PSR 2045−16, while for PSR 2022+51 the corresponding quantities were 392 MHz and 1280 pulses. In each case the data were recorded during an interval of about 10 min in one day. Mean pulse profiles and polarization parameters recorded at other times were not significantly different from those presented. Following Morris *et al.*[5], the horizontal axis is labelled in longitude (or alternately, phase), 360° equalling the pulsar period. In the lowest graph the total intensity, I, and the intensity of the linearly polarized component, $(Q^2+U^2)^{1/2}$, are plotted. The height of the small rectangle on the right represents the probable error ($\pm 2\sigma$) in the profiles resulting from random noise. Its width is equal to the sampling interval. The percentage linear polarization is plotted in the centre graph with $\pm 2\sigma$ error bars on every second point, and position angle is similarly plotted on the upper graph.

A number of results of interest are evident in these graphs. The percentage polarization is frequently very large—for PSR 2022+51 (Fig. 1*c*) the polarization of the leading component is essentially complete. Although the polarized component usually has peaks corresponding to peaks in the total intensity, the percentage polarization is much lower for these intense components than for the weaker regions between.

In general, the variation of position angle is continuous through the components of the pulse. In two cases, PSR 1133+16 and PSR 2022+51, the position angle varies at an approximately uniform rate through the pulse, while for PSR 2045−16 the variation is more complex but still continuous. For PSR 1237+25 the position angle is almost constant through the entire pulse (excepting the almost unpolarized central part) although the pulse has at least five separate components. The variation in polarization parameters across the pulse in these pulsars cannot be accounted for by combination of components, each having a particular position angle.

The continuous variation in position angle observed in PSR 1133+16 and PSR 2022+51 is very similar to that observed in PSR 0833−45 by Radhakrishnan *et al.*[3]. Adopting the pulsar model consisting of a rotating neutron star with an oblique magnetic axis[7–9], Radhakrishnan and Cooke[10] proposed that the radiation was originating in the vicinity of a magnetic pole with the swing in position angle resulting from a corresponding swing in the direction of the magnetic field as the star rotated. Komesaroff[11] has expanded this model in a quantitative way and shown that it can account for many of the features of pulsar radio emission. Ultra-relativistic particles moving out from the pole would, because of the curvature of the field line, radiate a narrow beam in the direction of the instantaneous particle velocity, this radiation being largely polarized in the plane containing the field line. Coherent processes are necessary to account for the intensities observed. Pacini and Rees[12] suggest that if the emission is originating close to the surface of the star then protons and not electrons must be responsible.

It has been shown[10] that differential Faraday rotation is not responsible for the variation in position angle across the pulse in PSR 0833−45. Morris *et al.*[5] have observed PSR 2045−16 at a frequency of 2650 MHz and found a similar variation in position angle to that shown in Fig. 1*d*. Combination of these two observations yields a value of $0{\cdot}10 \pm 0{\cdot}23$ rad/m^2 for the differential rotation measure between the leading and trailing peaks of the pulse. Any such differential rotation between parts of a pulse must take place within the velocity of light cylinder. Current models[13] indicate that gyrofrequencies are greater than, or of the order of, the frequency of radio emission within the velocity of light cylinder, so Faraday rotation in the usual sense would not be expected there.

It has been pointed out[5] that the position angle variation observed for PSR 2045−16 (Fig. 1*d*) is that expected when the line of sight passes very close to the magnetic pole. It is proposed that for PSR 1237+25 (Fig. 1*b*) the line of sight passes directly through the pole, the position of which coincides with the dip in the polarized component at the centre of the pulse. In this case no swing of position angle would be expected. There is some indication that the pulse profile complexity is correlated with separation of the line of sight from the magnetic pole, those cases where the separation is small having complex (many component) pulses.

The continuous variation of position angle through the pulse, even when the pulse profile is complex, combined with the absence of differential Faraday rotation, gives strong support to the model of pulsar emission proposed by Radhakrishnan and Cooke and by Komesaroff. It would appear impossible, from symmetry considerations, to account for these observations by models in which the double pulse structure originates in a dual lobe radiation pattern from a single particle beam[9,14]. Also, the high fractional polarizations observed for these slower pulsars do not appear consistent with the plasma turbulence model proposed by Ichimaru[15].

The observation that the peaks in the pulse profile have a smaller percentage polarization than the weaker regions is simply explained if the peaks of emission are radiation from particles of higher energy than those responsible for the rest of the pulse. Provided the frequency of observation is below the synchrotron critical frequency, the emission intensity is increased and the fractional polarization is reduced as the particle energy is increased. Multiple component pulses as observed for PSR 1237+25 and PSR 2045−16 (Figs. 1*b* and 1*d*) can then be accounted for as radiation from several different beams, whereas they are more difficult to explain in the Fresnel zone model[11]. Also, observed pulse widths are considerably larger than those predicted by the Fresnel zone argument, so it would seem that other factors must also be important.

Finally, it is pointed out that the high percentage polarizations observed make it essential to consider the total intensity rather than one linear component when discussing pulse shapes and amplitudes, spectra of pulse components, or related topics.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Unidentified Interstellar Microwave Line

DURING a search for $H^{12}C^{14}N$ (hydrogen cyanide) and its molecular isotope $H^{13}C^{14}N$, we have detected a new molecular line. The discovery was made in June 1970 using the 36-foot telescope of the National Radio Astronomy Observatory in Tucson, Arizona. The receiver was a 3-mm mixer radiometer developed by NRAO in conjunction with Jefferts, Wilson and Penzias of the Bell Telephone Laboratories. The single sideband noise temperature of the radiometer was 3000 K and it covered frequencies from 85 to 90 GHz. At these frequencies the telescope has a beamwidth of 1′ of arc and a beam efficiency of 50 per cent under optimum conditions. The filter bank receiver used consisted of forty filters spaced 1 MHz apart covering a velocity range of 130 km s^{-1}.

The calibration of the antenna beam efficiency was accomplished by making observations of Jupiter. The observations were made during June when daytime heating caused some variation in the gain of the antenna. This gain variation is primarily due to a broadening of the main beam. For those molecular clouds which are several minutes of arc in diameter, the effect of the variation in gain on observed antenna temperature should be small. The accuracy of the antenna temperature measurements is estimated to be ±30 per cent. Because of its proximity to the Sun, Orion had to be observed with the dome closed which attenuates the observed antenna temperatures by approximately a factor of two. Antenna temperatures given here are referred to a calibrated noise tube and are an average of several observations. This should smooth out the gain variations; it underestimates the actual antenna temperature, however. No correction was made for dome attenuation in the case of Orion.

As we are unable to make a positive spectral identification of this new line we shall refer to it as the X-ogen line (which means that it has an unknown extraterrestrial origin). The line appeared in the upper sideband of the receiver during a period when the local oscillator was tuned to receive the $H^{13}C^{14}N$ line at 86·3 GHz in the lower sideband. After determining the line frequency, the local oscillator frequency was increased in order to place the line in the lower sideband of the receiver. The line appeared again in the expected channels of the filter bank receiver. Comparison measurements were made off source to remove unbalance in the filter receiver and to check for interference. Additional baseline smoothing was accomplished by frequency-switching the receiver. As the X-ogen line was found in the sources in which the $H^{12}C^{14}N$ line appeared, the radiometer was checked for spurious responses. All responses were at least 30 dB (1/1,000) below the principal sidebands of the receiver, however.

In determining the rest frequency of the X-ogen line, comparisons were made with HCN and CO observations (Table 1). A comparison of velocities for several molecules and atoms in various interstellar clouds can be found elsewhere[1]. In a given source, the cloud velocity can be obtained from data on these molecules. The velocity can then be used to shift the observed X-ogen frequency back to obtain the corresponding rest frequency. There is a certain amount of discrepancy between velocities measured with different molecules, probably due to some degree to the different regions within a given cloud which are involved in absorption or emission from a particular molecule. The main source of error in our technique is probably due to the three unresolved HCN quadrupole components which may introduce a systematic error in the profile comparisons. As a result, the accuracy to which the X-ogen rest frequency can be determined is limited to ±2 MHz.

The frequency determined for the X-ogen line is 89·190±0·002 GHz which is 560 MHz higher than the $H^{12}C^{14}N$ (1→0) transition. In a limited survey of molecular clouds, X-ogen was found in Orion, W51, W3 (OH), L134 and in Sgr A (NH_3A), the NH_3 cloud located 3′ south of Sagittarius A (ref. 2). The spectra for the five sources are shown in Fig. 1 and the spectral parameters are listed in Table 1. The width of the line in Orion and several other sources is less than the corresponding line in $H^{12}C^{14}N$. The velocity agreement with $H^{12}C^{14}N$ and other molecules was generally within 7 km s^{-1}. In Sagittarius the line is very broad, extending from 0 km s^{-1} to +50 km s^{-1}. A similar broad feature is seen in $H^{12}C^{14}N$, $H_2{}^{13}C^{16}O$ and other interstellar molecular spectra.

It is unlikely that X-ogen is an atomic line because the only nearby atomic recombination line of any significance is H 59α at 89·199 GHz (ref. 3). In addition to the frequency discrepancy, H 59α would not be expected to have the intensity and sharp profile of the X-ogen line. X-ogen cannot be explained by Doppler-shifted $H^{12}C^{14}N$ because a velocity offset from the local standard of rest of 1,690 km s^{-1} would be required, highly improbable for a galactic gas cloud. We rule out transitions of any rare isotope of hydrogen cyanide because there are no known isotopic transitions at the X-ogen frequency. Finally, we do not believe that X-ogen is a "hot band" rotational

Table 1. ANTENNA TEMPERATURE AND VELOCITY OBSERVED FOR X-OGEN

Source	Position R.A. (1950)	Position Dec (1950)	Antenna temperature (K)	Line width (km s^{-1})	Central velocity (km s^{-1}) X-ogen	$H^{12}C^{14}N$	$H_2{}^{12}C^{16}O$ (ref. 1)	$^{12}C^{16}O$ (ref. 6)
W3 (OH)	2 h 23 m 17 s	61° 39′ 00″	4	8	−44	−49	−47·8	
Orion*	5 h 32 m 47 s	−5° 24′ 21″	6	7	12	7		10·5
L134†	15 h 50 m 50 s	−4° 26′ 00″	5	1	8		2	
Sgr A (NH_3A)	17 h 42 m 28 s	−29° 01′ 30″	6	34	27	26	34·7	
W51	19 h 21 m 27 s	14° 24′ 30″	5	12	61	55	65·4	

* Observed with the telescope dome closed (3 dB attenuation).
† Observed with 100 kHz filter spacing.

transition of HCN because of the excitation energy required (the lowest vibrational state is ν_2 at 569 cm^{-1}). To add to the identification problem, it is possible that we have not detected the main molecular isotope of X-ogen but a less common member instead.

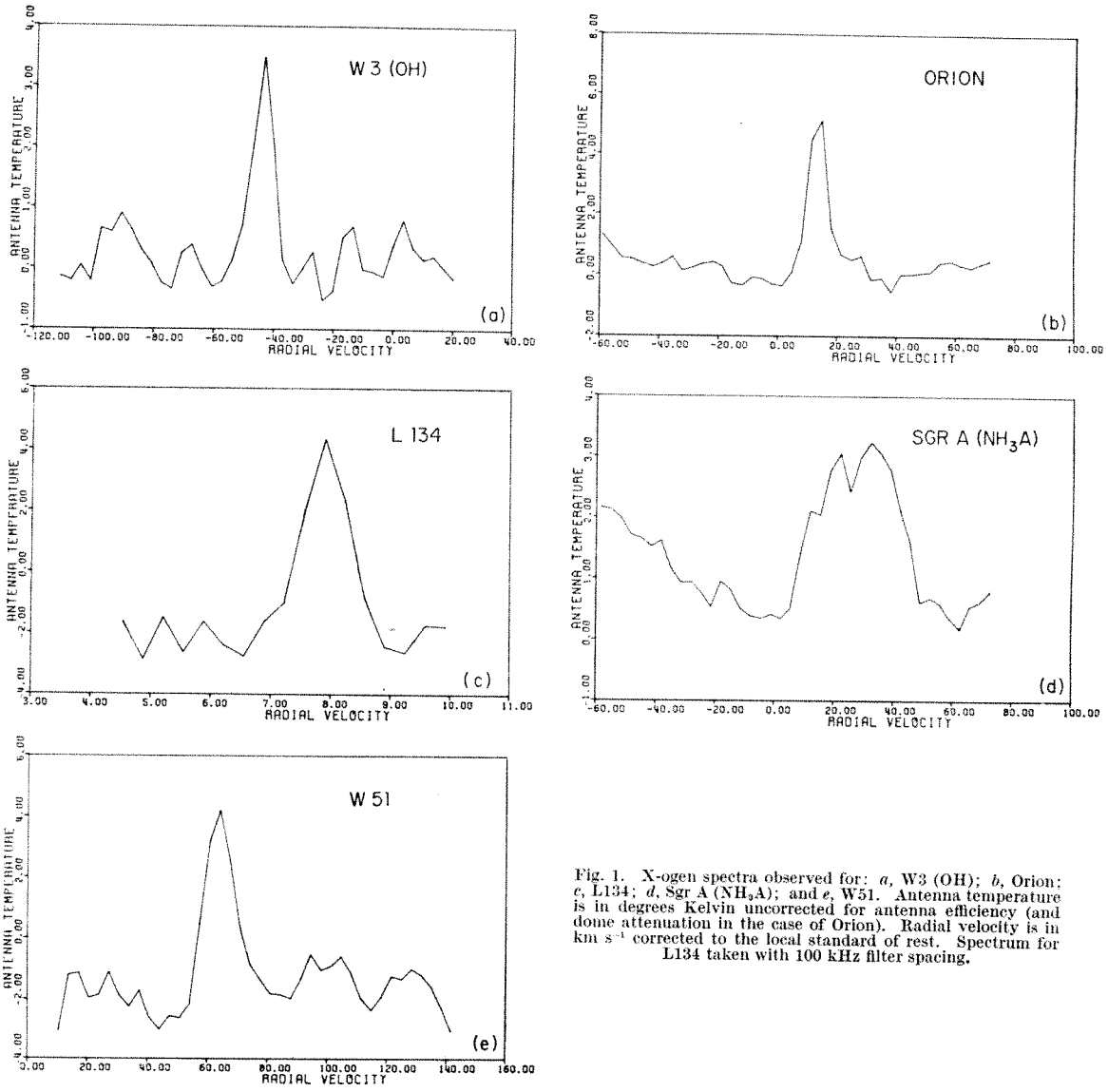


Fig. 1. X-ogen spectra observed for: *a*, W3 (OH); *b*, Orion; *c*, L134; *d*, Sgr A (NH_3A); and *e*, W51. Antenna temperature is in degrees Kelvin uncorrected for antenna efficiency (and dome attenuation in the case of Orion). Radial velocity is in km s^{-1} corrected to the local standard of rest. Spectrum for L134 taken with 100 kHz filter spacing.

Several molecules have been suggested which have transitions in the neighbourhood of 89,190 MHz. For example, the 7_{16}–6_{43} transition frequency of $H_2{}^{32}S$ is calculated to be 89,500 ± 300 MHz from infrared ground state combination differences (unpublished results of L. E. S.). The line strength for this transition is small, however, and the two levels involved require rotational energy of approximately 360 cm^{-1}; hence this H_2S transition would have to be undergoing maser action in order to be detected. From our present data the X-ogen emission seems to be fairly normal which tends to rule out H_2S as a possible identification. HNC (hydrogen isocyanide), an isomer of hydrogen cyanide, has been suggested by G. Herzberg as a possible identification because HCN and X-ogen are often found in the same cloud (see Table 1) and the X-ogen frequency is only 560 MHz higher than the $J = 1 - 0$ transition frequency of $H^{12}C^{14}N$. An *ab initio* calculation based on current knowledge of the HNC molecular geometry[4] places the $J = 1 - 0$ transition of $H^{14}N^{12}C$ in excess of 2 GHz above the X-ogen frequency and the corresponding transition of $H^{14}N^{13}C$ more than 1 GHz below. We cannot rule out HNC on this basis, however, because the structural information is somewhat imprecise for an accurate microwave calculation. Klemperer[5] has suggested the molecular ion $H^{12}C^{16}O^+$ and calculated the $J = 1 - 0$ transition frequency to be 89,246 MHz which is only 55 MHz above the X-ogen frequency. There is no apparent identification conflict with HCO because its nearest transition is expected to fall approximately 2·4 GHz below the X-ogen line.

Higher resolution spectra are needed to aid in the identification of X-ogen. Our present resolution and signal-to-noise ratio are not adequate to detect hyperfine splitting or similar spectral structure. Profile-matching of high resolution X-ogen spectra with CO spectra[6] should remove the possible systematic error in the rest frequency introduced by using HCN spectra. Mapping is necessary to learn the spatial extent of the X-ogen emission from each source and to check for anomalous emission.

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Absorption Lines in Quasi-stellar Objects with $z_{abs} > z_{em}$

OF the approximately twenty-five quasi-stellar objects which are known to have absorption lines[1,2], six have absorption lines with larger redshifts than the corresponding emission lines. In each case, $0{\cdot}01 \lesssim z_{abs} - z_{em} < 0{\cdot}03$. Four of the objects (PKS 1229−02, PHL 1222, PKS 0119−04, 4C 25·5) have only one emission line and one absorption line system. The two remaining objects (Ton 1530, B 194) each have one emission line system, but several distinct absorption line systems, only one of which has a larger redshift than the emission lines. It has been suggested that absorption lines in QSOs originate in clouds of gas which have been ejected from the QSO as a result of outbursts[3]. The lines having $z_{abs} > z_{em}$ are then interpreted as resulting from clouds which have a peculiar inward velocity that exceeds the mean outward motion of the system of clouds. In spite of the extremely large velocity dispersion required among the clouds to bring about this situation, it is conceivable that it might occasionally occur. But the relatively common occurrence of lines for which $z_{abs} > z_{em}$ suggests that the assumption that the usual state of a QSO is one in which matter is flowing outward may not be entirely valid.

It has been emphasized by Burbidge and Burbidge[1] that most of the absorption line redshifts in QSOs have $|z_{em} - z_{abs}| < 0{\cdot}03$. This has been interpreted to mean that the absorbing gas is closely associated with the QSOs. This interpretation is substantiated by the fact that most of the line profiles for which $0 < z_{em} - z_{abs} < 0{\cdot}03$ resemble the classical P Cygni-type profiles[4,5]. That is, these lines can be understood in terms of the scattering of resonance line radiation in an expanding medium with spherical symmetry. By extending this reasoning to the situation where $z_{abs} > z_{em}$, the most plausible explanation for such lines is that they are formed by gas which is moving toward the QSO. Because the absorption component of the lines is in all cases less intense than the adjacent continuum, it must be caused by gas which lies between the observer and the continuum source, which I assume to be located at the centre of the QSO and to have dimensions $< \sim 10^{16}$ cm (as indicated by the time scale of the variability of the continua of QSOs). Consequently, as long as the emission component of the lines is only broadened, and not redshifted, by the motion of the infalling gas, the difference $z_{abs} - z_{em}$ is a measure of the radial velocity of gas. Because $z_{abs} - z_{em} \sim 0{\cdot}02$ typically, then $v \sim 0{\cdot}02c$, directed inward.

Rees[3] has mentioned the alternative hypothesis that $z_{abs} > z_{em}$ may be caused by the transverse Doppler effect. For this to produce the observed redshift differences, the space velocities of the clouds must exceed $0{\cdot}1c$. Six QSOs indicate material velocities this large, while six objects have $z_{abs} > z_{em}$. Yet, if the transverse Doppler effect were indeed important, the number of objects for which $v > 0{\cdot}1c$ should be much greater than the number for which $z_{abs} > z_{em}$. I shall assume, therefore, that the non-zero redshift differences are true indicators of the radial velocities of the absorbing clouds.

What could cause these high inward velocities? It is doubtful whether any explosion or ejection phenomenon would directly accelerate the gas inward. The centres of activity in such objects are usually associated with the central regions, toward which the gas is falling. As indicated previously, cloud–cloud collisions may set up a velocity dispersion in gas which is being ejected from a QSO at high velocities such that occasional clouds have negative radial velocity with respect to the QSO. One would then expect, however, to observe $z_{em} > z_{abs}$ much more frequently than $z_{abs} > z_{em}$, but this is contrary to the observations. I propose that the high inward velocities are caused by the acceleration of gas due to the gravitational attraction of a large central mass. This basic idea has already been proposed in a different context by Lynden-Bell[6] for the nuclei of galaxies. He has considered this problem in some detail, so I shall not pursue the matter further. I am interested here, not in attempting to explain the general QSO phenomenon but in suggesting that absorption lines with redshifts larger than the emission lines may be a manifestation of the infall of substantial amounts of gas due to gravity.

If this interpretation is correct, a lower limit to the mass of a QSO can be obtained. Let us assume that clouds of gas, having angular momentum per unit mass h, are falling toward the central mass from essentially infinity. Ignoring the possible effects of cloud–cloud collisions, the angular momentum of the gas limits the velocity that the clouds may attain by preventing any approach to the centre closer than $r_{min} \sim h^2/2G\mathcal{M}$, where $\mathcal{M}$ is the central mass of the QSO. The equation of motion for the gas is $\dot{v}_r = -G\mathcal{M}/r^2$, from which it can be shown that $v^2 \sim 2G\mathcal{M}/r$. Except near the region where $r \sim r_{min}$, the space velocity v may be equated with the radial velocity, v_r, of the gas. Unless the injection of gas into the potential well of the central object occurs rather evenly in time, the profile of the absorption lines should reflect the changing velocity of the gas as it is accelerated. The time scale τ over which any portion of the gas has a characteristic velocity v, that is, over which the gas does not change its velocity by a factor of ~ 2, may be taken to be given by $\tau \sim v/2|\dot{v}| = 2G\mathcal{M}/v^3$. That is, for the velocity to remain unchanged over a longer time scale, one is restricted to flatter portions of the potential well; greater distances, in other words. Thus a given velocity remains unchanged over a longer period of time only for a larger mass. Now, it appears from the visual inspection of the spectra of at least one of the QSOs, Ton 1530, that no changes in the absorption lines have been detected over a period of several years (R. Lynds, private communication). For gas which has been accelerated to velocities of $0{\cdot}01c$, this lack of variability can be produced only by a central mass having $\mathcal{M} \gtrsim 10^7\ \mathcal{M}_\odot$. The corresponding minimum distance to which the gas has fallen is $r > 2 \times 10^{16}$ cm. These limits can be raised by more than an order of magnitude for objects with $z_{abs} - z_{em} \sim 0{\cdot}03$, like B 194 and PHL 1222, if they are observed to have undergone no variability since the first epoch plates were taken of these objects.

The absorption lines in QSOs for which $z_{abs} > z_{em}$ should be studied carefully to detect possible changes in

either linewidth or redshift with time, because the time-scale of such variations may lead to masses for the QSOs. This reasoning probably cannot be applied to objects for which $z_{em} > z_{abs}$ because the absorbing gas in these QSOs is moving outward, and therefore the likelihood that such gas is moving only under the influence of gravity is much less than in the opposite situation. The fact that Ton 1530 and B 194 have absorption redshifts for which both $z_{abs} > z_{em}$ and $z_{abs} < z_{em}$ indicates that the infall and outflow of material may be a continuing cycle. This suggests an evolution for the gas associated with QSOs in which it is accreted by gravitation, and then expelled by some mechanism such as particle pressure[7].

If the interpretation of the $z_{abs} > z_{em}$ phenomenon is correct, the need to postulate the creation of matter in the centre of QSOs[8] would be alleviated somewhat, because it would attest to both the influx of large quantities of gas toward the central regions and the existence of sufficiently large central masses to satisfy the energy requirements of the QSOs.

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Hoyle–Narlikar Theory of Gravitation

HARRIS[1] has given a treatment of the Bohr quantization of a spherically symmetric mass distribution surrounded by a gravitational field in the Einstein theory. One result is to provide a relationship between cosmological and elementary particle parameters.

This note concerns the Bohr quantization of the gravitational theory of Hoyle and Narlikar[2]. This theory derives from a direct interparticle action

$$S = \sum_{a<b}\sum \iint \tilde{G}(A,B)\mathrm{d}a\,\mathrm{d}b \qquad (1)$$

summed once over all pairs of particles a,b, $\mathrm{d}a\mathrm{d}b$ are world line elements and $\tilde{G}$ is a time-symmetric scalar two-point Green function satisfying the equation

$$g^{ij}\,\tilde{G}(X,A)_{;ij} + \tfrac{1}{6}R(X)\tilde{G}(X,A) = -(-g)^{-\frac{1}{2}}\,\delta^4(X,A) \qquad (2)$$

where R is the scalar curvature and g the determinant of g^{ij}.

The theory is Machian in the sense that the equations of motion do not exist in the limit of a one particle universe. We cannot therefore consider the single mass case of Harris, but must account for all masses $b \neq a$ in the universe. The mass of particle a is given by

$$m_a(X) = -\sum_{b\neq a}\int \tilde{G}(X,B)\mathrm{d}b \qquad (3)$$

and is a function of position.

Although the theory is nonlinear, so that the contributions from the right-hand side of equation (3) may not be superposed directly, such a treatment of Machian theories is often given as an approximation and has been discussed in this case by Hawking[3]. Although the advanced contribution to m_a diverges in this approximation, the retarded contribution is finite, and given by

$$m_a = N/H^2 \qquad (4)$$

where H is the Hubble constant and, in the steady-state cosmology, N is the constant particle density

$$N(X) = \sum_a \int \delta^4(X,A)[-g(X,A)]^{-\frac{1}{2}}\,\mathrm{d}a \qquad (5)$$

If we assume that in a rigorous treatment of the Hoyle–Narlikar gravitation theory the advanced contribution causes no embarrassment we may therefore proceed with the fully retarded part (4). Remembering that this is only a linear approximation, we substitute (3) and (4) into the total action (1)

$$S = \frac{N}{H^2}\sum_a \int \mathrm{d}a \qquad (6)$$

If we work with the conformally flat form of the cosmological metric, it is easily shown[3] that the right-hand side of (6) may be written

$$\frac{N^2}{H^2}\int_0^{1/H}\int_{-\infty}^{-1/H}\frac{4\pi r^2\,\mathrm{d}r}{(\tau H)^4}\,\mathrm{d}\tau$$

where (5) has also been used. This is immediately evaluated to give

$$\frac{N^2}{3H^3}\cdot\frac{4\pi}{3}\left(\frac{1}{H}\right) = \frac{N_0^2}{4\pi}$$

where N_0 is the total number of particles in the universe. The net action for each particle is then Bohr quantized

$$N_0/4\pi = nh \qquad (7)$$

Substituting this into (4) for the mass

$$m_a = 3nhH \qquad (8)$$

We now have to decide on the value of n. If the theory is Machian we expect $m_a \to 0$ as $N_0 \to 0$ so that n should be a positive power of N_0. We make the *ad hoc* assumption that n is an integer multiple of $\sqrt{N_0}$. This quantity is the mean fluctuation in the total number of particles, N_0, and frequently appears in this type of analysis, although as yet its significance is not understood.

Then

$$m_a = 3n_0\sqrt{N_0}\,hH = 3n_0\sqrt{N_0}\,hH/c^2$$

in e.g.s. units, where n_0 is a smaller integer.

Numerically this gives

$$m_a \sim 10^{-24}\ \mathrm{g}$$

or about the mass of elementary particles. This result becomes still more striking if we use the well known "coincidence"

$$\sqrt{N_0} \sim \frac{c/H}{4\pi e^2/m_e c^2}$$

(m_e = mass of electron). Then

$$m_a \sim \tfrac{1}{2}\frac{\hbar c}{e^2}\,n_0\,m_e$$

$$\sim \tfrac{1}{2}\times 137\,n_0\,m_e$$

which is just Nambu's formula[4], originally derived on empirical grounds to describe the mass spectrum of elementary particles.

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Energy Levels and Lorentz Invariance

An atom is in an excited state S′ with energy E'. It falls to a state S with lower energy E and emits a photon with frequency ν. According to a basic postulate of quantum mechanics, this frequency is related to the energies by

$$h\nu = E' - E \tag{1}$$

The theoretical calculation of E and E' is carried out in the rest-frame of the atom, and so it would appear that ν in equation (1) is the frequency observed in that frame. Conservation of momentum, however, demands that the atom recoils when the photon is emitted, and so there is not one rest-frame but two—the rest-frame of S′ and the rest-frame of S. It is true that the recoil is small and consequently the two rest-frames are nearly the same. But in spectroscopy wavelengths are sometimes given with 8-figure accuracy, and it is interesting to inquire whether such (or higher) accuracy is consistent with neglect of the small difference between the two rest-frames.

To discuss the recoil relativistically we must speak of 4-momentum, and it is convenient to take units for which $c = 1$ and to use imaginary time ($x_4 = it$). Latin suffixes range 1, 2, 3, 4. Let p'_r, p_r be the 4-momenta of the atom in the states S′, S, and let k_r be the 4-momentum of the emitted photon. Then

$$p'_r p'_r = -m'^2,\ p_r p_r = -m^2,\ k_r k_r = 0 \tag{2}$$

where m', m are the proper masses of S′, S. (Because $c = 1$, proper mass and proper energy are merely different names for the same thing.) Conservation of momentum and energy demands that

$$p'_r = p_r + k_r \tag{3}$$

or equivalently

$$p_r = p'_r - k_r \tag{4}$$

Squaring this and using equation (2), we get

$$m^2 = m'^2 + 2p'_r k_r \tag{5}$$

The frequency ν of the emitted photon is given by $ih\nu = k_4$, the fourth component of a 4-vector, and, if we are to make a significant spectroscopic prediction about this frequency, we must make up our minds what frame of reference to use. If we were concerned with the excitation of an atom (S→S′), it would be natural to use the rest-frame of S. But we are concerned with emission, and there can be no doubt that the rest-frame of S′ is the one to use—it is the mass-centre frame for the event. In this frame we have

$$p'_r = (0,\ 0,\ 0,\ im'),\ p'_r k_r = -m'h\nu \tag{6}$$

and so equation (5) gives

$$h\nu = \tfrac{1}{2}(m'^2 - m^2)/m' \tag{7}$$

If we knew the proper masses, m', m, we could calculate (without reference to equation (1)) the frequency ν, measured in the rest-frame of the atom before emission.

We have now before us two formulae for the frequency of the emitted photon, the formula (1) which will be found in any textbook on quantum mechanics[1] and the formula (7) obtained as above by a simple application of the conservation of 4-momentum, the only quantum feature being the assumption that the energy of a photon is $h\nu$. They cannot both be right, unless the two right-hand sides happened to be equal. That can hardly be the case, but they are nearly equal in practice. This near-equality has been fortunate, for it has permitted quantum theory to grow from strength to strength on the basis of a formula which, to a relativist, cannot but appear rather grotesque. I propose to compare the two formulae, that is, the formulas (7) and (1), rewritten as

$$h\nu_0 = E' - E \tag{8}$$

to avoid using the same symbol for different things. I shall call ν the true frequency and ν_0 the conventional frequency; E' and E are energy levels of the atom, calculated by quantum theory.

No comparison can be made until we answer the following basic question: What is the proper mass (equivalently, rest-mass or rest-energy) of an atom in a state S with energy E?

It appears that if $m_1, m_2, \ldots m_n$ are the proper masses of the particles of which the atom is composed, then the proper mass m of the atom is

$$m = M + W \tag{9}$$

where

$$M = m_1 + m_2 + m \ \ldots \ + m_n \tag{10}$$

and W is the (negative) binding energy or mass defect[2]. But equation (9) as it stands does not mean much. It might be taken as the definition of W, and the fact that W is negative merely implies that the mass of the atom is less than the sum of the masses of its constituent particles. In fact, equation (9) becomes significant only when we identify W with the energy E as in equation (8) and write for the two proper masses in equation (7).

$$m' = M + E',\ m = M + E \tag{11}$$

I have searched without success for an authoritative statement that $W = E$. Perhaps it is accepted without question (see ref. 3). These authors use equation (8) above without mention of the two frames of reference, but the difference between them may be covered by the approximation used.

Substitution from equation (11) in equation (7) gives

$$h\nu = (E' - E)[M + \tfrac{1}{2}(E + E')](M + E')^{-1} \tag{12}$$

and so by equation (8) the ratio of the true frequency to the conventional frequency is given accurately by

$$\nu/\nu_0 = [1 + \tfrac{1}{2}(E + E')/M](1 + E'/M)^{-1} \tag{13}$$

Now E/M and E'/M are in fact dimensionless small quantities of the same order of magnitude, and expansion of equation (13) gives

$$(\nu_0 - \nu)/\nu_0 = \tfrac{1}{2}(E' - E)/M + \mathrm{O}[(E/M)^2] \tag{14}$$

or, with equation (8)

$$(\nu_0 - \nu)/\nu_0 = \tfrac{1}{2}h\nu_0/M + \mathrm{O}[(h\nu_0/M)^2] \tag{15}$$

This may be called the fractional error arising from the use of the conventional frequency instead of the true frequency.

To appreciate the magnitudes involved, let us restore c and use c.g.s. units, so that

$$(\nu_0 - \nu)/\nu_0 = K + \mathrm{O}(K^2) \tag{16}$$

where

$$K = \frac{h\nu_0}{2Mc^2} = \frac{h}{2cM\lambda} \tag{17}$$

λ being the wavelength of the emitted photon, measured in the rest-frame of the atom before emission.

Consider in particular the spectrum of hydrogen. Because K is small, it is not necessary to carry many decimal places. In c.g.s. units we have

$$h = 6{\cdot}62 \times 10^{-27},\ c = 3{\cdot}00 \times 10^{10}$$
$$M = 1{\cdot}67 \times 10^{-24},\ \lambda = 6{\cdot}56 \times 10^{-5} \qquad (18)$$

this last being the most prominent line in the H-spectrum. Hence, because the last term in equation (16) is of the order 10^{-18}, the fractional error is

$$(\nu_0 - \nu)/\nu_0 = 1{\cdot}01 \times 10^{-9} \qquad (19)$$

Thus we may conclude that, in general terms, the error does not make itself manifest if we are satisfied with 8-figure accuracy in spectroscopy, but does appear in the ninth figure, at least in the case just considered.

I was led to consider the above matters in an attempt to review arguments by which one sets an upper bound to the proper mass of a photon. If μ is that proper mass, we are to modify the last of equation (2) to read $k_r k_r = -\mu^2$ and then proceed much as above with this modification. But I decided that it would confuse the issue to try to discuss two things at once, and so I have followed the usual hypothesis—the proper mass of a photon is zero—in order to bring out the relativistic inadequacy of the commonly accepted formula (1).

Because proper mass (equivalently, rest-mass, proper energy, or rest-energy) is the most important Lorentz-invariant scalar associated with any system, no relativist can feel happy about the formulae (11). They are patched up. The conservation equations (3) or (4) are very fundamental, and manipulation of those equations suggests that the basic scalar is not proper mass, but its square—in fact, that $m = M + E$ is merely a useful approximation to some more fundamental formula for m^2. Indeed, according to the covariant Bethe–Salpeter wave equation[4] it is m^2 that is to be quantized. But even that equation offends what may be called "Minkowskian aesthetics" in the matter of the "reduced mass" factors; in Minkowskian geometry one does not expect to see in any vital formula the sum of the magnitudes of 4-vectors $(m_1 + m_2)$, any more than one expects to find, in Newtonian mechanics, the sum of the magnitudes of two forces or of two momenta.

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Transformation to Rotating Coordinates

In the treatment of problems involving rotating coordinate frames one of two types of viewpoint is conventionally adopted. The assumption may either be made that the use of principles familiar in special relativity can be extended to the rotating coordinate case, or a general-relativistic treatment may be used, with use of an explicit coordinate transformation for uniform rotation in going from the inertial frame to the rotating frame.

The first approach leads to a "paradox" initially cited by Ehrenfest[1]. In this viewpoint, illustrated by the model of the rotating disk, distances in the radial direction are assumed to be unaffected by the rotary motion, for these are everywhere orthogonal to the direction of motion. Measuring scales used for circumferential measurements are assumed to be Lorentz-contracted by a factor $(1 - \omega^2 r^2/c^2)^{1/2}$, where ω is the angular velocity and r the radius, because such measurements are everywhere locally parallel to the direction of motion. This leads to the usual conclusion that an observer on the disk would find a circumferential measurement of $2\pi r(1 - \omega^2 r^2/c^2)^{-1/2} > 2\pi r$. This conclusion has since been adopted by many authors as a demonstration of non-Euclidean character of the geometry in non-internal coordinate frames[2–6].

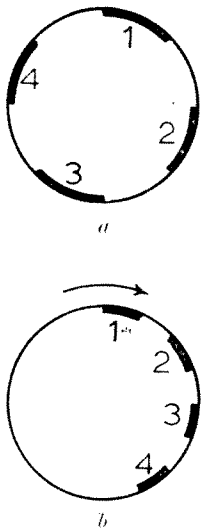


Fig. 1. *a*, Stationary disk; *b*, result of an arbitrary application of Lorentz contraction to periphery of disk in rotation.

The fallacy in the foregoing argument can be readily seen from the following example. Consider a circular disk, made possibly of transparent material, on the circumference of which a pattern of stripes is painted in four octants, as indicated in Fig. 1*a*. The disk is then set into rotation at a speed such that the rim is travelling at 86·6 per cent of the velocity of light in free space, for a Lorentz contraction factor of 1/2. The positions of the ends of the stripes on the rim can be observed at an instant of laboratory time. This is possible by means of a flash of light emitted above the plane of the disk which exposes the shadow of the stripes on a photographic film held stationary in the back of the disk; an alternative coincidence-detection observation may also be devised. An elementary application of the special-relativistic hypothesis could then lead to the expectation of a pattern as in Fig. 1*b*, which is clearly impossible on the basis of the symmetry of the disk. The special-relativistic assumption must therefore be discarded.

In deriving the equations of the electromagnetic field in a rotating reference frame, Schiff[7] used the explicit transformation to rotating coordinates

$$x = x_0 \cos \omega t_0 + y_0 \sin \omega t_0$$
$$y = -y_0 \sin \omega t_0 + y_0 \cos \omega t_0$$
$$z = z_0 \qquad (1)$$
$$t = t_0$$

The metric tensor of this transformation was used to obtain the current and charge-containing field equations from the covariant form

$$\frac{\partial}{\partial x^i}[\sqrt{-g}\, g^{im}\, g^{jn}\, F_{mn}] = \sqrt{-g}\, J^j \qquad (2)$$

The field equations which Schiff obtained were placed into standard vector notation in the coordinates of the rotating frame by Webster[8], who found

$$\nabla \cdot B = 0$$

$$\nabla \times E = -\dot{B}$$

$$\nabla \cdot E = \rho/\epsilon_0 + \nabla \cdot (v \times B)$$

$$\nabla \times B = \mu_0 J + \frac{1}{c^2}\dot{E} - \frac{1}{c^2}\{\nabla \times [v \times (E - v \times B)] + v \times (\nabla \times E)\} \quad (3)$$

where $v = r\omega$ and the equations are written in MKS units.

Equations (3) correctly approach the standard inertial-frame form of Maxwell's equations for $v \to 0$, but they may be expected to be incorrect for nonvanishing v. This expectation is based on the essentially Galilean character of the transformation equation (1). This Galilean character was inferred from the result of the following procedure: the Schiff–Webster calculation was carried out, but with the use of the classical Galilean transformation for rectilinear motion

$$\begin{aligned} x &= x_0 + vt_0 \\ y &= y_0 \\ z &= z_0 \\ t &= t_0 \end{aligned} \quad (4)$$

When the electromagnetic field equations were obtained in the Galilean frame represented by this transformation, and were placed into vector notation, the resulting equations were identical with equation (3). The rectilinear Galilean transformation was discarded by Einstein[9] in favour of the special-relativistic (Lorentz) transformation to a uniformly moving frame. This change was induced as a consequence of the experimental observation, by Michelson and Morley and others, of the isotropic constant velocity of light in inertial frames.

Measurements of the anisotropy of the velocity of light are capable in principle of verifying the correctness of the coordinate transformation to rotating coordinates, through the avenue of the metric tensor g_{ij}, which depends on the coordinate transformation law according to

$$g_{ij} = \frac{\partial x^a}{\partial x^i}\frac{\partial x^a}{\partial x^j} \quad (5)$$

For light propagating in the direction specified by the direction cosines n^i, the measured velocity of light will be[10]

$$v^i = \frac{-cg_{44}}{n^i g_{14} + (-g_{44})^{1/2}} \quad (6)$$

With measurements of sufficient accuracy, the correctness of a rotating-coordinate transformation can be verified by the use of equation (6), or equivalently by solution of the equation for the null-element of world line $ds^2 = 0$ for the propagation of light. The experimental configuration for such measurements of the velocity of light may take many forms and hence will not be discussed here. It is sufficient, however, that the experiments are possible in principle. Until the results of such measurements are available, we must recognize that we do not have a verified form for the law of transformation to rotating coordinates.

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Denudation of the Continents

Estimates of denudation often enter into geologic argument and have to be extrapolated backwards over geological intervals of time. The importance of human activity in the present-day erosional cycle has been pointed out by Douglas[1] and has been carefully evaluated by Judson[2] in a comparison of world-wide river load estimates with data from the nearly undisturbed Congo and Amazon basins. Judson concludes that man's intervention has increased the overall denudation rate from 9 to 24 billion tons a year: far more than enough to discredit the present rate as a geologic parameter. In this communication I attempt to verify Judson's pre-human estimate and to defend its general applicability to the past 300 million years.

Compared with man's erosion of the soil his effect on weathering has probably been small. The present rate of chemical weathering (indicated by the dissolved load of rivers) should therefore be about the same as it was when the soil was conserved and weathering roughly balanced denudation. A convenient index of chemical weathering is sodium, a widespread, mobile and fairly abundant element the distribution of which is reasonably well known. The pre-human denudation rate can then be calculated

$$\text{denudation rate} = \frac{\text{transport rate of dissolved sodium in rivers}}{\text{sodium leached from unit mass of source rock}} \quad (1)$$

Geochemical studies[3,4] indicate that the weathering of 100 tons of average continental surface rock supplies about 0·9 ton of sodium to the hydrological cycle; and the annual discharge of dissolved sodium in rivers is about 20×10^7 tons[3,5], of which 9×10^7 tons have been recycled from the sea through the atmosphere[3,4] and $1{\cdot}5 \times 10$ released by human activities[4]. This leaves a net discharge arising from chemical weathering of $9{\cdot}5 \times 10^7$ tons, and substituting in equation (1) we get

$$\text{annual rate of denudation} = \frac{9{\cdot}5 \times 10^7 \times 100}{0{\cdot}9} = 10{\cdot}5 \times 10^9 \text{ tons} \quad (2)$$

Sedimentary rocks decay by erosion and eventually by metamorphism. If, for a given formation, the rate of each process is proportional to s, the amount of sediment present, the decay should follow a law of the form

$$\frac{ds}{dt} = -k.s \quad (3)$$

according to which the surviving volume of a formation of age t would be

$$s = r.e^{-kt} \quad (4)$$

r being the original volume of the formation. If a formation is defined as consisting of the sediments formed in a year, then r represents the annual rate of sedimentation (= denudation). If the surviving volumes of all the sedimentary systems are known the volume of each system can be divided by the duration of the corresponding geological period, and the quotient or "survival rate" can be plotted against the average age of the system[6]. Then, if the points so obtained all lie on a curve $s = r.e^{-kt}$, it may be concluded that the sedimentation rate has

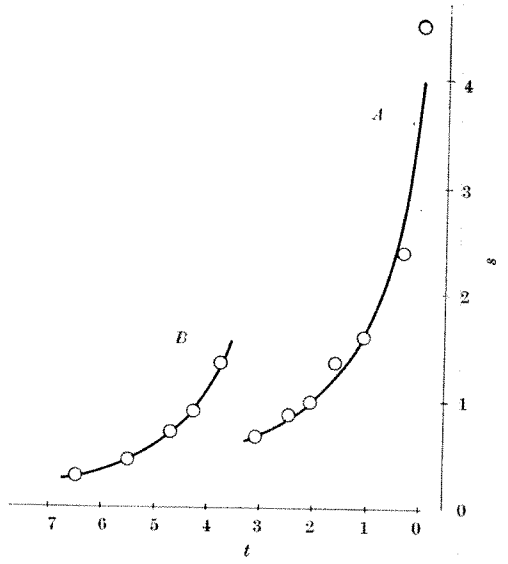


Fig. 1. Survival rate of sediments as a function of their age. s, Surviving volume (now) of each geologic system, expressed in km³ per year of the corresponding period; t, average age in hundreds of millions of years. A, $s = 2{\cdot}1\ (e^{-2{\cdot}0t} + e^{-0{\cdot}37t})$; B, $s = 1{\cdot}4\ (e^{-1{\cdot}7(t-3)} + e^{-0{\cdot}44\ (t-3)})$.

remained constant and it can be found by solving for r. Unfortunately, volume estimates are not common in stratigraphic literature; but enough data are available to make a rough calculation. According to Ronov and Yaroshevsky[7], the total volume of sediments on the continents and shelves is about 600×10^6 km³; this includes detailed estimates by Ronov and Khain[8] totalling 268×10^6 km³ for the Devonian, Carboniferous, Permian, Triassic and Jurassic systems. To get a tentative list of the volumes of all the sedimentary systems, I divided the balance of 332×10^6 km³ between the remaining systems in proportion to their maximum known thicknesses as reported by Holmes[9]. The deep sea sediments, here taken as 100×10^6 km³, were added to the Cretaceous, Tertiary and Pleistocene in proportion to the respective durations of these periods, on the assumption that most of the present ocean floor is post-Jurassic (personal communication from B. C. Heezen) and that it has been spreading at a uniform rate. The resulting volumes are shown in Table 1, and the corresponding survival rates are plotted in Fig. 1 as a function of age.

It seems that the geologic systems are thus divided into two distinct groups, pre-Carboniferous and post-Devonian, each conforming approximately to a decay law of the type predicted, though for reasons that are not clear it turns out to be binomial

$$s = \frac{r}{2}(e^{-at} + e^{bt}) \qquad (5)$$

For Carboniferous to Pleistocene, the best fit is given by

$$s = 2{\cdot}1(e^{-2{\cdot}0t} + e^{-0{\cdot}37t}) \qquad (6)$$

whence $r = 4{\cdot}2$ km³ per year; or, at a density of 2·6, about 11 billion tons. For the pre-Carboniferous group the best fit is

$$s = 1{\cdot}4(e^{-1{\cdot}7(t-3)} + e^{-0{\cdot}44(t-3)}) \qquad (7)$$

It has been suggested that the earlier Palaeozoic systems have been selectively preserved by burial under younger sediments (personal communication from R. M. Garrels). On the other hand, they might be the fossil remnants of an erosional pattern that could be reconstructed and analysed. Thus for the Devonian (now represented by 1·36 km³ per year of Devonian time), substituting in equation (5), the following equation can be written

$$1{\cdot}36 = \frac{v}{2}(e^{-2{\cdot}0 \times 3} + e^{-0{\cdot}37 \times 3}) \qquad (8)$$

where v represents the volume of Devonian sediments (per year of Devonian time) in existence 300 million years ago, assuming the post-Devonian erosional regime to have taken over at that point. The solution is $v = 8{\cdot}3$, and the pre-Carboniferous denudation rate can now be found by solving the equation

$$8{\cdot}3 = \frac{r}{2}(e^{-1{\cdot}7(3{\cdot}75-3{\cdot}00)} + e^{-0{\cdot}44(3{\cdot}75-3{\cdot}00)}) \qquad (9)$$

whence $r = 16{\cdot}6$ km³ per year. The mean value of r found in this way for the five pre-Carboniferous systems is 16·1, corresponding to about 40 billion tons a year: nearly twice the present denudation rate and four times the immediate pre-human one. It is the second term (e^{-bt}) in equation (5) that reflects the early history of these decay processes as seen today; and because its exponents in equations (6) and (7) do not seem to differ markedly in their coefficients, these two pre-Carboniferous models (selective preservation and rapid erosion) can probably not be distinguished by inspection of the curves.

Table 1. VOLUMES AND SURVIVAL RATES OF SEDIMENTARY SYSTEMS

System	Average age[9] (100 m.y.)	Duration (m.y.)	Max. known thickness[9] (km)	Volume acc. to Ronov[8] (10^6 km³)	Share of all continental and shelf sediments (10^6 km³)	Share of deep sea sediments (10^6 km³)	Total volume (10^6 km³)	Survival rate (km³ yr⁻¹)
Pleistocene	0·01	2	1·8	—	7	2	9	4·50
Tertiary	0·36	68	31·5	—	115	50	165	2·40
Cretaceous	1·03	65	15·5	—	56	48	104	1·60
Jurassic	1·58	45	13·3	62	62	—	62	1·38
Triassic	2·03	45	9·1	45	45	—	45	1·00
Permian	2·48	45	5·8	40	40	—	40	0·89
Carboniferous	3·10	80	13·9	53	53	—	53	0·66
Devonian	3·75	50	11·5	68	68	—	68	1·33
Silurian	4·20	40	10·3	—	37	—	37	0·93
Ordovician	4·70	60	12·1	—	44	—	44	0·73
Cambrian	5·50	100	12·1	—	44	—	44	0·44
Late Pre-Cambrian	6·50	100	7·9	—	29	—	29	0·29
					600[7]	100	700	

Table 2. ESTIMATES OF DENUDATION RATE (BILLIONS OF TONS PER YEAR

Basis of estimate	Present	Pre-human (Recent)	Carboniferous to Recent	Pre-Carboniferous
River-gauging[2] (world-wide)	24	—	—	—
River gauging[2] (selected basins)	—	9	—	—
Sodium cycle	—	10	—	—
Survival rate method	—	11	11	40 (?)

The results of these speculations are summarized in Table 2, together with Judson's estimates of present and pre-human denudation rates. There is fair agreement of the three independent pre-human rates, and there is no obvious sign of drastic variations since the Carboniferous. Before then the rate could have been greater by a factor of about 4, possibly on account of the absence of plant cover, but the evidence for this is inconclusive. Reliable measurements of the pre-Devonian and post-Jurassic

systems are needed for more accurate calculations of the erosional cycle in these periods.

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Numidian Flysch: an Oligocene—early Miocene Continental Rise Deposit off the African Platform

THE Numidian Flysch[1–6] is a thick (1,000–3,000 m) alternating deposit of brown shales and highly quartzose coarse sandstones of Oligocene–early Miocene age that occupies the external part of the Riff-Tellian Atlas-Southern Apennines belt in the Southern Mediterranean (Fig. 1). Mineralogical composition, facies pattern and palaeocurrents indicate that this extensive large wedge of grain flow and slump deposits represents former continental rise sediments at the foot of the cratonal slope of the African Platform[7,8].

The northern coeval counterpart of the Numidian is represented by the Kliwa Sandstones[9–12] in the Carpathians (Fig. 1), and possibly by the Maikop Sandstones in Crimea and Caucasus (personal communication from L. R. Contescu). These formations, also representing continental rise-type deposition, derived their quartz fraction[9–11] from the adjacent cratonal slope of the Russian Platform.

The Numidian foldbelt facing Africa, 2,000 km long, can be traced from Gibraltar to the southern part of the Italian Peninsula[8]. In Algeria the cratonal Numidian quartzarenites grade laterally into eugeosynclinal flysch deposits[6]. In Sicily during the Oligocene–early Miocene the tectonically mobile Panormide Cordillera[4,5,13,14] separated the external (southern) Numidian Flysch (miogeosynclinal sequence) from the internal (northern) Reitano greywackes (eugeosynclinal sequence).

The Reitano Flysch (Oligocene–early Miocene) culminates an allochthonous series constituting the principal nappe of Sicily. The Reitano mudstones and turbides[15] overlie a sequence comprising: (1) Argille scagliose (Upper Cretaceous–early Eocene oceanic pelites); and (2) an Eocene–Oligocene Preflysch sometimes containing andesetic[11] greywacke (turbides) derived from a northern active volcanic island arc. During the paroxysmal phase of the Upper Tortonian orogeny[8] this series was transported above the Mesozoic biohermal carbonate rocks of the Panormide Cordillera and above the Numidian Flysch. A subsequent deformation (base of Middle Pliocene ?) subdivided the Numidian Flysch into several thrust sheets[8].

For the past 20 years most workers[1,6] have considered that the metamorphic or crystalline basement of the internal tectonic land (Gignoux's "Tyrrhenides") or the hypothetical Verrucano-type sandstone of the Panormide Cordillera substratum were the sources of the Numidian Flysch[18–20]. This view, however, is incompatible with the observed mineral composition of the Numidian sandstones which in Southern Italy, Sicily and Tunisia are clean, pure quartzose sandstones lacking the lithic and feldspathic debris characteristic of the internal tectonic land or the phyllites and micaschists fragments characteristic of the Verrucano-type sandstones[21].

The mineral composition of the Numidian quartzarenites in both Sicily and Tunisia is uniform, with 90–95 per cent of the sand fraction being composed of single-grained

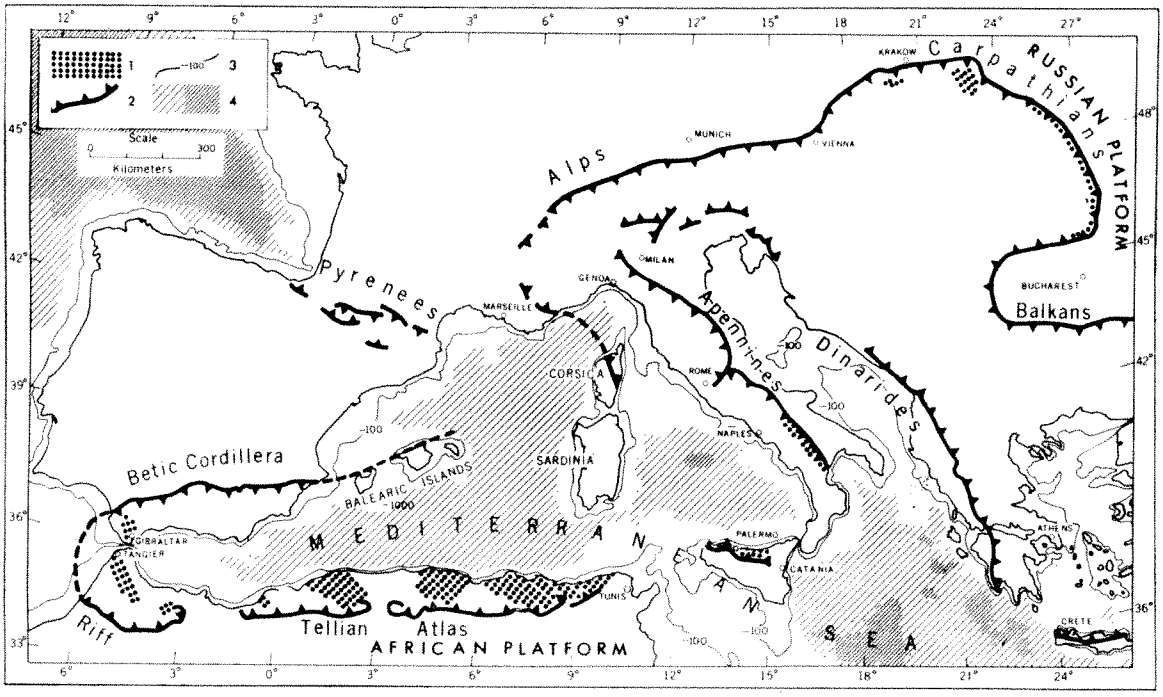


Fig. 1. Generalized tectonic sketch map of the Mediterranean showing the southern branch ("Perberides-Italo-Dinarides") and the northern branch (Alps-Carpathians) of Alpine mountain system (simplified and modified from ref. 30). Symbol legend: 1, general outcrop localities of Numidian Flysch (in southern branch) and Kliwa Sandstone (northern branch); 2, external sector of foldbelts; 3, offshore isobaths in fathoms; 4, depth respectively more than 1,000 fathoms (left symbol) and more than 2,000 fathoms (right symbol).

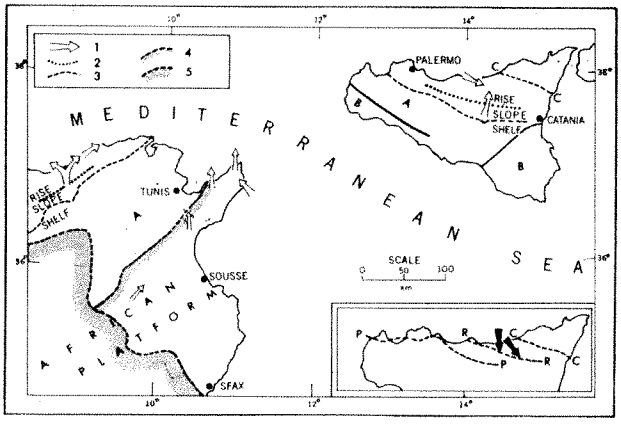


Fig. 2. Generalized map of Tunisia and Sicily showing distribution of facies and palaeocurrent directions in Oligocene–Lower Miocene sediments (Numidian Flysch and its shallower lateral equivalents) based on data in refs. 7, 8 and 31. The predominant directions of transport of Reitano Flysch are indicated (black arrows in inset). Symbol legend: 1, direction of sediment transport; 2, approximate base-of-slope position; 3, probable outer shelf edge; 4, inferred coastline during the Lower Miocene; 5, inferred coastline in pre-Lower Miocene time. *A*, Shelf facies with glauconitic sandstones; *B*, shelf facies with calcarenites and calcirudites; *C–C*, position of the internal massif nappe front (limestones and crystalline terranes of Calabride nappe); *R–R*, southern position of allochthonous Reitano Flysch; *P–P*, southern position of Panormide terranes.

"common" quartz that is usually rounded and has frosted surfaces, suggesting a probable aeolian origin. About 75 per cent of the transparent heavy minerals consist of zircon and tourmaline; rutile and garnets account for the remainder of the suite[7,15,22]. Moreover, the quartzarenites contain less than 5–10 per cent matrix and are often bimodal[7,15,16], with a coarse fraction mode of 0·5–4 mm and a fine fraction mode of 0·2–0·3 mm. This uniformly high mineralogical maturity and inherited bimodal texture, characteristic of inter-dune sands[23], indicate a provenance from older, rounded quartzose sediments transported on land and in shallow water for a long time before entry into the Numidian miogeosyncline.

All stratigraphic and sedimentological data that I have collected during the past 5 years indicate a southern derivation of the Numidian detritus from the pre-existing continental or epi-continental "Nubian Sandstone"[24] exposed over a large extent of the Saharan cratonic landmass along the southern edge of the Numidian trough.

In Sicily it is possible to recognize a lateral lithofacies continuum from south to north: facies 1, very coarse ultra-proximal sandstone, commonly friable and irregularly stratified; facies 2, finer proximal sandstone, well cemented with silica; facies 3, distal graded sandstone (turbidites and oceanic current deposits) mapped as the "Tavernola Formation". Facies 2 and 3 have been partly overthrusted above units of facies 1. Facies 2 probably represents deposits of coalesced submarine fans or continental rise deposits (recognized also by the presence of deep channels[26]), whereas facies 3 is interpreted as a near-axis trough deposit.

In Tunisia (Fig. 2), the following lithofacial distribution of Oligocene–Lower Miocene deposits is observed from south to north[7]: (1) continental facies, clean cross-stratified quartzose sandstones and laterite deposits; (2) shelf facies, quartzarenites with ripples and channels which indicate a northerly direction of sand transport ("Gres de Chérichira–Gres de Boghari"); a glauconitic sandstone outer-shelf subfacies is also present; (3) slope and rise facies, some 3,000 m of Numidian Flysch similar in all respects to the Numidian Flysch in Sicily; (4) basin facies, finely laminated quartzarenites (turbidites and perhaps oceanic contour current deposits similar to those of the Sicilian "Tavernola Formation") interfingering with the quartzarenites of the southern facies. The shelf facies, "Gres de Chérichia–Gres de Boghari", occasionally contains finer-grained quartzarenites than those of the Numidian Flysch. A sedimentary by-passing of coarse quartzose grains through some shelf-channels is inferred both in Tunisia and in Sicily[8].

It is worthwhile to note, for comparision, that similar palaeogeographic conditions and sedimentological features have been inferred for the Kliwa Sandstones intercalated in the Menilitic Shales of the Polish and Rumanian Carpathians[9–12]. This unit also occupies an external position near the foreland, and is exposed for about 800 km along the northern margin of the Carpathians (Fig. 1). The Kliwa Sandstones, consisting of a similar grain flow facies, are mature sands containing rounded and frosted quartzose grains[12]. As in Sicily[27] they locally include some diatomaceous horizons[28]. The Kliwa Sandstones pass laterally into sediments of the Krosno Flysch, lithic turbidite sandstones having compositional and sedimentary structures[29] similar to those of the Reitano Flysch.

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Variation of the Luminescence Lifetime of a Molecule near an Interface between Differently Polarizable Dielectrics

IN a recent paper[1] the energy transfer from an excited molecule S to an acceptor A was considered as a retardation effect. In classical terms, the field of S induces A to oscillate and the induced field of A, arriving at S, slows

down this oscillator. This effect may be studied, too, without A being an absorber, but simply a polarizable molecule, oscillating in phase with the radiation field of S at the location of A. Then the field which is produced by A and acting at S accelerates or slows down this oscillator depending on the phase relation to its motion; thus the decay time of the luminescence of S will be prolonged or shortened. This phase relation depends on the time needed for the propagation of the electromagnetic field from S to A and from A to S.

Let us look at a molecule S at a distance d from the planar interface between different dielectric bodies. It induces oscillating dipoles in all volume elements in these bodies and the field at S produced by these dipoles may be obtained as the sum of the contributions of each volume element. Neglecting interactions between induced dipoles the summation is easily possible and the following results are obtained

$$\frac{1}{\tau_d} = \frac{1}{\tau_d \mathrm{pa}} \cdot \sin^2\theta + \frac{1}{\tau_d \mathrm{pe}} \cdot \cos^2\theta \qquad (1)$$

oscillator at angle θ with normal on interface.

$$\frac{\tau_\infty}{\tau_d \mathrm{pa}} = 1 + \frac{3}{4} a \left(\mathrm{f}(x) - \frac{\sin x}{x} \right) \qquad (2)$$

oscillator parallel to interface.

$$\frac{\tau_\infty}{\tau_d \mathrm{pe}} = 1 + \frac{3}{2} a \left(\mathrm{f}(x) + \frac{\sin x}{x} \right) \qquad (3)$$

oscillator perpendicular to interface.

$$\frac{\tau_\infty}{\tau_d \mathrm{is}} = 1 + a\,\mathrm{f}(x) \qquad (4)$$

isotropic distribution. With the abbreviations

$$\mathrm{f}(x) = \frac{4 \sin x}{x^3} + \cos x - \frac{4 \cos x}{x^2} + x \left(\sin(x) - \frac{\pi}{2} \right) \qquad (5)$$

$$x = \frac{4\pi n_1 d}{\lambda} \qquad (6)$$

$$a = \frac{\alpha\pi}{n_1^2} q_\infty \; ; \; \alpha = \frac{3}{4\pi} \frac{n_2^2 - n_1^2}{2n_2^2 + n_1^2} \qquad (7)$$

τ_d is the relaxation time and λ the vacuum wavelength of the luminescence light; n_1 is the refractive index of the medium surrounding S and n_2 is the refractive index of the other medium. q_∞ is a quantum yield defined by the probability that a molecule in the luminescent excited state will emit a quantum of luminescent light, the environment being extended to infinity ($d = \infty$) (this quantity q_∞ must be distinguished from the easily accessible quantum yield of luminescence, which is number of emitted to number of absorbed quanta). According to (1), (2), (3) and (4) the luminescence decay time of a molecule must be markedly affected by the proximity of the interface between dielectrics.

A similar effect, the variation of the decay time of the fluorescence of a molecule in front of a metal mirror, was reported in refs. 2–4. (The experiments described in ref. 4 were proposed by F. P. Schäfer, first measured with clearly positive results by W. Sperling and then measured with increased precision by K. H. Drexhage, using equipment developed by F. P. Schäfer. Many implications of the effects were considered in ref. 5. K. H. Drexhage does not give credit to the above mentioned investigators associated with the early phase of that work.) The decay time was obtained there by superimposing the direct and reflected radiation field of the oscillating dipole, and by integrating the Poynting vector over a large sphere about the dipole. This method, when applied to the case of the reflexion at the interface between dielectrics, is much more involved than the method discussed here and in ref. 1. Drexhage[4] has applied the method in ref. 2 to that case and has measured τ_d independently.

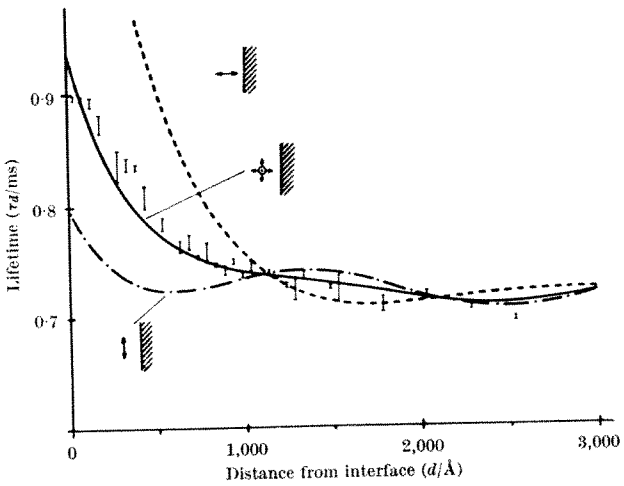
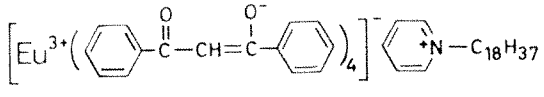


Fig. 1. Lifetime τ_d of the luminescence of europium complex I as a function of the distance d from the interface between monolayer system and air. Theoretical curves according to equations (2), (3), (4), with $n_1 = 1{\cdot}52$; $n_2 = 1$; $\tau_\infty = 0{\cdot}72$ ms and $q_\infty = 1$. — · — · —, Oscillator parallel to interface; - - - - -, oscillator perpendicular to interface; ———, isotropic distribution; bars, experimental results with root mean square deviation.

For experimentally realizing these effects the monolayer assembling technique[5,6] was used in the work cited in refs. 2–4 and in this article. This technique enables us to hold a fluorescent molecule at precisely adjustable distances from an interface (metal surface or surface of a medium with different refractive index) and to measure the decay time of the luminescence.

A monolayer of europium complex I



was deposited on a glass plate and superposed by tripalmitine. By exciting this arrangement with a flash of 366 nm light the intense red fluorescence ($\lambda = 612$ nm) of this complex is observed. The fluorescence follows an exponential decay law over at least two decades with a decay time of roughly a millisecond. Fig. 1 shows the decay time τ_d as a function of the distance d between monolayer of I and the interface of the monolayer system and air.

We assume the same value, $n_1 = 1{\cdot}52$, for the refractive index of tripalmitine and glass. The quantum yield of the fluorescence is $q_\infty = 1$. This value results from energy transfer experiments where I is used as a sensitizer[5] and from experiments of the decay time of the luminescence of I in front of a metal mirror[2,3]. By introducing the value $n_2 = 1$ (air) the curves are obtained from equations (2), (3) and (4) if the oscillator is assumed to be oscillating parallel to the plane of the layers, perpendicular and isotropic respectively. The experimental points correspond very well with the theoretical expectation for isotropic distribution.

The de-excitation of a molecule and appearance of a quantum of luminescent light is a discrete process and it seems surprising at first glance that the probability of this process may be changed considerably by placing the molecule in front of an interface which is quite far removed (in molecular dimensions). According to the above, this can be interpreted by representing the field, which determines the probability of observing the light quantum in a given space and time interval, by the classical electromagnetic field equations, showing that the probability of de-excitation of a molecule and appearance of a quantum of luminescent light does not depend only on the molecule but also on its broad environment. The result is a very direct manifestation of the wave particle duality of light.

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Effect of Low Magnetic Fields on the Catalysed Parahydrogen Conversion Rate over Certain Rare Earths

The purpose of this communication is to report a change in rate of the parahydrogen conversion if the catalyst is placed in a magnetic field of a few oersteds. The change is a decrease of several per cent, and it has been observed over Pr_2O_3, Nd_2O_3, Sm_2O_3 and Yb_2O_3. It has not so far been observed over Eu_2O_3, Gd_2O_3, Tb_2O_3, Dy_2O_3, Ho_2O_3, Er_2O_3 or Tm_2O_3. But Tb_2O_3 supported at low surface concentration on high-area Al_2O_3 shows the effect.

In a typical experiment high purity rare earths are calcined in air at 800° C overnight. From 10 to 100 mg is then placed in the flow reactor and heated for 1 h in purified hydrogen at 550° C. Praseodymia and terbia require special treatment and are heated in hydrogen at 800° C for 12 h. A 1 : 1 *ortho-para* hydrogen mixture is then passed over the catalyst at from 25 to 50 ml. per min at 1 atmosphere pressure and at 25° C. Zero extrinsic field conversions average about 20 per cent of equilibrium and are monitored by differential thermal conductivity. The effect of placing the catalyst in a magnetic field is measured by assembling the reactor in the gap of a large electromagnet or, for fields of less than 40 oersteds (3,000 A m^{-1}), in a coreless solenoid of 5,000 turns. Fields are monitored by a commercial Hall effect instrument sensitive to 0·1 oersted (7·95 A m^{-1}).

In the conditions of the experiment no catalyst (except possibly the alumina-supported Tb_2O_3) shows any measurable hydrogen–deuterium equilibration; the applied field causes no change of hydrogen flow rate, no temperature effect and no shift of equilibrium. The changes in conversion rates appear to occur instantaneously, or almost so, on application of the field, and they are completely reversible. No such effects have been observed with the diamagnetic rare earths.

Fig. 1 shows the rate changes observed over supported Tb_2O_3 on so-called gamma-Al_2O_3 as a function of extrinsic field applied to the catalyst. This sample, prepared by impregnation of alumina with an aqueous solution of terbium nitrate, contained 0·14 atom per cent of Tb_2O_3 on a specific surface of 160 m^2. The rate changes are expressed as the fraction $(k_H-k_0)/k_0$ where k_H is the specific rate in a field H and k_0 that in zero extrinsic field. The value of k_0 was about 1×10^{-8} mole o-H_2 formed per second per cm^2 (of catalyst surface). This is probably accurate within a factor of two, a major source of uncertainty lying in the specific surface of the Tb_2O_3. The rate changes are quickly and accurately determined by turning the magnet current on or off. The determination is thus essentially differential and, consequently, the precision is high. The minimum change of rate detectable was about ±0·5 per cent of the zero field conversion rate.

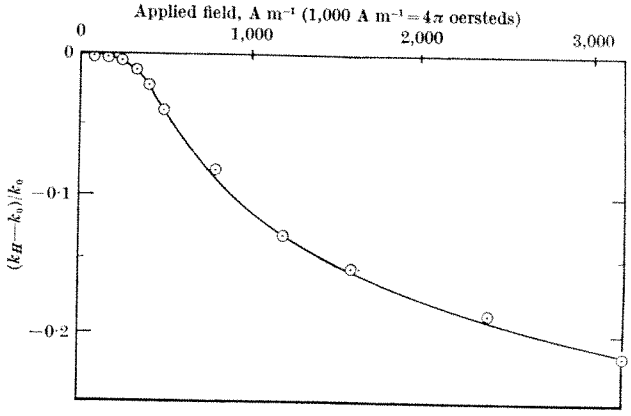


Fig. 1. Fractional change in the parahydrogen conversion rate over Tb_2O_3/γ-Al_2O_3 when the catalyst is placed in a magnetic field.

Fig. 1 shows the rate changes observed up to a field of 3,180 A m^{-1}. At much higher fields the rate rises as previously described for Cr_2O_3 above the Néel point[1,2], although the rise is much less for the supported Tb_2O_3 than for the pure rare earths[2]. It appears that the negative low-field effect described is not related to the much larger positive effects observed at high fields.

It will be noted in Fig. 1 that the effect of the extrinsic field changes rapidly, with field, in the region of 400–800 A m^{-1}. This suggests that, in this region, the Earth's magnetic field may be sufficient to influence the conversion rate. That this is indeed the case is readily shown by reversing the current in the solenoid. With the solenoid vertical and the angle of declination about 60°, reversal of the current gives a net resultant field change of about 80 A m^{-1}. It was found that, at 429 A m^{-1}, reversal of the current gave a readily perceptible change of rate, the greater change being found when the field produced by the solenoid was in the same direction (or nearly so) as the Earth's field. Field reversal gave no effect below 160 A m^{-1} and little or none above 8,000 A m^{-1}.

If the interaction of a molecule and a surface may thus be influenced by the Earth's magnetic field it would appear that a re-examination might be warranted of the possible role of terrestrial magnetism in such diverse areas as the navigational instincts of birds and the exclusive occurrence of L-amino-acids in natural proteins.

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BIOLOGICAL SCIENCES

Evidence for a New Haemoglobin Chain (ζ-Chain)

The possibility of a human embryonic haemoglobin was first indicated by the work of Drescher and Kunzer[1]. Two such haemoglobins, Hb-Gower 1 and Hb-Gower 2, have been discovered[2,3] and their structures are ε_4 and $\alpha_2\varepsilon_2$, respectively. The ε-chain is a distinct haemoglobin chain synthesized during embryonic development. It has therefore been suggested[4] that there is a separate genetic locus determining the structure of the ε-chain, just as there

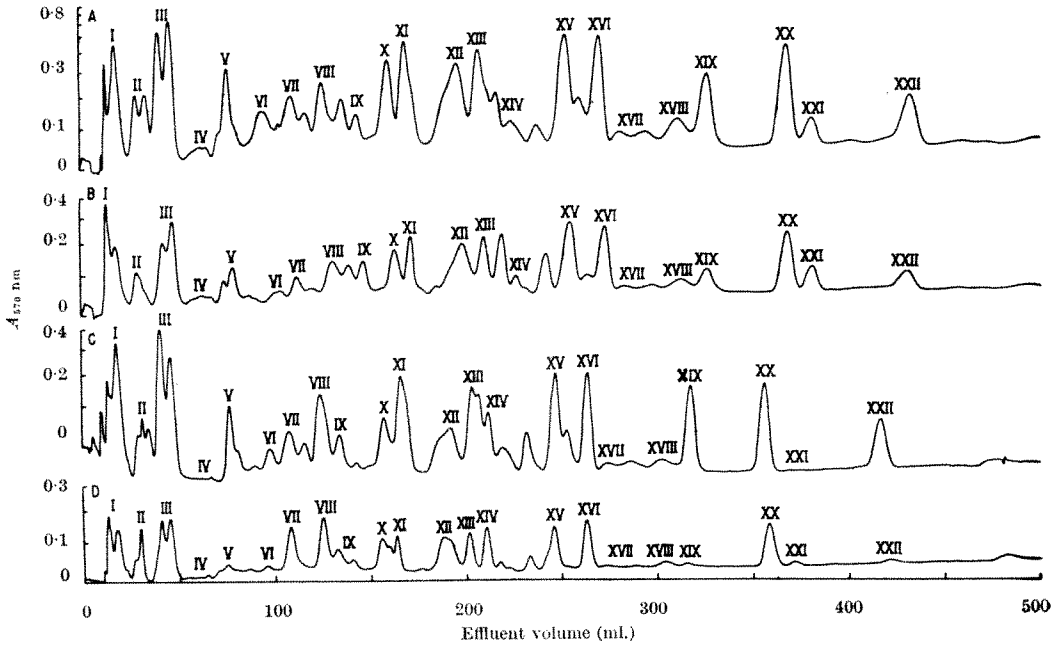


Fig. 1. Peptide patterns of complete tryptic hydrolysates: *A*, 3 mg of Hb-Portland 1 ($\gamma_4\zeta_2$); *B*, 2·4 mg of γ-chain from Hb-Portland 1; *C*, 2·2 mg ζ-chain of Hb-Portland 1; *D*, 2 mg of the γ-chain of haemoglobin F. Column was 0·9 × 17 cm Spinco 15 A resin; temperature 50° C. Development was with a linear gradient of 500 ml. of pyridine–acetate buffer beginning at *p*H 3·1, 0·2 M pyridine and ending at *p*H 5·0, 2·0 M pyridine (250 ml. of each). Column flow of 30 ml./h and ninhydrin flow at 15 ml./h.

are separate loci determining the structure of the α, β, γ and δ-haemoglobin chains[5].

The existence of a third embryonic haemoglobin, Hb-Portland 1, has been indicated; it is a tetramer (molecular weight 66,000) which is composed of a pair of γ-chains plus a pair of non-α-chains, previously designated as x-chains[6,7]. Haemoglobin Portland 1 was originally observed in a newborn infant with multiple congenital anomalies and complex autosomal chromosomal mosaicism[6,7], but it has since been found in several newborn infants with D_1 trisomy and in very small amounts even in newborn normal infants[8]. Here we present evidence that the x-chain is in fact a new human haemoglobin chain which we designated as the ζ-chain.

Tryptic peptide patterns of Hb-Portland 1 and of its γ and ζ-chains were obtained according to Jones[9]. The γ and ζ-chains were separated from Hb-Portland 1 globin by countercurrent distribution. The chains were then aminoethylated and digested with trypsin. In Fig. 1 the peptide patterns of the individual chains from Hb-Portland 1 and the γ_F-chain were compared with the peptide patterns produced by globin from Hb-Portland 1. These chromatograms illustrate that Hb-Portland 1 is a composite of normal γ plus ζ-chains.

The peptide zones shown in Fig. 1 were collected and, where necessary, were further purified by chromatography. The amino-acid composition of each Hb-Portland 1 peptide was determined using the Spinco amino-acid analyser. The following nine possible new peptides (ζT-peptides) were thus revealed and their composition is shown inside the parentheses. Two new peptides were obtained by the further chromatography of zone I: ζT-A (Lys, Thr, Ser, Leu) and ζT-B (Arg, Asp, Thr_1, Ser, Glu_3, Gly, Ala, Ile_2, Leu); one from zone II, ζT-C (Lys, Thr, Ser_2, Glu, Ala, Val_2, Leu_2, Phe); one from zone VII, ζT-D (Lys, Thr_2, Ser, Gly, Ala_2, Leu); one from zone VIII, ζT-E (His, Ser, Glu, Ala, Leu_2, Tyr); one from zone XI, ζT-F (Lys, His, Thr, Ser, Glu, Pro, Leu_2, Phe); two from zone XV, ζT-G (Arg, Ile, Leu) and ζT-H (Lys, His, Ser, Gly, $Ala_{1\ or\ 2}$, Cys, Leu_2); and one from zone XIX, ζT-I (Lys, His, Ser, Gly, Ala). These nine peptides differ from any of the tryptic peptides of the α, β, γ and δ-chains, indicating that the ζ-chain is not identical to any of these four haemoglobin chains. A peptide, ζT-J, was isolated from zone XXII and found to consist of one arginyl residue and one tyrosyl residue. Its composition and chromatographic behaviour indicate that it is identical to the tryptic peptide T-14 at the carboxyl-terminus of the α-chain. It is present, however, in sufficiently high amounts to rule out the possibility that it may arise from contamination with α-chains and should therefore be regarded as an integral part of the ζ-chain. In addition to these peptides, several tryptic peptides were isolated which had compositions identical to tryptic peptides of the γ-chain.

The peptide pattern of the ζ-chain differs from the peptide patterns of the α, β and δ-chains and the amino-acid composition (Table 1) of the ζ-chain is quite different from any of these chains. Similarly, comparison of the peptide patterns (Fig. 1) and amino-acid compositions (Table 1) of the ζ-chain and γ-chain reveals differences. Furthermore, recent studies on the structure of human embryonic haemoglobins by Szelenyi *et al.*[10], and A. Yoshida (personal communication) elucidated the amino-acid composition of two peptides that are unique to the ε-chain. These two peptides differ from any of the nine new tryptic peptides of the ζ-chain. A comparison of the amino-acid composition of the ε-chain[11] with that of the ζ-chain (Table 1) reveals further differences between the chains. This firmly establishes the existence of this new chain and indi-

Table 1. AMINO-ACID COMPOSITION OF THE α, β, γ, δ AND ζ-HAEMOGLOBIN CHAINS

Amino-acid	α*	β*	γ*	δ*	ζ†
Lysine	11	11	12	11	10
Histidine	10	9	7	7	7
Arginine	3	3	3	4	4
Aspartic acid	12	13	13	15	12
Threonine	9	7	10	5	11
Serine	11	5	11	6	12
Glutamic acid	5	11	12	12	11
Proline	7	7	4	6	5
Glycine	7	13	13	13	10
Alanine	21	15	11	15	14
S-Aminoethylcysteine	1	2	1	2	1
Valine	13	18	13	17	12
Methionine	2	1	2	2	2
Isoleucine	0	0	4	0	5
Leucine	18	18	17	18	18
Tyrosine	3	3	2	3	3
Phenylalanine	7	8	8	8	8
Tryptophan	1	2	3	2	‡

* Values from the literature.
† Values from a 22 h and 70 h hydrolysis of the aminoethylated globin of the chain.
‡ Not determined.

cates that there is a separate ζ-chain locus determining its structure.

Further work on the ζ-chain of the Hb-Portland 1 is currently under way on haemoglobin samples obtained in Hong Kong from Chinese stillborn infants with erythroblastosis fetalis, due to homozygous α-thalassaemia.

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Note added in proof. Two recent papers provide similar conclusions about the uniqueness of the ζ-chain (D. J. Weatherall, J. B. Clegg and W. H. Boon (*Brit. J. Haematol.*, **18**, 357; 1970); and D. Todd, M. C. S. Lai, G. H. Beaven and E. R. Huehns (*Brit. J. Haematol.*, **19**, 27; 1970).

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Formate, a New Component of Bacterial Exopolysaccharides

THE occurrence of non-carbohydrate substituents in bacterial exopolysaccharides has been well documented. Acetate and pyruvate have frequently been detected either separately or together in the same polysaccharide[1,2]. More recently, a polysaccharide secreted by *Alcaligenes faecalis* var. *myxogenes* was shown to contain succinate[3]. Although formate is known as a bacterial metabolite it has not previously been reported as an integral component of bacterial extracellular polysaccharides. This may be partly because of the lack of suitable methods for detection or estimation of formate and also because of the known lability of O-acyl groups on carbohydrates.

The basic structure of the polysaccharide forming the extracellular slime or capsule of *Klebsiella aerogenes* type 54 (strain A3) was established by chemical methods[4]. A repeating unit structure was identified as β-cellobiosyl-α-glucuronosyl-1→3-fucose. When the polysaccharide from this strain was hydrolysed with phage-induced depolymerase enzymes, two products were obtained[5]. Each was identified as a tetrasaccharide with the same structure as the repeating unit of the polysaccharide, but one of these oligosaccharides was acetylated while both contained an unidentified substitutent.

The polysaccharide did not contain pyruvate or succinate. Unlike O-acetyl groups, the unknown component was not removed by mild alkaline treatment (0·01 M KOH at 20° C for 16 h), but was absent after mild acid hydrolysis (0·05 M H_2SO_4 at 100° C for 15 min). Hydroxamic acid derivatives were prepared from acid hydrolysates of the polysaccharide by the method of Thomson[6] and compared with authentic compounds by descending paper chromatography in butan-1-ol–acetic acid–water (4 : 1 : 5, upper phase) for 20 h. Spots were revealed by spraying with 10 per cent ferric chloride solution. After drying at room temperature, two spots were present with R_F values 0·51 and 0·43 respectively. These were exactly equidistant with acetylhydroxamic acid and with formylhydroxamic acid respectively. To confirm that formate was a substituent of the polysaccharide and of oligosaccharides derived from it, the enzyme formate dehydrogenase was prepared from *Pseudomonas oxalaticus*[7]. This enzyme is specific for the NAD-linked oxidation of formate and is unaffected by the presence of acetate. Assay of mild acid hydrolysates of the polysaccharide or the oligosaccharides obtained by depolymerase treatment gave reduction of NAD in the presence of formate dehydrogenase. By comparison with standards, the formate content of samples was calculated to be in the approximate ratio of one mole formate per mole of fucose or glucuronic acid. Calculated on a dry weight basis the polysaccharide contained 46·5 per cent glucose, 19·1 per cent fucose, 20 per cent glucuronic acid, 6·3 per cent acetate and 6·2 per cent formate. Some loss of formate could be expected because of the volatility of the free acid obtained after acid hydrolysis of the samples. A similar result was obtained for the acetylated tetrasaccharide. The non-acetylated tetrasaccharide also contained one molar equivalent of formate. It is also possible to estimate the presence of formate by reduction to formaldehyde and use of the chromotropic acid reagent[8]. Using this method, the formate content of the non-acetylated oligosaccharide was in good agreement with the value obtained by the enzymic method.

The sugar which is formylated is not known, but one residue can definitely be excluded. Treatment of either tetrasaccharide with a β-glucosidase preparation liberated one mole of glucose in each case. The other product was a trisaccharide containing either formate or formate and acetate. Further enzymic hydrolysis was not possible. Attempts to isolate a formylated sugar by graded acid hydrolysis have also proved unsuccessful.

Preliminary experiments indicate that formate is present in the extra-cellular polysaccharides of several other *K. aerogenes* strains. It is not present in all strains, however, and it remains to be seen how widespread is the occurrence of formate in polysaccharides of other bacterial species or of non-bacterial origin.

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Transduction in *Mycobacterium smegmatis*

SEVERAL people have studied lysogeny and lysogenic conversion in the advanced prokaryote, *Mycobacterium*[1–3], and presented evidence for reciprocal genetic exchange from the lysogenic to the non-lysogenic state. Biochemical and physiological changes induced by lysogenization of *M. phlei* with mycobacteriophage B2 have been demonstrated by Juhasz[4]. We wish to report the isolation of a mycobacteriophage capable of mediating transduction in *Mycobacterium smegmatis* strain SN2, thus providing an opportunity to study the genetics of mycobacteria.

M. smegmatis strains SN2 and EB165 were provided

by Dr R. Bönicke (Borstel Research Institute for Experimental Biology and Medicine, Borstel, Germany). Luria broth[5] supplemented with 'Tween-80' (0·05 per cent) was used for propagation of bacterial cultures, and Bordet–Gengou broth base[6], solidified with 2 per cent Difco agar, was used for phage titration. All cultures and phages were propagated at 37° C. The minimal medium used was a half-strength preparation of medium 56 (ref. 7). *M. smegmatis* SN2 was treated with ultraviolet light to a survival of 1 per cent, or with N-methyl-N′-nitro-N-nitrosoguanidine to a survival of 1–5 per cent. The standard procedures[8,9] for isolation of auxotrophs were modified to suit the nature of mycobacteria by addition of 'Tween-80' to minimize clumping. After mutagenic treatments, cells were plated and screened for auxotrophs, which were all very stable after isolation; the reversion frequency to prototrophy in the case of single auxotrophs was of the order of 1×10^{-9} to 1×10^{-10}.

Ten independent isolations of mycobacteriophages were made from soil samples by an enrichment technique according to the method of Froman *et al.*[6]. Of the ten phages isolated against *M. smegmatis* SN2, one phage, I3, forms turbid plaques on strain SN2 but clear plaques on strain EB165. The phage has a wide host range on related mycobacteria. Phage adsorption is independent of Ca^{2+}, Mg^{2+} or tryptophan in the medium or of the growth phase of the bacterium. The average burst size is about 60, with a latent period of 250–270 min. Phage were propagated by a soft-agar layer technique[10] and using a bubbler tube with aeration[11].

For transduction studies, recipient cultures grown overnight on a shaker were centrifuged, and resuspended in warmed, fresh Luria broth. They were infected at a multiplicity of infection of 1–2 with phage lysate free of chloroform. Details of transduction are presented in Table 1.

Table 1. TRANSDUCTION OF *M. smegmatis* SN2 MUTANTS BY PHAGE I3 PROPAGATED ON WILD STRAIN SN2

Phage lysate	Transductants per ml. Glycine	Histidine	Arginine	Alanine	Adenine
Experiment	$2{\cdot}4 \times 10^2$	$4{\cdot}7 \times 10^2$	$1{\cdot}4 \times 10^2$	$1{\cdot}2 \times 10^2$	$2{\cdot}4 \times 10^2$
Treated with antiserum	0	0	0	0	0
Treated with deoxyribonuclease	$2{\cdot}4 \times 10^2$	$4{\cdot}6 \times 10^2$	$1{\cdot}3 \times 10^2$	$1{\cdot}2 \times 10^2$	$2{\cdot}4 \times 10^2$

5×10^8 cells of the mutants of SN2 were incubated at 37° C for 45 min with I3 phage (1×10^9 plaque forming units) propagated on wild type strain SN2. Aliquots (0·1 ml.) of washed cells were plated on minimal media. Transductants were stored after incubation of the plates for 4–5 days at 37° C.
The phage lysate was pretreated in turn with antiserum (K–10) and deoxyribonuclease (50 μg/ml. at 37° C for 30 min).

Transduction has been demonstrated for histidine, glycine, arginine, alanine and adenine markers. Colonies remain sensitive to phage after transduction. Treatment of phage with phage antiserum destroys the transducing ability. At a low multiplicity of infection, the frequency of transduction is very much reduced. To eliminate the possibility of transformation, lysate was pretreated with deoxyribonuclease[12]. This did not reduce the number of transductants detected.

These results can be extended to the study of the chromosome of *M. smegmatis* with respect to various functions. Although few markers have been transduced so far we intend to look for transduction of markers for sugar fermentations, synthesis of other amino-acids and resistance to streptomycin and the drug isoniazid, to which only mycobacteria are susceptible.

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Detection and Identification of Monoamine Oxidase Inhibitors in Biological Samples

THE monoamine oxidase (MAO) inhibitor drugs are now widely used in psychiatric practice for the management of various types of neurotic and psychotic depressions; unfortunately, these therapeutically valuable drugs are implicated in an increasing number of severe and sometimes fatal accidents caused either by gross overdosage, producing symptoms of hypernoradrenalinaemia[1] or more frequently by interacting with other drugs such as sympathomimetic amines[2–5], amine precursors[6–9], central nervous system depressants[10–12] and also with foods containing tyramine, DOPA and other amines[13].

In most poisoning cases involving MAO inhibitors, the toxicologist is in a difficult position because the inhibited enzyme is resynthesized very slowly, so that the toxic effects of the drug or of its association with other compounds may occur several days after the inhibitor has been almost completely excreted; consequently, at the time of the analysis he will very often recover smaller traces of the drug than can be detected by current chemical methods based on the development of a coloured reaction with relatively non-specific agents, such as dimethylaminobenzaldehyde or Folin Ciocalteu reagent.

We wish to describe a very sensitive and specific method for the detection and identification of these drugs, based on the inhibitory action of these substances on the activity of MAO; this activity is demonstrated by use of a system including nitro blue tetrazolium and an indole amine substrate, tryptamine. The enzyme oxidizes tryptamine into an aldehyde which in turn reacts with the dye to form a blue formazan[14–16].

Rat liver mitochondria provide the MAO; after homogenization of fresh rat livers in 0·3 M sucrose, mitochondria are isolated by the method of Hawkins[17] and stored at −10° C. A suspension of MAO is prepared by diluting 1 ml. of the mitochondria preparation with 3 ml. of 0·1 M phosphate buffer, *p*H 7·4. The different MAO inhibitors in the free base form—harmine, iproniazid, isocarboxazid, nialamide, pargyline, phenelzine, pivhydrazine and tranylcypromine as well as isoniazid, a weak MAO inhibitor chemically related to the most potent hydrazine derivatives—are extracted by vigorous shaking with freshly distilled ether from an aqueous solution at *p*H 10. The organic solvent is dried with solid sodium sulphate, evaporated under nitrogen to near dryness and transferred to a silica gel G thin layer plate which is then developed in chloroform and ethyl acetate (1 : 1).

After evaporation of the solvent, the plate is sprayed with the enzyme suspension and incubated for 15 min in a moisture tank kept at 37° C. The plate is then developed for MAO activity by spraying with a solution containing 10 mg of nitro blue tetrazolium, 10 mg of tryptamine HCl and 1 mg of sodium sulphate in 5 ml. of 0·1 M phosphate buffer, *p*H 7·4. The plate is incubated

Table 1. THIN-LAYER CHROMATOGRAPHY OF THE DIFFERENT MAO INHIBITORS ON SILICA GEL G PLATES IN ETHYL ACETATE : CHLOROFORM (1 : 1)

Drug	R_F
Nialamide	0·00
Harmine	0·04
Iproniazid	0·07
Tranylcypromine	0·11; 0·88
Phenelzine	0·50; 0·68; 0·83
Pivhydrazine	0·58
Isocarboxazide	0·70
Pargyline	0·79

for 2 h at 37° C, in the moisture tank without being moved. Areas of MAO inhibition appear very distinctively as white spots on a dark blue background, at different heights on the plate according to the respective R_F of the drug concerned (Table 1).

All the drugs tested, except isoniazid, inhibited dye reduction. Apart from its specificity, this method has a very high sensitivity permitting the detection of 10 ng of these drugs.

The chromatography of phenelzine and tranylcypromine reveals the presence of respectively three and two active distinct spots. Studies to elucidate the chemical nature of these compounds are now in progress.

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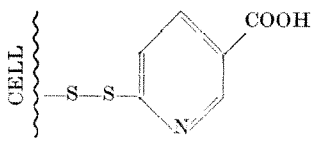
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Effect of 6,6′-Dithiodinicotinic Acid on the Dissemination of Ehrlich Ascites Tumour

PREVIOUS studies have shown that 6,6′-dithiodinicotinic acid (carboxypyridine disulphide, CPDS), while reacting with —SH groups of Ehrlich ascites tumour cells, does not enter these cells to any appreciable extent[1,2]. The reaction of CPDS with cell —SH groups leads to the formation of stable mixed disulphides[3,4].



as in the case with other disulphides of analogous structure[2]. Other types of cells, such as human lymphocytes and blood platelets[4], or mouse kidney (unpublished observations), are similarly affected by CPDS. The effect of CPDS treatment on the mammalian cells studied is two-fold: (*a*) the external —SH groups are blocked, and (*b*) this blocking introduces one carboxyl group on the cell surface for each —SH blocked. This interaction is described in more detail elsewhere[3]. Because —SH groups are not ionized at physiological *p*H, but carboxyl groups are, the net charge of the cell is made more negative by this treatment. It is known[3] that this modification does not effect respiration and glycolysis of Ehrlich ascites cells.

CPDS, when injected intraperitoneally in Swiss mice, in single doses as high as 1 g/kg, has no visible toxic effects. Daily injections of 450 mg/kg (in two daily doses), for 21 consecutive days, also had no visible effects. In all cases a solution of the sodium salt of CPDS was injected, prepared by dissolving the acid in sodium bicarbonate solution.

The mixed disulphides formed from cell —SH groups and 6-mercaptonicotinic acid (by the action of CPDS on cells) are split by thiols such as glutathione or cysteine. It can therefore be expected that in the living animal this —SH blocking group may be removed by physiologically available thiols. To estimate the approximate life of these mixed disulphides in the living mouse, ascites bearing mice were injected intraperitoneally with 6 mg of CPDS/mouse (in bicarbonate solution). The mice were killed at different times after injection (1–20 h), the ascites fluid was withdrawn. the Ehrlich ascites were separated by centrifugation and washed, and the amount of 6-mercaptonicotinic acid combined with the cells was determined, as described elsewhere[3]. The results indicated that the —SH groups of ascites cells in these mice remained blocked for about 4 h. This was taken as starting point for our studies on the effect of CPDS on the dissemination of Ehrlich ascites tumour.

As already reported[5,6], a marked dissemination of cancer cells to the lungs takes place in mice transplanted intracerebrally with Ehrlich ascites cells. We have used this system to study the effect of CPDS on the dissemination of this tumour, and applied the technique described by Rosso and his colleagues[5], adapted to our particular strain of tumour. A constant number of Ehrlich ascites cells (about 200,000) was injected into the brain of each mouse. After 7 days, the lungs were excised, and minced lung fragments (about 80–100 mg per implant) were transplanted subcutaneously in the retroscapular area of normal Swiss mice, on both sides. After 30 days the tumours were excised and weighed. The presence of a tumour weighing 100 mg or more was taken as evidence that in the donor mouse the tumour had spread from the brain to the lungs. Retroscapular tumour weights up to about 7 g were found in our experiments. The control group of mice was given intraperitoneal injections of physiological saline (0·1 ml.) every 4 h for 7 days (and nights) starting 1–3 h after the intracerebral injection of tumour cells. The test group was given intraperitoneal injections of CPDS (2 mg/mouse, or about 100 mg/kg) in sodium bicarbonate solution (0·1 ml.) at the same intervals. The results of these experiments are reported in Table 1.

Clearly the extent of dissemination of the tumour from the brain to the lungs is substantially reduced by CPDS. In each of these experiments the extent of

Table 1. EFFECT OF 6,6′-DITHIODINICOTINIC ACID ON DISSEMINATION OF EHRLICH ASCITES TUMOUR

Experiment	Saline*	6,6′-Dithiodinicotinic* acid
I	23/36 (18)	8/46 (23)
II	28/44 (22)	7/46 (23)
III	14/48 (24)	3/44 (22)

* Ratios between the number of subcutaneous tumours and the number of transplants with 80–100 mg of lung fragments obtained from mice bearing intracerebral tumours 7 days after intracerebral transplant of tumour. The numbers of animals used are in parentheses (Swiss mice, 18–20 g). For details, see text.
$P < 0.001$, evaluated for the sum of all the experiments ($\chi^2 = 37.7$; d.f. = 1).

spreading in the treated group was only a quarter of that of the control group. I hope to determine whether this is the result of blocking of the —SH groups, or the modification of the charge of the cell surface or both. Studies on the spread of cancer cells from implant sites other than brain (for example, bone marrow) will also contribute to establishing the mechanism of action of CPDS.

It is interesting to note that, when CPDS was injected only once or twice daily, even in total amounts larger than those of the experiments reported, no decrease in tumour dissemination occurred. This confirms the assumption that the dissemination of Ehrlich ascites cells is prevented only as long as the concentration of CPDS in the organism is sufficient to maintain the blocking groups on the cell surface sulphydryls.

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^{137}Cs Levels in Human Adult Population in California, 1960–69

THE presence in humans of caesium-137, a radioactive product of nuclear fission, was demonstrated in 1955[1] and ubiquitous distribution has since been described in population samples in Great Britain[2–4], Europe[5–9], Japan[10] and North America[11–21]. The long physical half-life of ^{137}Cs (30 years), coupled with its ease of absorption into the human body, has stimulated numerous studies of its metabolic behaviour and continuing evaluations of its hazard to human health. This report presents the results of 1,052 measurements of ^{137}Cs levels in 454 persons during the decade 1960–69.

The subjects, of both sexes, were all more than 20 years old and lived in or near Los Angeles, California. The quantitative measurements of ^{137}Cs, together with naturally occurring potassium-40, were made by analyses of the gamma spectra obtained with a "total body counter" consisting of four sodium iodide crystal scintillation detectors in a steel-lined room, and a multichannel pulse height analysis system, as described previously[12]. The accuracy of the technique ($\sigma = \pm 4$ per cent for ^{40}K and ± 10 per cent for ^{137}Cs) agreed closely with the accuracies reported for fifteen other "body counters"[22].

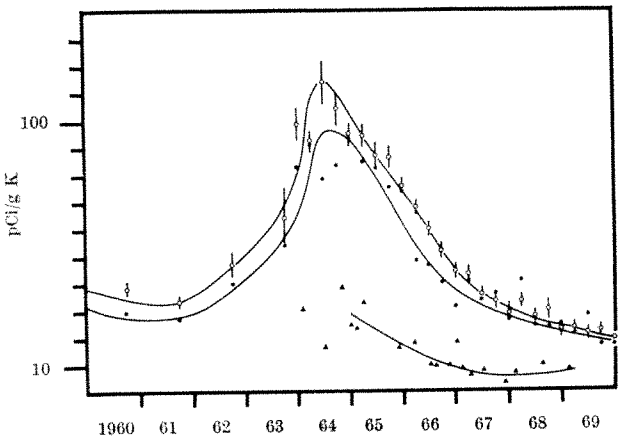


Fig. 2. Mean values of ^{137}Cs activity in human males (○) and females (●), and a hospital diet (▲) in southern California, tabulated quarterly. The vertical lines represent the standard error of the mean values ($\sigma/N^{1/2}$).

Fig. 1 shows the trend of individual values, expressed as picocuries of ^{137}Cs per g of K ($Ci \times 10^{-12}$/g K) over the ten year period. The peak of bodily contamination during 1964–65 occurred about 12–15 months after the cessation of detonations of nuclear devices in the atmosphere by the United States and the Soviet Union. Subsequent nuclear testing programmes conducted in the atmosphere by France and China have not reversed the downward trend since 1965, but have significantly slowed the rate of decrease particularly in 1968–69. This effect of recent injections of ^{137}Cs into the environment is seen more clearly in the flattening of the curves after late 1967 in Fig. 2, which presents the mean values of ^{137}Cs/g K for men and for women, tabulated by quarterly periods. In fact, if the quarterly mean values are replotted on semi-logarithmic graph paper, the points from mid 1965 through 1967 can be described quite well by a single declining exponential function, with a slope corresponding to a half-time of about 17 months. Since early 1968, however, the decline in body burdens has departed from this exponential rate and, indeed, values of ^{137}Cs/g K for early 1970 have remained at about the same level as in 1969. The consistently higher concentration of ^{137}Cs observed in men, compared with women, has previously been reported by a number of other investigators. Fig. 2 also gives the concentrations of ^{137}Cs in 7 day pooled, regular diets obtained bimonthly from a local hospital[23]. The ^{137}Cs/K ratios in the human subjects were about three times greater than the ratio in the dietary samples. Whether or not they truly represented the diets of the persons we

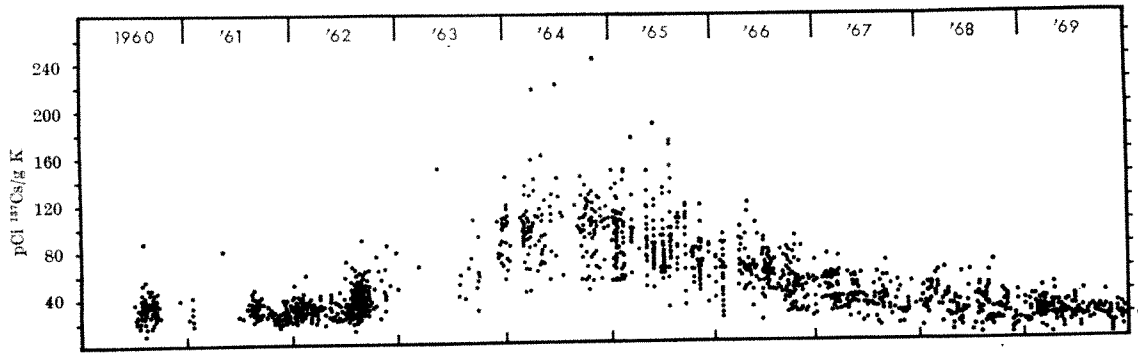


Fig. 1. ^{137}Cs levels in human population in Southern California, 1960–69.

measured is, of course, unproven, but the finding agrees with the three-fold difference in $^{137}Cs/K$ ratio reported for humans and their diets by Gustafson[18]. It is interesting that the plots of the ^{137}Cs concentrations in each of eight individuals which were measured periodically over a span of 6–10 years were essentially similar to the curves of quarterly mean values for the population shown in Fig. 2.

What has been the radiation dose sustained by members of this particular population as a result of the continued presence of ^{137}Cs in their bodies during the past ten years? A reasonable estimate can be made by calculating the absorbed radiation dose to a hypothetical "standard man" in whom the level of ^{137}Cs fluctuated according to the curve for quarterly mean values for males shown in Fig. 2. A total body mass of 70 kg with a potassium content of 135 g was assumed. The concept of "absorbed fraction" introduced by Ellet, Callahan and Brownell[24] and developed in detail for target body organs in the MIRD pamphlets[25] was used, together with the equilibrium absorbed dose constants for ^{137}Cs and ^{40}K. The total body concentration of ^{137}Cs, combined over the ten year period, was obtained by planimeter measurement of the area under the mean curve for males in Fig. 2. The calculated absorbed doses to total body, to marrow and to the gonads were between 8 and 10 mrads for the 10 year period. These values include the doses delivered by all beta particles, primary photons, conversion electrons and X-rays from the disintegration of ^{137}Cs nuclei. To place this radiation dosage in perspective, the total dose delivered simultaneously by the natural ^{40}K continually present in the body, calculated by the same method, came to 156 mrad. In our population sample, no individual had a ^{137}Cs burden greater than twice the quarterly mean value, from which we infer that very few persons in the area received as much as 20 mrad and we conclude that the average dose attributable to ^{137}Cs internal contamination was about 10 mrad, or about 6 per cent of the radiation received from the body potassium. This is close to the United Nations Scientific Committee estimate[26] of the long-term worldwide ^{137}Cs contamination, of 15 mrad absorbed dose as a consequence of the nuclear explosions carried out before 1966. Although the major part of this dose has now been delivered, ^{137}Cs will be with us for many years to come. Programmes to monitor it must therefore continue.

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Effect of Lithium on Foot Shock Aggression in Rats

Lithium has been shown to be effective in the treatment of mania[1–3], and it reduces aggressive behaviour in Siamese fighting fish and small rodents[4]. I have investigated the action of lithium on aggressive behaviour in rats subjected to foot shock. This type of aggression has been well described already[5,6].

Sprague–Dawley male rats (280–350 g, Charles River Laboratories) were used. The testing apparatus consisted of a clear plastic box, 23 × 20 × 20 cm, with a grid floor through which graduated electric current was delivered. Animals received either 5 mequiv/kg of lithium chloride or the same volume of physiological saline by intraperitoneal injection on five consecutive days. At this time there is a significant change in brain serotonin metabolism[7].

To measure the flinch–jump threshold[8], each rat was placed in the test box and a series of unavoidable shocks were delivered to its feet. Ten series of ten stimulations at shock intensities varying regularly in steps from 0·1 to 3 mA were given. Shocks lasted 1·0 s and were presented 30 s apart at 2 min intervals. One of three responses was recorded to each shock: no response, "flinch" (a startle or crouch behaviour in which animals' feet did not leave the grid), or "jump" (removal of two or more paws from the grid). Twenty rats were tested after receiving lithium and twenty after receiving saline.

In the foot shock aggression experiment, pairs of rats matched for size and weight were placed in the test box. Twenty pairs had been pretreated with lithium chloride and twenty with saline. After behavioural habituation to the cage, pairs were subject to increasing intensities of shock to the feet. At each intensity, shocks were delivered thirty times a minute, lasting 1·0 s for a total of 1 min. This was repeated five times at 2 min intervals. There was a 5 min interval between different intensities. The latency to fighting behaviour was measured with a stopwatch.

The jump threshold was defined as the average shock intensity at which the jump response occurred in ten series. For control animals injected with saline, the mean value for the jump response was 1·08 mA with standard deviation of 0·10. For lithium treated animals the mean was 1·10 mA with an s.d. of 0·15 (Table 1). Thus there was no significant difference between animals treated with saline and lithium as far as the jump response to foot shock was concerned. There was also no significant change in the flinch threshold.

The threshold for foot shock aggression was defined as the mean latency to attack in seconds for the five series of shocks at each intensity. Latency to aggression was defined as 60 if no fighting occurred within 1 min. There was a significant difference in the latency to fighting

Table 1. THRESHOLD INTENSITY FOR JUMP RESPONSE IN MILLIAMPS (mA) IN RATS PRETREATED WITH LITHIUM CHLORIDE (5 MEQUIV/KG) OR PHYSIOLOGICAL SALINE FOR 5 DAYS

Treatment	No.	Mean jump threshold MA
Lithium	20	1·10 ± 0·15
		N.S.
Saline	20	1·08 ± 0·10

N.S., not significant.

Table 2. THRESHOLD TO ATTACK IN SECONDS AT DIFFERENT INTENSITIES OF FOOT SHOCK TO PAIRS OF RATS PRETREATED WITH LITHIUM CHLORIDE (5 MEQUIV/KG) OR SAME VOLUME OF PHYSIOLOGICAL SALINE FOR FIVE CONSECUTIVE DAYS

Treatment	No. pairs	Intensity MA	Threshold to aggression seconds ± s.d.
Saline	20	1·5	30 ± 6
		1·75	22 ± 8
		2·0	12 ± 4
		2·25	5 ± 2
Lithium	20	1·5	60*
		1·75	60*
		2·0	52 ± 25*
		2·25	36 ± 12*
		2·5	24 ± 6
		2·75	10 ± 2

* Difference from saline treated animals significant $P < 0.001$.

behaviour between saline pretreated and lithium pretreated rats at all intensities studied (Table 2). There was a marked tendency for rats treated with lithium not to fight at intensities which provoked fighting in those treated with saline. Moreover, rats treated with lithium sustained high levels of shock intensity without fighting.

The prolongation of the latency to attack in pairs of rats treated with lithium and subjected to foot shock indicates that lithium can have an inhibiting effect on aggressive behaviour induced by pain. That this effect is not due to an altered threshold to painful stimulation is demonstrated by the findings in the flinch jump experiment, when there was no alteration in the intensity of stimulation required to produce the flinch jump in lithium pretreated animals.

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Epidermis and Pheromone Production in Males of the Desert Locust

SYNCHRONOUS sexual maturation among crowded males of *Schistocerca gregaria* is achieved through a pheromone produced by the cells of the epidermis[1,2]. The synthesis and release of the pheromone have been correlated with marked histological changes which occur in the appearance of the epidermis during maturation[2]. I have examined the development of the epidermis in males of *S. gregaria*, and have found that the changes associated with maturation are more interesting than previously thought.

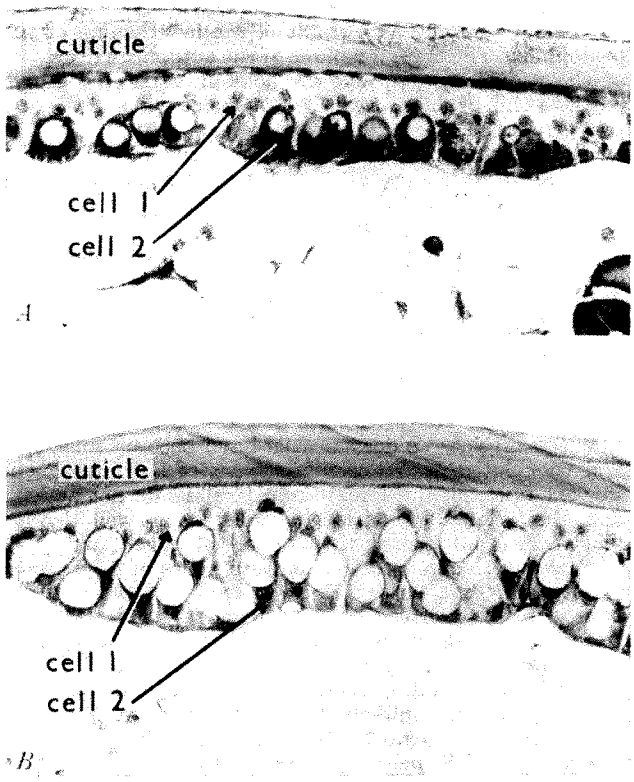


Fig. 1. Transverse sections of the third abdominal tergite of males of *S. gregaria*. *A*, Male locust at the onset of maturity (16 days) showing original epidermal cells (cell 1) and developing acidophils (cell 2). ×64. *B*, Mature male locust (21 days) showing the acidophils with large vacuoles. The acidophils are also lengthened, giving the epidermis a multi-layered appearance. ×64. All material fixed in Bouin's solution and stained with Mallory's triple stain.

Desert locusts were reared under conditions described previously[3], and males were killed at various stages of adult life—from emergence to sexual maturity (28 days). The third abdominal tergite was fixed in Bouin's solution, embedded in ester wax (60° C), and sectioned at 10μ. The sections were stained with Mallory's triple stain.

As reported earlier[2,3], the epidermis of the newly ecdysed male has the appearance of a simple squamous epithelium: the cells are narrow and the nucleus occupies the majority of the cell. During the following 14 days the cells increase slightly in volume, an increase which seems to be associated with the completion of cuticle formation. Until the onset of sexual maturity the epidermis undergoes little modification. As sexual maturity advances, the integument assumes the characteristic yellow coloration, and the epidermis is transformed from a simple epithelium into a compound epithelium of large vacuolated cells, which earlier workers have interpreted in terms of elongation and vacuolation of the original epidermal cells[2,4]. This transformation is caused not by the alteration of the original epidermal cells, but by the appearance, growth and vacuolation of additional cells. Between the basement membrane and the original epidermal cells (Fig. 1*A*, cell 1) appear new cells (Fig. 1*B*, cell 2) which are clearly differentiated from the original epidermal cells. The cytoplasm of the new cells reacts strongly with the acid fuchsin part of Mallory's stain, and they appear as deep-red cells among the neutral-staining epidermal cells. The site of origin of the acidophils is not known, but they are not apparent in the

epidermis of the immature male. The vacuole appears in the acidophil almost as soon as the cell itself can be distinguished, and grows in the distal part of the cell, pushing outwards into the epidermal cells which separate the acidophils from the cuticle (Fig. 1*A*). The compound layer of epidermal cells and acidophils increases greatly in thickness largely as a result of the lengthening of the acidophils. The overall volume of the cytoplasm decreases as the vacuole grows, and the acidophils become drawn out into thin columns with their vacuoles perched distally away from the basement membrane (Fig. 1*B*). In regions where the acidophils are very numerous many do not elongate—presumably as a result of competition from bordering cells—and the cytoplasmic stalk remains short. This creates the impression of a multi-layered epidermis of vacuolated cells (Fig. 1*B*). As the vacuole reaches its final volume the remaining cytoplasm of the acidophil is reduced to a thin band with the nucleus pressed against the vacuole. The result of the dramatic growth of the acidophils and their vacuoles is that the original epidermis is disorganized and compressed against the cuticle. The overall impression obtained by comparing only preparations of newly ecdysed males and fully mature males is that the original epidermal cells have become thickened and vacuolated[2,4]. In certain regions of the integument (for example, pleural folds) the acidophils do not appear, and the original epidermis remains as a well defined layer. It is interesting that Loher[2] observed two types of nuclei in the epidermis of mature males of *S. gregaria*: perhaps this can be explained in terms of the two cell types I have described.

In *S. cancellata* there are scent glands in the abdominal tergites of the mature male: these comprise a glandular cell and a duct cell, in addition to the normal epidermal cells[5]. It seems unlikely that the structures found in *S. gregaria* correspond to those in *S. cancellata*, while the integument of *S. paranensis*[6] seems to be identical with that of *S. gregaria*. Further work is being carried out on the integument of the male desert locust to clarify the site of origin of the acidophilic cells, and the mechanism by which the pheromone is released.

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Characteristics of Spontaneous Activity in Tsetse Flies

THE word "spontaneous" is often applied to the activity of tsetse in the field without it being certain that such activity is not environmentally stimulated. During a laboratory study of circadian rhythms in adult tsetse, however, it has now been possible to confirm that flies do move in the near environmental vacuum of constant temperature, humidity, darkness, noise and, probably, smell.

Five characteristics of such apparently spontaneous activity have emerged: (1) an underlying, endogenous, circadian rhythm exists which is clearly demonstrable in constant darkness, but which is obscured in constant light; (2) the amount of activity increases roughly exponentially during the course of starvation (compare refs. 1 and 2); (3) there are very long intervals when no detectable movement occurs; (4) when activity does occur it is performed intensely for brief periods of less than about a minute; (5) the intervals between these bursts may be very regular. This communication draws attention to the last three points; the first two will be reported in detail elsewhere.

Tsetse (*Glossina morsitans orientalis* Vanderplank) were obtained as pupae from the Tsetse Research Laboratory, Langford, Bristol, and reared at $25 \pm 1°$ C and 60 ± 5 per cent relative humidity in a 12 h light : 12 h dark cycle. In these conditions unfed (teneral) flies live about 3–5 days, and fed (post-teneral) flies about a week after their last blood meal.

Activity was measured in rocking-box actographs ($180 \times 85 \times 43$ mm) coupled to a multi-channel event recorder. With a chart speed of 152 mm/h and a trace width of about 0·25 mm, the smallest interval that could be discerned between bouts of activity was about 3 s, and activity of about 20 movements/min could not be distinguished from more intense activity. Tsetse were active in this system exclusively in short bursts separated by long intervals. A burst of activity was usually effectively continuous, but often included very brief breaks, such breaks generally being at least ten-fold shorter than the interval to the next burst.

Table 1. EXAMPLES OF THE FORM OF SPONTANEOUS ACTIVITY IN TSETSE EARLY AND LATE DURING STARVATION

Type of fly and light regime	Mean interval between bursts of activity (min)		Mean length of bursts of activity (s)	
	Early	Late	Early	Late
♂ teneral (DD)	214	45	15	9
♂ teneral (DD)	79	16	28	24
♀ teneral (DD)	39	16	10	10
♀ post-teneral (DD)	147	38	15	22
♀ post-teneral (LL)	64	13	42	34
♀ post-teneral (LL)	100	78	29	27

Early refers to records taken on the first 1–2 days of starvation; late refers to records taken 2–4 days later. Teneral = unfed; post-teneral = fed; DD, constant darkness; LL, constant light; LD, alternating 12 h light: 12 h dark.

Six typical examples of tsetse activity as it changes during starvation are analysed in Table 1. These examples are selected from ninety-six individual activity records covering a total of some 700 fly-days. Because of the high variance caused by the effects of lights-on and lights-off in a light : dark cycle, data have been computed from records taken in constant light or constant darkness only. Further, in order to allow for the variance caused by circadian changes in activity, all means are calculated from entire 24 h periods. The individual differences between the intervals early and late in starvation are highly significantly different (Mann–Whitney U test, $P < 0{\cdot}01$). The distribution of the lengths of the activity bursts for

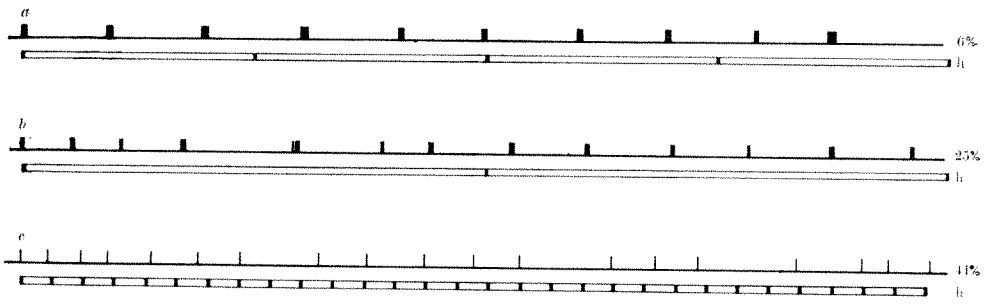


Fig. 1. Records of tsetse activity showing marked periodicity in the bursts of activity. Upper line in each pair is tracing of activity record, lower line is time scale in h. *a* and *b*, Unfed males (DD); *c*, fed female (LL).

each fly is extremely skew, with the lowest class always the largest; confidence limits for the means are therefore not quoted, but in every case the ranges overlap almost completely. The absolute maximum burst lengths observed for these flies were 30, 95, 30, 70, 70 and 54 s, respectively. Changes in the intensity of activity evidently occur primarily by shortening the interval between the bursts of activity; the length of the bursts themselves remains relatively constant. Blow-flies apparently change their activity in an analogous manner[2].

Although considerable variation in the length and form of the activity bursts occurred between individual flies, the general principle of discrete packets of activity separated by much larger intervals was universal. This sporadic nature of tsetse activity in the laboratory confirms inferences from the field[3,4]. In the laboratory, the bursts are performed in a more or less periodic manner at any given time. For example, the standard deviation of the mean intervals taken over any 24 h of record was usually around 75 per cent of the mean. Over shorter periods, much clearer, visually striking periodicities occurred; examples are shown in Table 2 and Fig. 1.

Table 2. EXAMPLES OF PERIODICITY IN THE SPONTANEOUS ACTIVITY OF TSETSE

Type of fly and light regime	No. of intervals	Mean interval (min)	Coefficient of variation (per cent)
♀ post-teneral (DD)	9	147	48
♀ post-teneral (LL)	27	100	41*
♀ post-teneral (LL)	24	10	25
♂ teneral (DD)	12	9	25*
♂ teneral (LD)	14	15	19
♂ teneral (DD)	8	22	15
♂ teneral (DD)	11	18	9
♂ teneral (DD)	8	22	6*

$$\text{Coefficient of variation} = \frac{\text{s.d.} \times 100}{\text{mean}}$$

* Records shown in Fig. 1; other abbreviations as for Table 1.

The duration of the activity bursts, whose maximum length appears to be of the order of 1–2 min, is strikingly similar to the known course of amino-acid consumption during enforced flight. Proline, which seems to serve as an immediate source of rapidly mobilizable energy reserve for flight, is depleted from its maximum resting level to its minimum by 2 min of such treatment[5]. Furthermore, about 30 min to 2 h of rest are required for the complete recovery of the proline concentration; this period is close to the observed duration of the intervals between activity bursts.

These similarities, and the sort of periodicity shown in Fig. 1, are suggestive of rather simple physiological mechanisms controlling spontaneous flight. At least two complications arise, however. First, a lack of any apparent correlation between interval length and burst length does not suggest a simple relationship of the sort found between glycogen and flight duration in *Drosophila*[6]. Second, whereas proline reserves increase for the first 3 days after a blood meal and then decrease rapidly until death[5], no sort of parallel to this is detectable in the activity pattern. Such considerations imply that a simple, mechanistic interpretation may be premature.

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Nitrification and Induction of Nitrate Reductase in Nitrogen-deficient Algae

IT has been known for a long time that nitrogen-deficient algae can reduce added nitrate very rapidly[1,2]. In accordance with this observation, Morris and Syrett[3,4] were able to demonstrate, in *Chlorella* grown in ammonium, the formation of nitrate reductase after the nitrogen source had been exhausted. This seems to contradict the well established fact that nitrate reductase in algae and all other plants studied is an adaptive enzyme which is formed only in the presence of its substrate[5].

While working on the metabolism and enzymes of nitrogen-deficient algae we have been able to confirm the findings of Morris and Syrett[3,4] and to show that when nitrogen is deficient cellular nitrogen compounds are oxidized to nitrate and nitrite, which, in turn, induce the synthesis of nitrate reductase.

Ankistrodesmus braunii (Naegeli) Collins (strain 202–7c from Pringsheim's culture collection at Göttingen), *Chlorella fusca* Shihira et Krauss (=*pyrenoidosa*, strain 211–11m), and *Chlorella vulgaris* Beijerinck (strain 211–8m) were grown in the conditions described by Czygan and myself[6], but with 8×10^{-3} M NH_4Cl and 8×10^{-3} M KCl instead of KNO_3 and NaCl. To measure nitrate reductase activity in cell free extracts, the cells were broken[7] and centrifuged for 10 min at 10,000*g* and 4° C. Our test system contained 1 ml. of the supernatant, 51·0 μmoles KNO_3, 0·5 μmole FAD, 3·75 μmoles sodium dithionite, and 0·05 M phosphate buffer, *p*H 7·5, to make a final volume of 3·0 ml. The concentration of nitrite was determined after 30 min at 25° C.

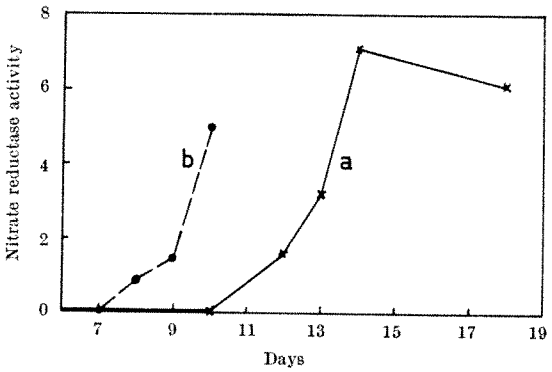


Fig. 1. Nitrate reductase activity in extracts of *Ankistrodesmus braunii*. The figure shows nitrogen depleted (*a*) after 10 days of growth in nutrient solution containing NH_4Cl, or (*b*) by transferring the algae into nitrogen free medium after 7 days of growth. Light intensity was 4,000 lux. Nitrate reductase activity is given in 10^{-3} μmoles of nitrite formed/mg soluble protein/min.

Fig. 1 shows the formation in *Ankistrodesmus braunii* of nitrate reductase after depletion of the ammonium nitrogen in the nutrient solution, and when the algae were transferred to a nitrogen free medium. At the same time, nitrate and nitrite can be detected in the cells (Table 1). It should be stressed that we have never observed any formation of oxidized nitrogen compounds, nor any nitrate reductase activity, in the presence of ammonium ions in the nutrient medium. Thus we assume that this activity is due to an oxidation of cellular

Table 1. FORMATION OF NITRATE AND NITRITE BY NITROGEN-DEFICIENT *Ankistrodesmus braunii*

a			*b*		
Days	Nitrate	Nitrite	Days	Nitrate	Nitrite
10	0	0	0	0	0
12	0	3	1	0	2
13	10	5	2	0	3
14	100	3	3	30	4

The table shows depletion of nitrogen (*a*) after 10 days of growth in nutrient solution containing NH_4Cl, or (*b*) by transferring the algae into nitrogen free medium after 7 days of growth. Light intensity was 4,000 lux. Nitrate and nitrite contents ($\times 10^{-3}$ μmoles/ml.) are of cell free extracts.

organic nitrogen compounds which takes place only when nitrogen is deficient in the light, or in the dark in the presence of glucose. We found similar reactions in *Chlorella fusca* and *Chlorella vulgaris*.

The oxidation in algae of cellular nitrogen compounds to nitrate and nitrite is in agreement with observations made with microorganisms and higher plants. Thus heterotrophic nitrification without chemosynthetic carbon dioxide fixation has been found in certain bacteria and fungi[8]. An oxidation of hydroxylamine has been demonstrated in chloroplasts[9] and extracts from higher plants[10]. Yamafuji and Osajima[11] isolated from algae dehydrogenases for ammonia, hydroxylamine, hyponitrite and nitrite. The significance *in vivo* of these enzymes and the occurrence in green plants of reactions leading to the oxidation of organic and inorganic nitrogen compounds, however, have so far remained doubtful, in spite of occasional reports on the formation in the light of nitrate in higher plants grown in the absence of nitrate[12].

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Copper Tolerance in the Marine Fouling Alga *Ectocarpus siliculosus*

INTRASPECIFIC variation in marine algae in response to habitat conditions has been proposed[1] and, in some cases[2,3], demonstrated. During an investigation into the ship-fouling properties of the brown alga *Ectocarpus siliculosus* (Dillw.) Lyngb. we have found evidence of differential response to dissolved copper which may be associated with the habitats from which the populations were taken.

The populations studied were located at Rhosneigr, Anglesey, an open uncontaminated rocky shore, and from the hulls of two ocean-going freighters treated with copper-based antifouling preparations. Unialgal cultures were established from excised pieces of filament taken from ten plants in each population. The material was grown together in a modified enriched seawater medium[4]. No soil extract or chelating agents were incorporated.

Known volumes of plant material (0·05–0·07 ml.) were measured by means of slow centrifugation (4,000 r.p.m.) for 10 min in 'Wintrobe' blood sedimentation tubes[5] and used as inoculum in successive media containing different concentrations of dissolved copper (as $CuCl_2$). The cultures, all 100 ml., were grown at 10°–15° C under continuous fluorescent 'White' light of 2,700 lux for 5 weeks. Volume assessments of total plant material were made weekly and the percentage increase or decrease in volume was noted; the plant material was always centrifuged in the appropriate medium. Each experiment was run in duplicate.

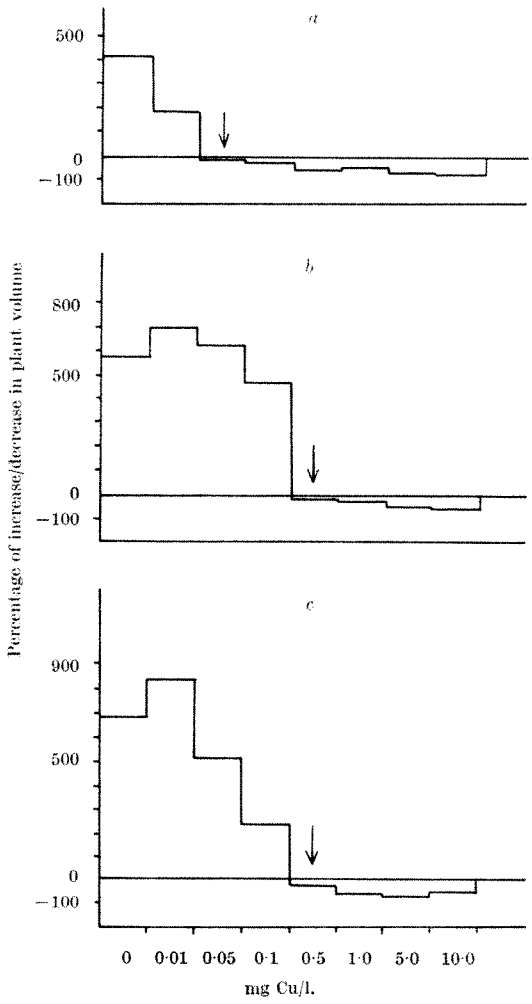


Fig. 1. Response to dissolved copper after 35 days in culture by populations of *Ectocarpus siliculosus* from (*a*) Rhosneigr, Anglesey, and (*b*) and (*c*) the hulls of two ocean-going freighters. Arrows indicate critical toxicant concentration for each population.

Results showed close agreement and the mean values in Fig. 1 indicate that the ship-borne populations of *E. siliculosus* are more tolerant to dissolved copper than the Rhosneigr population by a factor of 10.

This bioassay method proved satisfactory and the concentration of toxicant at which there is no increase or decrease in plant volume (critical toxicant concentration) seems to be more useful in practice than a subjective estimate of plant vigour.

The different responses to copper by shore and ship-borne populations of *Ectocarpus* are in evident agreement with well established cases of heavy metal tolerance in higher plants[6] and fungi[7]. Copper has long been used as a base for antifouling preparations and the faster growth of plants of both ship-borne populations in low copper concentrations than in control conditions suggests that they were tolerant to the metal.

It is interesting that *E. siliculosus* and *Enteromorpha* spp., the most frequently encountered ship-fouling plant taxa, are polymorphic, euryhaline, pollution-tolerant and cosmopolitan in distribution. But the characteristics which enable these species to exploit a wide range of habitats and environmental conditions seem likely to be based at least as much on a high intrinsic genetic variability as on a general physiological toughness.

The adaptive aspects of these findings are likely to be important in several marine ecological problems, for they disclose a situation analogous to DDT resistance in insects. This work also goes some way towards explaining the

incomplete success of antifouling preparations and the difficulty of developing an infallible product. The economic implications to the shipping industry and to antifouling research in the era of the super-tanker are self-evident.

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Basic Rectangle of the Mandible

The arrangement of teeth in the lower jaw of man (Hominidae) may be distinguished from that of apes (Pongidae) by means of the "basic rectangle of the mandible"[1]. Leakey defined the rectangle and suggested that, in the Pongidae, the sides "are nearly twice as long as the upper and lower ends"; whereas in the Hominidae "the basic rectangle is seen to be nearly a square"[1]. Leakey presented several illustrative diagrams but he did not otherwise quantify his measurements.

The basic rectangle may be treated more precisely by computing an index derived from measurements of the sides of the rectangle. When the antero-posterior length of the rectangle times 100 is divided by its breadth, an index results which I call the index of the basic rectangle of the mandible (BRM Index). I measure the length of the rectangle as the shortest distance between the anterior alveolar margin of the left central incisor and a straight edge placed in contact with the posterior surfaces of the third molar teeth. The breadth is the distance between the points of contact of the two third molars with the straight edge.

In order that mandibular shape may be compared among higher primates, and that comparisons among fossil primates may be more meaningful, I now present data on the BRM Index for nineteen genera of living primates including *Homo sapiens* (Table 1). (Representative rectangles are shown in Fig. 2.) Measurements of length and breadth of the rectangle were taken on mandibles of known sex of adult *Pan troglodytes*, *Gorilla gorilla gorilla*, *Pongo pygmaeus pygmaeus*, *Hylobates concolor*, *Cercopithecus nictitans*, *C. aethiops*, *Nasalis larvatus*, *Colobus badius*, *Macaca radiata*, *M. nemestrina*, *M. fascicularis*, *M. mulatta*, *M. arctoides*, *Papio* sp., *Callimico goeldii*, *Saimiri sciureus*, *Callicebus moloch*, *Aotus trivirgatus*, *Ateles fusciceps*, *Pithecia pithecia*, *Cacajao rubicundus*, *Alouatta seniculus*, and *Tarsius syrichta*. The classification is that of Napier[2]. The mean index and its standard error were computed separately for males and females of each species. When male and female indices did not differ ($P > 0{\cdot}05$), the sexes were combined. There was no significant difference among the five macaque species for either sex, so all female macaques were combined and all male macaques were combined. The measurements for *Homo sapiens* in Table 1 were taken on mandibles of known age and sex from American Negroes, aged 19 to 60 years.

As Leakey has suggested[1], mandibular shape in living man differs clearly from that in living pongids as indicated by the BRM Index. Table 1 shows that the index in some Cebidae and the tarsier, however, overlaps the index for *Homo sapiens*. All the coefficients of variation are less than 10, suggesting that none of the samples represents a mixed population; the low coefficient of variation in some genera may represent inadequate sampling.

Human mandibles were considered with respect to age and race. Adult Negro mandibles did not differ with age. (The coefficient of correlation for the BRM Index with age,

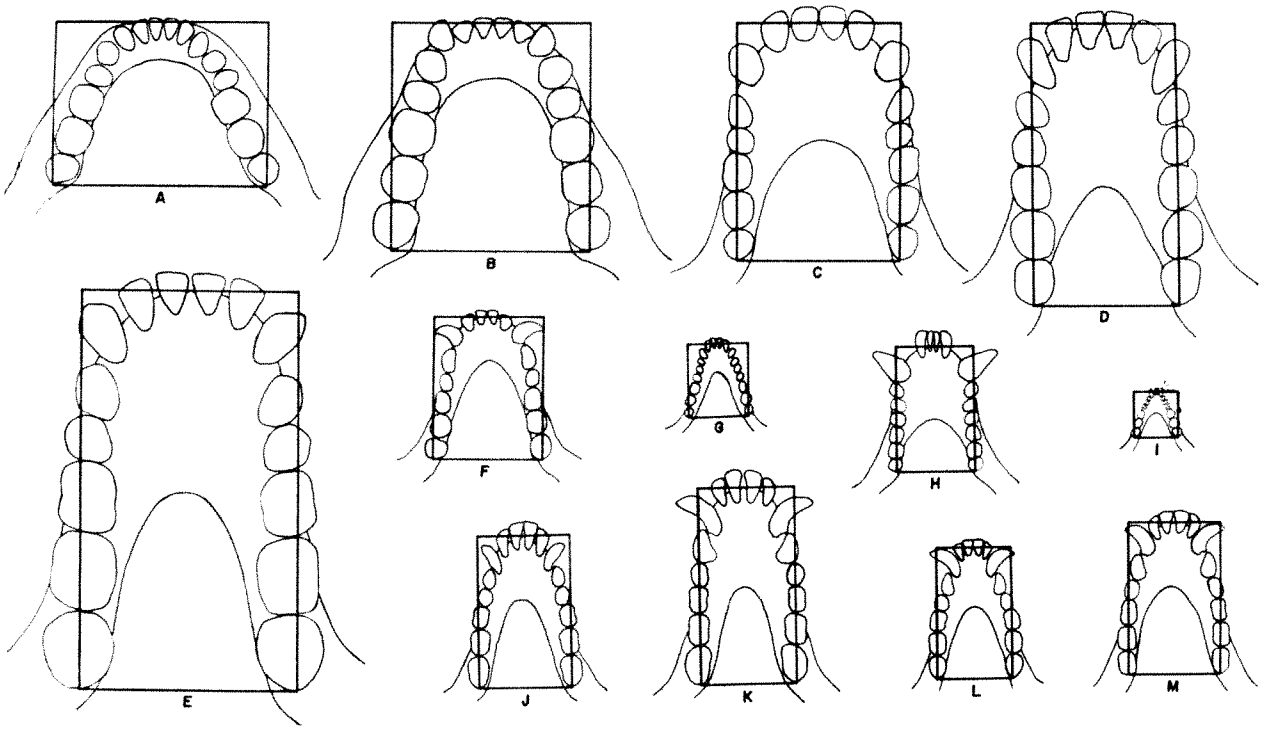


Fig. 1. The basic rectangle of the mandible in representative genera of living primates (all to the same scale). The BRM Index for each is given in parentheses. *A*, *Homo sapiens*, white (77); *B*, *Homo sapiens*, negro (116); *C*, *Pan*, (145); *D*, *Pongo* (196); *E*, *Gorilla* (185); *F*, *Hylobates* (133); *G*, *Callicebus* (120); *H*, *Cacajao* (160); *I*, *Tarsius* (104); *J*, *Macaca radiata*, female (166); *K*, *Macaca mulatta*, male (205); *L*, *Cercopithecus aethiops*, male (175); *M*, *Cercopithecus nictitans*, male (168).

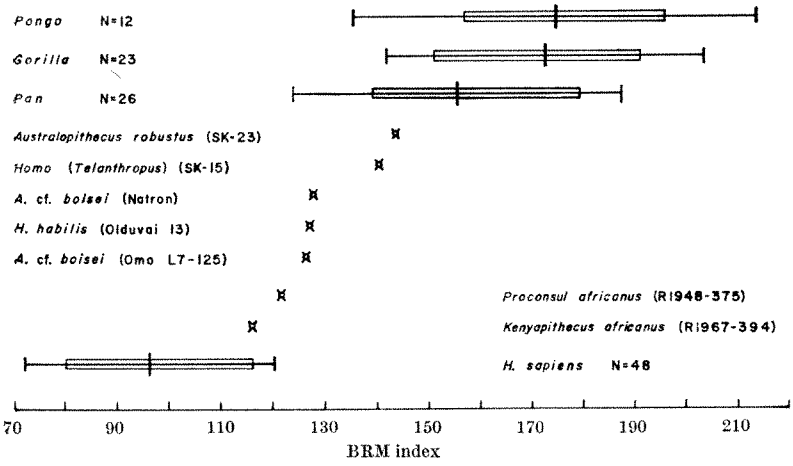


Fig. 2. The index of the basic rectangle of the mandible (BRM Index) in living and fossil hominids and pongids. The total length of each figure indicates the estimated population range using limits of three times the standard deviation on either side of the mean; the enclosed space represents the observed range of variation; and the cross line marks the mean; N is the sample size in each case.

$r = -0{\cdot}12 \pm 0{\cdot}16$.) There was, however, a striking racial difference. A sample of twelve American White mandibles had an average BRM Index of $86{\cdot}7 \pm 2{\cdot}2$, much lower than that for American Negro reported in Table 1 ($P < 0{\cdot}0001$). Racial differences are illustrated in Fig. 1*A* and *B*. It should be noted that while the Negro dental arch has been referred to as U-shaped[3], even in the extreme case illustrated in Fig. 1*B*, the rows of molar teeth are not parallel.

Table 1. INDEX OF THE BASIC RECTANGLE OF THE MANDIBLE IN LIVING PRIMATES

Genus	Sex	N	$\bar{x} \pm$s.e.	*V*	o.r.
Homo	M+F	48	$96{\cdot}6 \pm 1{\cdot}2$	8·4	80–116
Pan	M+F	26	$155{\cdot}6 \pm 2{\cdot}1$	6·9	139–179
Gorilla	M+F	23	$172{\cdot}9 \pm 2{\cdot}2$	6·0	151–191
Pongo	M+F	12	$174{\cdot}7 \pm 3{\cdot}7$	7·4	157–196
Hylobates	M+F	13	$138{\cdot}2 \pm 1{\cdot}8$	4·8	130–153
C. nictitans	M+F	21	$153{\cdot}0 \pm 2{\cdot}2$	6·5	137–175
C. aethiops	F	14	$168{\cdot}4 \pm 1{\cdot}3$	2·9	158–175
C. aethiops	M	19	$177{\cdot}2 \pm 1{\cdot}9$	4·7	164–191
Nasalis	M+F	6	$182{\cdot}5 \pm 3{\cdot}8$	5·0	172–193
Colobus	M+F	9	$202{\cdot}4 \pm 2{\cdot}6$	3·9	189–214
Macaca	F	31	$180{\cdot}6 \pm 2{\cdot}0$	6·1	156–208
Macaca	M	31	$198{\cdot}6 \pm 2{\cdot}3$	6·3	171–229
Papio	F	3	$211{\cdot}7 \pm 6{\cdot}0$	4·9	200–220
Papio	M	3	$232{\cdot}3 \pm 7{\cdot}2$	5·4	218–241
Callimico	M+F	5	$116{\cdot}4 \pm 1{\cdot}0$	2·0	114–120
Saimiri	F	10	$117{\cdot}7 \pm 1{\cdot}0$	2·6	113–121
Saimiri	M	11	$126{\cdot}5 \pm 1{\cdot}2$	3·1	122–136
Callicebus	M+F	22	$129{\cdot}2 \pm 1{\cdot}5$	5·4	117–145
Aotus	M+F	16	$129{\cdot}3 \pm 1{\cdot}7$	5·4	118–141
Ateles	M+F	12	$140{\cdot}5 \pm 1{\cdot}5$	3·8	131–151
Pithecia	M+F	9	$148{\cdot}9 \pm 1{\cdot}9$	3·7	139–155
Cacajao	M+F	12	$153{\cdot}5 \pm 1{\cdot}1$	2·6	148–161
Alouatta	M+F	21	$168{\cdot}9 \pm 1{\cdot}8$	4·9	150–184
Tarsius	M+F	9	$112{\cdot}8 \pm 2{\cdot}5$	6·6	103–122

N = sample size; $\bar{x}$ = mean; s.e. = standard error of the mean; *V* = coefficient of variation; o.r. = observed range of the sample; M = male; F = female.

Table 2. SIZE AND SHAPE OF THE BASIC RECTANGLE OF THE MANDIBLE IN HOMINOIDEA

	Homo sapiens		*Pan*		*Gorilla*		*Pongo*		*Hylobates*	
	M	F	M	F	M	F	M	F	M	F
N	39	9	13	12	15	8	6	6	6	7
Length										
$\bar{x}$	53·9	51·3	70·0	68·5	94·5	83·9	90·1	75·5	37·9	39·2
s.e.	0·7	0·5	1·2	0·7	1·0	1·3	0·9	0·6	0·4	0·8
V	7·6	3·0	6·3	3·6	3·8	3·9	2·6	2·1	2·3	5·3
M–F		†	nonsig.			‡		‡	nonsig.	
Breadth										
$\bar{x}$	55·8	54·0	44·2	44·9	54·4	49·3	50·3	44·7	27·8	28·1
s.e.	0·6	0·6	0·6	0·7	0·7	1·2	0·7	1·5	0·5	0·4
V	6·1	3·2	5·1	5·2	4·8	6·0	3·5	8·5	4·6	4·0
M–F		*	nonsig.			†		‡	nonsig.	
Index										
$\bar{x}$	97·0	95·1	158·7	152·5	174·1	170·6	179·3	170·0	136·3	139·7
s.e.	1·4	1·2	3·5	2·1	2·6	3·9	2·2	6·9	2·6	2·6
V	9·2	3·6	7·9	5·0	5·9	6·5	3·0	9·9	4·7	4·9
M–F	nonsig.		nonsig.		nonsig.		nonsig.		nonsig.	

N = sample size; $\bar{x}$ = mean; s.e. = standard error of the mean; *V* = coefficient of variation; M–F = significance of difference between male and female means: nonsig., $P \gtrsim 10$ per cent; *, $P < 2{\cdot}5$ per cent; †, $P < 1$ per cent; ‡, $P < 0{\cdot}1$ per cent. All measurements are in mm.

Sex differences in the human mandible are well known[4,5]. As expected, the length and breadth of the basic rectangle are greater in the human male than in the female, but sexual dimorphism is lacking in the shape of the arch as expressed by the BRM Index (Table 2). The index of all the apes also lacks significant sexual dimorphism, as do the length and breadth measurements of the chimpanzee (*Pan*) and the gibbon (*Hylobates*). Differences in index between male and female occur, however, in some platyrrhine and catarrhine monkeys.

Estimates for population ranges of the BRM Index of living hominids and pongids, using limits of three times the standard deviation on either side of the mean, are shown in Fig. 2. Estimated values are also presented for several fossil hominids and pongids, based on casts and photographs. The dental arch of australopithecines is less "parabolic" than that of *Homo erectus* or *Homo sapiens*; thus indices for some australopithecines fall within the estimated population range for living pongids. The indices for both *Kenyapithecus africanus* and *Dryopithecus* (*Proconsul*) *africanus* fall almost within the estimated population range for American Negro. While post-mortem distortion may have altered the shape of the dental arch in some fossil mandibles, the similarity in the BRM Index of the two Rusinga Island mandibles in Fig. 2 supports the suggestion[6], arrived at on the basis of other evidence, that mandible No. 394 is most similar in its overall morphology to *Dryopithecus* (*Proconsul*) *africanus*. If "*Kenyapithecus*" *africanus* is linked "clearly with the family Hominidae"[1] on the basis of shape of the basic rectangle, then *Proconsul africanus* must be also, although most would agree the latter is not. Whether the shape of the dental arch is expressed as the basic rectangle, the anterior mandibular index[7], or the degree of posterior divergence[8], it is clear that in Miocene hominoids the shape was quite different from that in living apes, and in many early hominids the shape was different from that in living man. The functional and taxonomic significance of these differences has not yet been explained adequately.

I conclude that, whereas the basic rectangle of the mandible may be useful taxonomically among living primates, it does not serve to distinguish between pongids and hominids when fossil hominoids are considered. The shape of the basic rectangle, therefore, does not provide evidence for placing *Kenyapithecus africanus* within the Hominidae.

I thank Dr J. L. Angel and Dr R. Thorington (US National Museum), Dr J. C. Moore (Field Museum of Natural History, Chicago), Dr R. G. Van Gelder and Dr Sydney Anderson (American Museum of Natural History), and Dr S. L. Washburn (University of California, Berkeley) for permission to study material in their collections. I also thank Mr R. L. Susman for technical assistance. This work was supported in part by a University of California research grant.

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Book Reviews

ROCKET RECORDS

The Papers of Robert H. Goddard

Including the Reports to the Smithsonian Institution and the Daniel and Florence Guggenheim Foundation, Vols. 1–3. Edited by Esther C. Goddard in association with G. Edward Pendray. Vol. 1: 1898–1924. Pp. xx+1–560. Vol. 2: 1925–1937. Pp. x+561–1104. Vol. 3: 1938–1945. Pp. x+1105–1707. (McGraw-Hill: New York and London, 1970.) 1,440*s*; $150.

"THE dream of yesterday is the hope of today and the reality of tomorrow," wrote Robert H. Goddard in 1904 at the age of 21; and for the next forty years he strove to bring the liquid-fuel rocket from dream to reality. At his death in 1945, Goddard left more than 6,000 pages of orderly and continuous records, of which a generous selection—chiefly correspondence, technical reports and entries from Goddard's daily diary—appears in these volumes, chronologically arranged and well printed. Mrs Esther Goddard and her co-editor, Dr Pendray, are to be warmly congratulated on what is not only a worthy monument to the life work of her late husband, but also a fascinating record of an inventor's career for anybody interested either in rocketry or in the history of technology.

Robert H. Goddard was born at Worcester, Massachusetts, in October 1882. His parents moved to Boston soon afterwards, but in 1898 they returned to Worcester, where Goddard remained for much of his life. His family was quite prosperous, and he was allowed to indulge in some boyish inventions, such as trying to fly hydrogen balloons made of thin aluminium sheet and firing special arrows 300 feet into the air. He was away from school because of illness between 1899 and 1901, and did not gain his first degree until he was 25; this was followed by a doctorate at Clark University in 1911. After research in physics, and a serious attack of tuberculosis, Goddard was appointed assistant professor in physics at Clark University in 1915 and full professor in 1920. He retained this appointment until 1943, although on leave of absence from 1934.

Goddard's first patent for a liquid-fuel rocket was issued in July 1914, and a month later he completed a theoretical paper on the problem of reaching great altitudes by rocket. He calculated that, with perfect efficiency and an initial acceleration of about $3g$, an initial mass of about 1,000 pounds would be enough to send a mass of 1 pound to the surface of the Moon. In September 1916 Goddard asked the Smithsonian Institution for a grant towards practical work on liquid rockets: the answer was "yes", largely on the recommendation of Dr C. G. Abbot, then director of the Smithsonian Astrophysical Observatory. The Smithsonian Institution continued to finance Goddard until 1930, when he began receiving grants from the Guggenheim Foundation which continued to the end of his life. It was in March 1926, nine years after the Smithsonian grants began, that Goddard succeeded in launching the first liquid-propellant rocket, at Auburn, Massachusetts. The rocket, which weighed 5 kg at launch, was powered by gasoline and liquid oxygen, and flew to a height of 12 m and a distance of 55 m. Goddard built steadily on this small beginning, continually improving the design of combustion chambers, nozzles, propellant flow systems and methods of stability and control. In 1932 came the first flight with control by gyroscopic stabilization and vanes in the exhaust jet, and in 1935 the first supersonic flight. Subsequently he worked on turbopumps for propellant delivery, variable-thrust motors and nozzle-cooling techniques. Goddard did not develop very large rockets, because he was primarily concerned with upper-atmosphere sounding rockets rather than weapons; but nearly every feature of the German V2 was covered by his patents and first used in his rockets.

For forty years Goddard worked alone, apart from a few assistants, in face of scepticism from the public and most scientists, uncertainty about next year's finance, and repeated failures of the rockets. But he never wavered in perseverance or enthusiasm, and the laconic diary entries scarcely vary as the years roll by: "1916 June 24: Tried to fire short nozzle in small tank in morning. . . . 1944 Aug. 25: Tried starts morning and afternoon". What inner drive kept him going so steadily, free from doubt or despondency? Goddard does give one clue to help us in answering this unanswerable question. His thoughts first turned towards rockets and space in 1898 after reading H. G. Wells's *War of the Worlds*, with its story of Martian invaders and its "wonderfully true psychology"; and the experience that directed his life came in the following year, on October 19, 1899, to be precise, when he climbed a cherry tree at the family home, Maple Hill: "It was one of the quiet, colorful afternoons of sheer beauty which we have in October in New England, and as I looked toward the fields at the east, I imagined how wonderful it would be to make some device which had even the possibility of ascending to Mars, and how it would look on a small scale, if sent up from the meadow at my feet". From then on, October 19 was marked each year in his diary as "Anniversary Day", when he would if possible return to the cherry tree, almost as if he were a knight of old renewing his vows. Shelley's lines,

> I do remember well the hour which burst
> My spirit's sleep: a fresh May-dawn it was . . .

apply to many single-minded and inner-directed artists or inventors, and Goddard is among them, even if his May morning was an October afternoon. But Goddard also conforms to Edison's dictum that genius is 1 per cent inspiration and 99 per cent perspiration, for he was careful and methodical as well as enthusiastic: the fatal explosions that marred so many early experiments with rockets are conspicuous by their absence in his work. On the other hand, his methodical approach did tend to narrow his interests. He lacks the breadth of vision shown by the greatest inventors like James Watt, and, in spite of his insight into the future of space flight, Goddard's writings are a little too plain and workmanlike, and deficient in verve or excitement. Nevertheless Goddard deserves a high place among the single-minded inventors who have concentrated on a single vehicle and have driven it successfully along that hard road from hope to reality.

D. G. KING-HELE

EHRENFEST'S CHARACTER

Paul Ehrenfest

Vol. 1: The Making of a Theoretical Physicist. By Martin J. Klein. Pp. xvi+330. (North-Holland: Amsterdam and London, 1970.) 70*s*; $8.40.

THIS first volume of the biography of Paul Ehrenfest takes the reader from the date of his birth in Vienna in 1880 to just beyond the end of the First World War in 1920.

This time span includes the heroic period of the quantum theory and the atomic models with Planck, Einstein, Bohr, Sommerfeld, Lorentz, Boltzmann and others as the principal figures in theoretical physics. Paul Ehrenfest differed from these men because he had never acquired the skill to carry through any extensive calculation and emotionally he was possibly not suited to do so. His strength was directed towards critical analysis, asking the pertinent question, clearing up obscurities, getting to the bottom of a problem. This was often done with the aid of the "Socratic dialogue", verbal or in letters, with many of the outstanding theoreticians of the time. There is nothing novel in this as a method of research in theoretical physics, but the intensity and determination which Ehrenfest applied and the enthusiasm he was able to stimulate in his participants were unusual. This side of Ehrenfest's character was also the reason that he became famous as an inspiring teacher and lecturer. An impressive example can be found in his biographer's account of the inaugural lecture given by Ehrenfest when appointed professor at the University of Leyden in 1912.

A third of this volume is devoted to Paul Ehrenfest's personal biography: his youth in Vienna, the years of study at its university where L. Boltzmann's influence first directed him to statistical mechanics, the highly important stay of eighteen months in Göttingen and the five years' period in Russia. During these "Wanderjahre", Ehrenfest became acquainted with the European physicists of eminence and their problems. Being married to a Russian mathematician and the father of two girls, it became urgent for him to find an academic position with a steady income. After a disappointing search, help came from an unexpected side: Lorentz chose him as his successor.

It is characteristic of Ehrenfest's seriousness of mind and of his self discipline that he kept extensive notes not only on personal matters but of discussions, scientific questions and ideas occurring to him so as to make sure to reflect further on them at a later date. These notebooks and diaries provided his biographer with a mine of information, permitting him to create a true image of Paul Ehrenfest (pp. 90–91, a programme for little Tatyana's future intellectual development, is most typical).

The principal section of the volume is concerned with the history of atomic physics and the contribution Ehrenfest has made to it. There are the following chapters arranged chronologically: the critic of statistical mechanics, theoretical miscellany, the essential nature of the quantum hypothesis and the adiabatic principle.

The volume finishes with a chapter "Einstein und Ehrenfest". It gives the volume a charming ending full of glimpses into the bonds between the two friends and shows the human side of the awe-inspiring phenomenon of Einstein.

On reading this book, one cannot help admiring the erudition of the author, the agreeable style of his language and his ability to present physics in clear terms with didactic skill. This volume can be warmly recommended and I am looking forward to the second volume.

E. Bretscher

COSMIC RADIATION

Proceedings of the Eleventh International Conference on Cosmic Rays

Budapest, August 25–September 4, 1969. Vol. 1: Origin and Galactic Phenomena. Edited by A. Somogyi. (*Acta Physica* Supplement, Vol. 29.) Pp. 571. (Akadémiai Kiadó: Budapest, 1970.) 220*s*.

If in no other way, this book is remarkable both for the speed with which it has been produced (it is only a year since the conference which it reports took place) and for the high quality of its appearance, which does not seem to have suffered at all from the speed of its preparation. These factors must owe much to the production of the volume as a supplement to a journal (*Acta Physica*) where both the editors and publishers are familiar with producing the printed word from manuscripts quickly and accurately—perhaps there is a lesson here for those contemplating the publication of other conference reports.

This volume is the first of four to be published covering the Proceedings of the Eleventh Conference on Cosmic Rays, and is restricted to those papers dealing with the origin of cosmic radiation and galactic phenomena. The full texts of all the relevant original contributions are included, and although in several cases these contributions deal with work which has also been reported elsewhere, this completeness has resulted in a most valuable review book and source from which other relevant papers can be found. It is impossible to do justice to a book of this kind in a limited review; all that can be said is that this volume comprehensively covers the field which it is reporting, and if the other three volumes cover their fields in a similar detailed manner, then the set will become an essential and much used part of every astrophysics library. Although the price of these volumes will probably be far too high for the individual astronomer to buy them, they are good value when compared with the cost of similar volumes produced by some other publishers, and this sort of comprehensive review is in any case much more suited to the libraries of academic institutions than to the individual's private collection.

John Gribbin

PROBLEMS OF EPISTEMOLOGY

Studies in History and Philosophy of Science

Edited by Gerd Buchdahl and L. L. Laudan. Vol. 1, Nos. 1 and 2. Quarterly. (Macmillan (Journals): London, May and August 1970.) 100*s* per annum; 30*s* per issue.

This new quarterly, according to the editors' manifesto, intends to promote studies leading to "an understanding of the scientific enterprise", which (as they rightly emphasize) demands an approach that "must be simultaneously historical and philosophical". They are sorry for the "gap that has grown between the two disciplines" of history and philosophy of science, and which they somehow hope to bridge by providing a meeting place for philosophically minded historians and historically minded philosophers.

In entertaining such optimistic expectations, they do not seem to realize that the place of philosophical thinking in science has undergone a radical change since the beginning of the nineteenth century. In the preceding period of formation of modern science, philosophical reflexion was—for good reasons—an inherent part of the scientific activity of the great pioneers; by the end of this period, when science had acquired its own method and put it on a solid foundation, and at the same time, under the pressure of accumulated experience and increasing refinement of the techniques of investigation, had divided into a number of specialized disciplines, makers of philosophical systems—again for obvious reasons—were left irrevocably behind. What scientists still need is a continued analysis of the basic assumptions underlying their activity, but this epistemological analysis requires such an intimate knowledge of the most technical aspects of scientific investigation that it can only be carried out usefully by the scientists themselves. This situation has not, of course, prevented people from talking glibly about the foundations and the meaning of science without taking the precaution of acquiring the necessary experience; but their self-styled description as philosophers of science should not deceive any but themselves about the true status of their verbal exercises.

History of science, on the other hand, has a vital part to play in epistemological research: for no understanding of the sources of human knowledge can be complete without a reliable record of the actual evolution of scientific ideas. It has also to struggle with methodical difficulties; but it is successfully shedding the infantile impediments of chauvinism, hero worship, silly anecdote-mongering, and reaching the mature sophistication that will allow it to fulfil its task responsibly. To associate it, at this critical stage, with the worst dilettantism of our time is—the purest of intentions notwithstanding—to disserve rather than to help it. Indeed, to illustrate my point I have only to take the first number of this journal. It consists of two long essays: the first, by J. E. McGuire, on "Atoms and the Analogy of Nature", is an able, well documented and well argued, historical study of a significant problem of epistemology debated in Newton's time—history of science at its best; the other, entitled "In Defence of Classical Physics", is another of Professor P. K. Feyerabend's extravaganza—philosophy of science, so called, at its worst: bombastic, pointless chatter about dimly perceived issues long since settled by the physicists, and completely out of touch with the serious problems they are actually facing. If this first number is representative of the editors' policy, one must fear that one half of the journal will just be dead weight needlessly appended to its valuable historical content.

Fortunately, the second issue goes some way to temper such apprehensions: its contents—including papers of great interest and substantial essay reviews—are entirely of historical character and of high standard. Let us hope, therefore, that the historians will keep the philosophers under control, and all will be well. L. ROSENFELD

VARIABLE STARS

The Dwarf Novae

By John S. Glasby. Pp. 293. (Constable: London, August 1970.) 60*s*.

MR GLASBY is director of the Variable Star Section of the British Astronomical Association; he is an able and indefatigable observer of variable stars in general and of dwarf novae in particular. His new book is an exhaustive review of what is known, or has been thought, about the dwarf novae, and contains extensive bibliographies; it consists, in approximately equal proportions, of a synthesis of published papers and a description of the individual stars.

In the descriptive section, the author is on his own ground—indeed, many of the illustrative light curves are drawn from his own observations. But he is less at home in reviewing the observational and interpretative attempts of professional astronomers. There are about half a dozen important mistakes: for instance, evolving stars are said to have begun hydrogen burning in their centres (p. 84), and doppler shifts are not expected unless the motion is directly in the line of sight (pp. 85, 234). The author also forgets for whom he is writing: thus, he explains stellar magnitudes at the outset, but later writes gaily about the Hertzsprung–Russell (or Russell–Hertzsprung, as he insists on calling it) diagram, break-off points, zero-age main sequences, Kelvin time-scales, and Markov chains, all without previous explanation. There are also several careless mistakes. Not only is there a considerable number of inconsistencies in the text, and discrepancies between the text and several of the diagrams, but the values quoted on different pages for such quantities as orbital periods, spectral types and radial velocities sometimes conflict. The Crossley reflector, already credited with an aperture of 80 inches on page 213, grows still further by page 243.

In view of the occurrence of short-period light variations in at least some of the dwarf novae, it is a little surprising that Glasby makes no reference to any visual observations of such changes; and none of the visual light curves indicates any—not even that of U Gem, a system showing conspicuous eclipses when at minimum light. Furthermore, the number of observations used in plotting the magnitude variations seems, in many cases, to have been scarcely greater than the number of inflexions drawn in the light curves: it would have been better if Glasby had plotted the actual observations rather than just the curves.

In spite of careless grammatical mistakes and poor punctuation, the volume makes a substantial, if indigestible, meal for those interested in dwarf novae; it can be recommended as an introductory review, and will be of special interest to the amateur observers upon whose work our knowledge of the dwarf novae so largely depends.

ROGER GRIFFIN

SILICATE WEATHERING

Chemical Weathering of the Silicate Minerals

By F. C. Loughnan. Pp. x + 154. (Elsevier: New York and Barking, Essex, 1969.) 100*s*.

LOUGHNAN has written a book that will be welcomed by students of geology, geochemistry, soil sciences and various other related disciplines. There is really no other book like it in the field of chemical weathering of silicates, and in the present period of public concern about ecology, this volume ought to provide the needed quantitative information about the mechanisms of weathering of rocks and the formation of soil. In many ways, the present volume is comparable with W. D. Keller's *The Principles of Chemical Weathering* (1957), but although Keller's work had the same central theme, it was more a collection of lecture notes than a proper textbook. Loughnan has indeed written a cohesive textbook, perhaps more suitable for research students than for final year undergraduates.

The book has six chapters. The second chapter, on the structures and properties of the primary and secondary minerals, includes some basic, but selective, crystallographic information which will be appreciated by students and teachers alike. The third chapter, on the chemistry of weathering, very successfully proves that the basic laws of chemical equilibria can indeed explain the observed chemical weathering phenomena. The fifth and sixth chapters abound with many examples, quite a few from Loughnan's own well-known work in this field.

The references are well selected, but most are pre-1966. In the age of sea floor spreading, it is a pity that chemical weathering under specifically oceanic conditions is not discussed. SUBIR BANERJEE

BASIC PALAEOMAGNETISM

History of the Earth's Magnetic Field

By David W. Strangway. (Earth and Planetary Science Series.) Pp. viii + 168. (McGraw-Hill: New York and Maidenhead, August 1970.) 67*s*.

THERE are many differing views on the precise nature of the principle of uniformitarianism but, philosophy apart, the fact remains that man's success in extending his knowledge of the Earth backward in time for millions of years is one of his most remarkable achievements. From the magnificent intellectual edifice constructed by the great nineteenth-century geologists to the revised vision of the Earth embodied in the new global tectonics, man has steadfastly refused to be bound intellectually by the limitations imposed by his geologically infinitesimal life span.

And of all the properties of the Earth which have been traced through geological time, the most significant and important is undoubtedly the geomagnetic field as recorded in the Earth's rocks, for our whole modern view of the Earth as an internally dynamic body ultimately stems from the establishment of continental drift as a viable hypothesis, largely as a result of palaeomagnetic study. Naturally enough perhaps, the conceptual end results of palaeomagnetism—continental drift itself, sea floor spreading, geomagnetic dynamos, and the like—have attracted more than their fair share of attention from "popular" writers. On the other hand, palaeomagnetism *per se*, the foundation of the modern view, has received almost none.

To redress this imbalance somewhat, Strangway has written this introductory book which covers, in a simplified yet coherent and eminently readable way, all of the topics one would expect to encounter in a book on basic palaeomagnetism and rock magnetism. In reading it I could not help but reflect, somewhat sadly, that North America has a definite edge over Europe when it comes to producing books at this level. It is, I suspect, largely a question of public need. With more than two thousand universities and colleges, a system of higher education which tends to the non-specialist, a high school situation in which one in three seniors studies Earth science, and a noticeably more scientifically literate population, there is a large market for this sort of book in the United States. It will sell proportionately far less in Britain—which is a pity, and certainly no reflexion on its quality nor on the achievement of its author. PETER J. SMITH

STATISTICAL MECHANICS

Elementary Statistical Mechanics

By G. A. P. Wyllie. (Hutchinson University Library.) Pp. vii + 148. (Hutchinson: London, June 1970.) 35*s* boards; 15*s* paper.

THIS book is a simplified version of a lecture course, delivered over several years, by the author at the University of Glasgow. The standpoint of E. T. Jaynes, regarding the quantitative measure of information, is used to establish the canonical distribution as the least informative description of a system whose exact constitution and average energy are prescribed. The book is elementary in the sense that it requires only a knowledge of mathematics at first year university level and an elementary knowledge of quantum mechanics.

A large range of physical problems is covered which some readers may welcome. But I would have welcomed more time spent on the fundamental theory even at the expense of a restriction of the applications. A student reading the book, with no previous knowledge of the subject, will probably find it difficult to decide which is the basic theory. It was particularly irksome to find that there are no equations or subsections in the book numbered, the latter even being difficult to find on the page. There are some problems scattered throughout the text but no collections of problems at the ends of the chapters.

J. E. HEBBORN

SPECIALIZED CRUSTACEANS

The Taxonomy, Morphology and Ecology of Recent Ostracoda

Edited by John W. Neale. Pp. ix + 553. (Oliver and Boyd: Edinburgh, 1969.) 220*s*.

DURING the latter part of the nineteenth century and the early part of this century, the Ostracoda shared in the great outburst of attention that was paid to the world fauna. Then interest flagged. To the geologist, partly as a result of the economic pressures on stratigraphic studies, fossil forms continued to be studied intensively, but to the zoologist the stigma of "too small and highly specialized" became attached. Of recent years there has been a resurgence of interest. The palaeontologist, particularly in relation to palaeoecology, has ventured into the fields of functional morphology and ecology of recent ostracods, while a number of zoologists, though starting from different concepts, have converged onto the same interests. This renewed interest among workers scattered over the world has stimulated international meetings, and this volume constitutes the papers presented at a very successful symposium held at Hull during July 1967.

The title of the book gives an indication of the range of papers, which extend from such fields as numerical taxonomy through detailed morphological studies to distributional ecology. The editor has not only achieved a logical grouping of the papers but has also added clear and valuable summaries of the discussions which took place at the meetings and a most helpful general index as well as a separate index to genera and species. The papers are not all in English, but one would not expect this with the publication of an international symposium which one hopes will have an international circulation.

Naturally the standards of the papers vary, but are in general high. To criticize the volume is to criticize the work of experts, and it must always be remembered that the approach of the palaeontologist differs from that of the zoologist so that it is always possible for the one to criticize the work of the other. It does seem a pity, however, that no papers are included on some of the recent work on marine planktonic groups, though no doubt this is a reflexion of the interests of those present at the meeting. As Kilenyi points out, the approach to his paper on ostracod distribution tends to be more palaeoecological than ecological and this is also true of other ecological papers included. Fortunately for the zoologist there are exceptions to this as, for example, the work of Whatley and Wall (southern Irish Sea) and Williams (Anglesey coast) where, among other factors, the authors establish specific association with species of algae. A zoologist may find the work of a geologist lacking where Adamzek only uses *Cyclastrope hendersoni* as a standard for the carapace of a filter feeding ostracod and ignores the other Asteropidae, let alone the Cytherellidae. On the other hand, the geologist may wonder what use Rome's exquisite work on the caudal furka is when he will not find traces of this structure in his fossils.

This volume is in no way a textbook, but to the newcomer to the group it forms a guide to present trends in research, and to the general zoologist or palaeontologist it shows the growing relationship of studies of this group to other fields. Above all, the specialist ostracodologist will find it a unique source of a large number of important papers on new developments in his subject. E. J. ILES

PEDIGREE OF THE VERTEBRATES

Cytology and Evolution

By E. N. Willmer. Second edition. Pp. x + 649. (Academic: New York and London, June 1970.) 257*s*.

THIS book, welcomed in its first edition as a scholarly treatment of a field into which few had ventured, now reappears under the same title but with its range substantially modified. It still opens with an account of the basic cell types revealed by tissue culture, and of their possible relation to the course of early metazoan evolution. In the second half, however, there is now a thorough

redevelopment of Hubrecht's view that vertebrates evolved from nemertines; a view "more or less ridiculed by zoologists", as Willmer challengingly admits.

He presents the nemertine case with the expected wealth of detail, but weakens it by not subjecting it to a sufficiently rigorous analysis and by introducing a substantial element of special pleading. Moreover, when an argument is deliberately based primarily upon considerations of cellular organization, it is difficult to give proper weight to other aspects of animal design and evolution. Thus, the widely held view that vertebrates arose from an echinoderm/chordate (deuterostome) line is summarily dismissed, without benefit of discussion, on the ground that it depends on "somewhat unlikely reasoning" (as though no such charge could lie against the nemertine theory), while the suggested relationship of nemertines to protochordates is discussed with reference to the Enteropneusta, but with no mention of the Pterobranchia. Yet the sessile habit and ciliary feeding of the latter are very relevant, especially in conjunction with the presence of a temporary post-anal tail in the larva of *Saccoglossus horsti*.

The argument runs into no less trouble when histological detail is being considered. Willmer finds it curious that the principal lateral nerves of nemertines are not present in enteropneusts, but it would surely be surprising if they were, considering that the intraepidermal nerve net of the latter is more primitive in organization than the centralized system of flatworms. And the rigours of zoological analysis are wholly swept aside with the suggestion that the insulin-secreting follicles of Langerhans of the lamprey larva may be vestigial nephridia; a thought (admittedly subsidiary to the principal argument) evoked in the author by reflexions on the existence of enteronephridia in some highly specialized earthworms.

Willmer has designedly produced an "unorthodox and unusual" book, intended to provoke interest and investigation and not to be a comprehensive text. It certainly transmits his enthusiasm to his readers, and will give zoologists lively material for many an honours seminar.

E. J. W. Barrington

ASPECTS OF REPTILES

Traité de Zoologie

Anatomie, Systématique, Biologie. Publié sous la direction de Pierre P. Grassé. Tome XIV: Reptiles, fascicule 2: Caractères Généraux et Anatomie. Pp. 680+xxxii. (Masson: Paris, 1970.) 225 francs.

For many years there were very few available texts making comparative surveys of aspects of reptilian biology, and several of these were rather old. This, the first of two reptile "fascicules" in Grassé's *Traité*, is the latest of several works to appear in the past few years. It is nevertheless immediately welcome, for it covers aspects for which there is no other recent survey available.

The title page indicates that the volume is on anatomy, but there are chapters on locomotion and snake venom and in other chapters there are many welcome references to biochemistry and physiology. Otherwise, skin, skeleton, musculature, venom apparatus, nervous system, sense organs, blood and vascular system, respiratory and alimentary systems are covered. Several of the chapters are by persons who have carried out research in the areas concerned and these have many original figures. Most of the chapters, however, are compilations from the literature and have borrowed illustrations, but are no less useful as sources of information on that account. As elsewhere in the *Traité*, all the illustrations are redrawn giving a pleasing uniformity of style. This sometimes has drawbacks, however; Phisalix's figure of the skull of *Coluber hippocrepis* (Fig. 92) has some inaccuracies, bears an out of date name, and does not clearly illustrate some of the well made points in the text which it accompanies.

Few of the references are later than 1966; this is, no doubt, an indication of the time required to assemble and edit twenty-one contributions. The chapter on the eye has some lapses, perhaps because of the death of the author before completion; I was nevertheless astonished to find no reference to the books by Franz, Walls or Rochon-Duvigneaud (the author of the chapter!), all published more than 25 years ago.

In several respects the book should have been more carefully edited. I was rather frequently irritated at being unable to find in the bibliography a work cited in the text. Lapses in the spelling of the names of authors and reptiles are so common that anybody using the work as a source of information would be well advised to check these. For example, a snake found in France is variously cited and indexed as *Natrix maura* and *Tropidonotus viperinus*, almost side by side on p. 252; there are three spellings of *Thelotornis* in the text, two repeated in the index and none of them is correct. *Feylinia* is assigned to the family Dibamidae on pp. 415 and 417, a placement which I have not seen before.

In general, the five principal groups of living reptiles are characterized and compared, and variations at family and lower levels are indicated but not discussed. The book gives a sound introduction to a wide range of topics. Every institution in which there is an interest in reptiles should have a copy on the library shelf—at more than 6*d* per page I doubt that it will find its way onto many private bookshelves.

Garth Underwood

ANIMAL GROWTH HORMONES

Control of Growth and Metamorphosis

Part 2 of *Animal Hormones: A Comparative Survey*. By P. M. Jenkin. (International Series of Monographs in Pure and Applied Biology: Zoology Division, Vol. 47.) Pp. xviii+383. (Pergamon: Oxford and New York, August 1970.) 100*s*; $13.50.

In 1962 Penelope Jenkin published the first part of her "Comparative Survey of Animal Hormones"; but she was overtaken by the growth of published knowledge and was able to cover only "Kinetic and Metabolic Hormones". Now, after eight years, part two has appeared. This was intended to deal with all morphogenetic hormones—but in the event has had to be limited to hormones concerned in the control of growth, moulting and metamorphosis. But it forms a self-contained whole. Although the author assumes familiarity with part one, and provides many cross-references to this, it is none the less a valuable book in its own right. As in the earlier volume, the author has added enormously to her labours by comparing the hormonal regulation, for every topic that comes up for consideration, throughout representatives of the entire animal kingdom, so far as that is known. This has taxed the author; and it taxes the reader—but it was well worth doing.

In the description of the moulting of arthropods much weight is attached to "apolysis", the "freeing of the epidermal cells from the old exoskeleton", as the crisis of moulting. Apolysis is a useful self-explanatory term—so long as it is borne in mind that it is an abstraction. The first sign of moulting is the active synthesis of nucleoproteins in the epidermal cells which usually, but not always, leads on to mitosis. So far as a given cell is concerned, mitosis necessitates separation from the cuticle; but mitosis begins at different times in different regions of the epidermis. Separation is such a gradual process that it is impossible in practice to pin down the time of apolysis. And yet the "actual instar" is here defined as the form of the arthropod over the period from one apolysis to the

next. While it is still covered by the old cuticle it is said to be "pharate" or masked. If one could actually observe the "actual instar" it would show very striking changes in its form, as in an embryo, starting like a soft and folded leaf within a bud and assuming its proper form and appearance only at ecdysis when the old skin is shed. It is a little difficult to understand why the vague time point of apolysis along this continuous process of growth should be selected for separating the instars, which by definition have definitive forms.

But that is a detail. The book as a whole is a product of thought by the author and it demands thought from the reader; it is to be recommended on these grounds both to the advanced student and to the reflective investigator.

V. B. WIGGLESWORTH

MORPHOGENESIS FOR STUDENTS

Tissue Interactions during Organogenesis

By Etienne Wolff. (Documents on Biology, 1.) Pp. xiv + 225. (Gordon and Breach: New York and London, July 1970.) 175*s*, $21.00 boards; 62*s* 6*d*, $7.50 paper.

THIS is an interesting attempt by Etienne Wolff and a group of his past and present colleagues to bring a much needed comfort to the advanced undergraduate student. They have aimed to bridge the gap between textbook and the flood of original literature by a series of didactically written reviews by workers experienced in the subjects on which they write.

An opening survey of problems of determination by Wolff stresses the recent brilliant work on transdetermination in insects from Hadorn's laboratory. This, together with an account of the value of immunochemistry in studies of induction and cytodifferentiation (by Y. Croisille), gives a more general setting to the other seven reviews which all deal with the morphogenesis of avian organs. Those chosen are kidney (by Wolff), liver (le Douarin), skin (Sengel), neurocranium (Benoit and Schowing), lung and stomach (Dameron, Sigot, Marin). The emphasis is, in most cases, upon the *in vitro* analysis of tissue interactions which these workers have exploited so profitably.

The authors have succeeded admirably in presenting their material in a clear and simple way. The manner is excellent, but the matter, though important, is a little restricted. Even if bird material alone were considered for inclusion, the analysis of morphogenesis in limb and eye would have had worthwhile lessons to offer.

D. R. NEWTH

NEUROHYPOPHYSIAL HORMONES

Pharmacology of the Endocrine System and Related Drugs

The Neurohypophysis. (International Encyclopaedia of Pharmacology and Therapeutics, Vol. 1, Section 41.) Pp. viii + 486. (Pergamon Oxford, London and New York, May 1970.) 120*s*; $16.

THIS excellent volume is a valuable addition to the ambitious series published by Pergamon Press, sections of which are gradually appearing.

When applied to the neurohypophysis, the title of the series is misleading. Encyclopaedia it may be; international—judging from the list of its contributors—it certainly is; but pharmacology is only part of its business. It is true that there are chapters on the chemistry of the neurohypophysial hormones, and on the relationship between structure and action, topics which might be termed pharmacological; it is true too that about one third of the contributors work in departments of pharmacology. But the volume contains, in addition, full accounts of the comparative physiology, storage and release, biosynthesis and fate in the body of the hormones it treats. As would be expected, therapeutics is represented only by two short articles on clinical pharmacology at the end.

It must be difficult, in writing such a book, to know what to leave out. It would be impossible to include every reference and mention every possibility without greatly extending its compass. The contributors have managed to select wisely and to build their arguments round the important facts rather than attempting to include everything. The full lists of reviews at the end of each chapter direct the reader to more comprehensive accounts. As would be expected, there is some overlap now and then between chapters, but the editors, themselves distinguished workers in the field, have managed to produce a section which is well proportioned, all of whose parts fit reasonably well together.

One trouble about a book like this is that it is bound to be out of date by the time it is published. In rapidly expanding fields, large numbers of papers are written and important findings are reported almost weekly, while the slow businesses of writing, editing, printing and binding go remorselessly on. In one chapter, for instance on a subject where progress is fast, out of some 250 references there are only five as recent as 1968, and one of these is cited as being "in press". The reader will not find here the latest developments. But he will find intelligently written accounts of the basic physiology and pharmacology of the subject.

This is not a book for the physician, but it will be of great value to every teacher or experimental worker in endocrinology who can afford it.

J. M. WALKER

THYROID ACTIVITY

The Thyroid and the Autonomic Nervous System

By David Leak. Pp. ix + 148. (Heinemann (Medical): London, July 1970.) 35*s*.

THE thyroid is currently a biochemist's gland, and there is something of a gap in knowledge between the synthesis, output and transport of its hormones to the cell receptors, and the final expressions of the gland's level of activity under normal or clinical conditions.

Dr Leak has made a careful and thoroughly critical review of the way—or of one of the ways—in which the thyroid hormones may produce their effects on body function. The evidence is difficult and often conflicting—not least, as he emphasizes, because of differences in response in the various species in which these problems have been studied, and doubts as to their relevance to the situation in man. In addition, it has often been assumed that the reduction of an effect by adrenergic blockage implies that the effect is mediated through the autonomic system whereas the reduction may result simply from this system independently, causing changes which are additive to the effect examined; and Leak seems to make this assumption himself when discussing the ocular effects of thyroid overactivity.

It is unfortunate also that he refers to the levels of thyroid stimulating hormone as being usually increased in hyperthyroidism relying on a reference based on work in 1957 before the long acting thyroid stimulator was recognized. These points, and a curious predilection for treatment with D-thyroxine, are, however, minor limitations to a useful review which defines clearly the evidence for an increased sensitivity to, rather than production of catecholamines in conditions of increased thyroid activity. Even if it does not solve all the problems, this book will help considerably in clearing the ground for their closer study.

E. ERIC POCHIN

Correspondence

Medvedev's Complaint

SIR,—The excerpt from Zhores (that is, Jaurès) Medvedev's book (*Nature*, **227**, 1197; 1970) strikes me as subtle communist propaganda. When I read in your editorial on page 1177 that a Russian scientist was not permitted to travel abroad, lost his job and was for some weeks kept in a psychiatric institution, I was duly incensed. Unfortunately, my indignation continued ebbing with every line of Medvedev's confession.

A few months ago, I was invited to give a lecture in France. I applied to the National Science Foundation for travel funds. After a long delay, my request was turned down. I did not complain to my Congressman, or to the senators from our state, or to the Head of the Foundation, or to the French Ambassador in Washington, or the US Ambassador in Paris, and so on. When the time of his lecture came, Medvedev read the latter to his friends; I did not. Why did I acquiesce in my defeat? Because the official who refused my application had, under the existing law, full right to reject my plea, as had the official who kept Professor Medvedev back.

It would be easy to clarify my position but, naturally, I do not want anyone to suffer from too much light.

Yours faithfully,

J. J. BIKERMAN

15810 Van Aken Boulevard,
Shaker Heights,
Ohio 44120.

Food for Thought

SIR,—The rapidly growing science of nutrition is at a stage at which accurately characterized standard reference materials are urgently needed to provide a common basis for the great number and variety of studies that are being conducted in all parts of the world. Dietary control in animal experiments[1] is only one of many applications to be made of such materials.

About twenty substances pertinent to work in nutrition are included among the standard substances issued by the World Health Organization and by various governmental and other agencies in different countries. These currently available reference materials are issued in specimens in the milligram-to-gram range; such quantities suffice for purposes of identification and analytical control, but are too small for many applications in nutrition where gram-to-kilogram quantities are required. Furthermore, the present materials are usually certified for only a small number of constituents, whereas for many critical nutritional studies it is essential to know the content of many residual and trace constituents, particularly trace elements.

Immediate application can be foreseen for about eighty-five standard reference materials in the following categories: (1) essential nutrients in pure form; (2) basic food products of both natural and synthetic origin; (3) actual or simulated plant and animal tissues and fluids; (4) products involved in or resulting from plant or animal metabolism.

The expense of producing so many standard reference materials in large specimens would be so great that the operation would be feasible only if undertaken on a world-wide basis. For this reason I propose that concerned individuals, scientific societies, and standardizing agencies join in requesting the logical international agencies—the World Health Organization and the Food and Agriculture Organization—jointly to undertake a programme of International Standard Reference Materials. I shall be glad to furnish specific suggestions regarding such a programme to anyone who may be interested.

Yours faithfully,

ARCHIBALD T. MCPHERSON

4005 Cleveland Street,
Kensington, Maryland 20795.

[1] *Nutrition Reviews*, 27, 299 (October 1969).

Misleading Units

SIR,—In a review of mycotoxins (*Economie et Médecine Animales*, **11**, 75; 1970) C. W. Hesseltine gives concentrations of mycotoxins "par billion". I am convinced that they should be written "par milliard". For example, the sentence: "La limite de sensibilité des analyses utilisées s'élevait à 2–5 par billion d'aflatoxine", was almost certainly understood by Hesseltine in the United States as 2–5 per 10^9, whereas in most of the world this French sentence would be read as 2–5 per 10^{12}. Therefore I would like to appeal to toxicologists, and in particular to American colleagues, not to use the misleading term parts per billion (p.p.b.). It is possible to express the same concentrations in parts per million (p.p.m.), which is equally understandable to toxicologists from all over the world.

Yours faithfully,

TEODOR JUSZKIEWICZ

Department of Pharmacology and Toxicology,
Veterinary Research Institute,
Puławy, Poland.

Radiation Hazards

SIR,—Baines (*Nature*, **227**, 869; 1970) quotes some of our data for Australia and New Guinea to support his views on radiation hazards to South Pacific communities. The quotation, however, is hardly relevant. In fact the results of our measurements on fallout from nuclear weapons tests in French Polynesia—the source of Baines's concern—have been assessed by the Australian National Radiation Advisory Committee and found to have no significance as a hazard to health of the Australian community[1].

Measurements on shorter-lived fission products in fallout over Australia and New Guinea[2] following the French nuclear weapons tests of 1966, 1967 and 1968 indicate that:

(1) the highest γ-radiation dose to the whole body estimated from measured fallout from all tests in the three series was 3·3 mrad;

(2) the highest radiation dose to thyroid for young children due to ^{131}I in milk consumed during any 12 month period between 1966 and 1968 was estimated at 226 mrad.

As we pointed out when reporting the measurements, these radiation doses are not only the highest but are also upper limits. Thus weathering, shielding and other effects would reduce whole body doses by a factor of 3 to 5, while decay of ^{131}I before the milk is consumed, and other factors, would result in substantially lower thyroid doses. In any case, these upper limits on estimated dose are far less than dose levels which would be expected to be of biological significance[3]. Although measurements are still in progress on fallout in Australia from the 1970 series of tests in Polynesia, the data accumulated up to the present indicate

levels of fallout which are lower than from previous series.

Reference to widely reported inventories of ^{90}Sr in the stratosphere of the Southern Hemisphere[4,5], and to measurements on monthly deposition of ^{90}Sr in Australia[6], indicates that:

(1) stratospheric burden of ^{90}Sr in the southern hemisphere decreased from some 1,200 kCi in 1963 to 50 kCi in mid-1968, with annual deposit of ^{90}Sr in Australia falling from 2·1 mCi/km² in 1964 to 0·4 mCi/km² in 1968;

(2) high yield nuclear weapons tests in Polynesia in August–September 1968 added some 130 kCi to a total stratospheric burden of 180 kCi ^{90}Sr and an annual deposit of ^{90}Sr in Australia of 0·6 mCi/km² in 1969.

As already reported[6,7], the 1968 test series in Polynesia contributed a substantial proportion of the total monthly ^{90}Sr deposit in Australia in late 1968 and early 1969 but the magnitude of the total deposit remained small.

When planning the Australian programmes for monitoring fallout from tests in Polynesia, consideration was given to possible problems of contamination of migratory fish and birds, which may enter the Australian diet. Evidence available to us on the migration habits of tuna caught in Australian waters suggested that only yellowfin, comprising less than 1 per cent of the total catch, might originate from the Tuamotu Archipelago. Measurements on yellowfin taken in Australian waters showed no change in concentration of fission products following tests series in Polynesia.

Yours faithfully,

W. J. Gibbs
J. R. Moroney
D. J. Stevens
E. W. Titterton

Australian Atomic Weapons Tests Safety Committee,
PO Box 50, Ascot Vale, Victoria 3032, Australia.

[1] Australian National Radiation Advisory Committee, *Report to the Prime Minister, March 1969, on Biological Aspects of Fallout in Australia from French Nuclear Weapons Explosions in the Pacific (July–September 1968).*
[2] Gibbs, W. J., *et al., Austral. J. Sci.*, **29**, 407 (1967); **30**, 217 (1967); **31**, 383 (1969).
[3] Australian National Radiation Advisory Committee, *Report to the Prime Minister, November 1965.*
[4] Telegadas, K., *USAEC Report HASL-184*, 153 (January 1968).
[5] Krey, P. W., *et al., USAEC Report HASL-210*, 145 (July 1969).
[6] Gibbs, W. J., *et al., Austral. J. Sci.*, **32**, 238 (1969).
[7] Cambray, R. S., *et al., UKAEA Report AERE-R 6212* (November 1969).

Obituary

Dr E. H. Rodd

Ernest Harry Rodd died on July 22, 1970. He was born in 1888, and educated at Christ's Hospital and the Central Technical College, South Kensington, which he entered in 1906 to begin his close association with Professor H. E. Armstrong until the latter's death in 1939. His main research work here, on crystal morphology in the benzene series, was of topical interest in connexion with the then recently published views of Barlow and Pope on valency volumes. On the final closure of the old Central laboratory and the dispersal of Armstrong's research students, Rodd worked for a while at the National Physical Laboratory before joining, in 1917, the research staff at Levinstein Ltd, Blackley, Manchester, later to become part of the British Dyestuffs Corporation and eventually the headquarters of the Dyestuffs Division of Imperial Chemical Industries Ltd. Here he took a full share in the work of the rapidly expanding research department, as emphasis passed from the manufacture of known products to the invention and eventual manufacture of new ones.

In order to promote closer relations between scientists working in the Dyestuffs Division and those working in the universities, in 1929 the Dyestuffs Group Research Committee was set up, the academic members being Sir Jocelyn Thorpe, Sir Robert Robinson and Sir Ian Heilbron, with Rodd as secretary. As the work of this and other liaison committees grew, Rodd became fully employed as academic liaison officer, a post he filled with distinction until he retired more than twenty years later. During this period he became well known in the laboratories of practically all the universities and technical colleges in the UK and several abroad.

After his retirement his name became familiar to a world-wide circle of chemists as the creator and editor of *The Chemistry of Carbon Compounds*, the ten volumes of which were published between 1951 and 1962, an outstanding achievement after a normal lifetime's work in industry. He was particularly pleased when it was decided to publish a revised edition, and right up to the day of his death he took a very active and constructive interest in its progress.

Announcements

University News

Dr N. Uri, a member of the Explosives Research and Development Establishment, Waltham Abbey, and **Professor E. M. Evans,** of British Resin Products Limited, have been appointed visiting professors in the Department of Chemistry of the **City University.**

Professor Malcolm B. Wilkins, University of Nottingham, has been appointed Regius professor of botany in the **University of Glasgow.**

Professor Olaf Rundquist, on sabbatical leave from Hamline University, St Paul, Minnesota, and **Professor J. G. Pike,** professor of mechanical engineering at the Royal Military College of Canada, have been appointed visiting professors at the **Heriot-Watt University.**

Professor M. S. Longuet-Higgins has been appointed visiting professor in the Department of Mathematics, **University of Surrey.**

Dr Paul S. J. Spencer, University of Aston in Birmingham, has been appointed professor of applied pharmacology in the Welsh School of Pharmacy, **University of Wales Institute of Science and Technology.**

Appointments

The following appointments have been made to the **MRC advisory boards:** *Biological Research Board,* **Professor P. M. B. Walker,** already a member of the board, will succeed **Professor R. A. Gregory** as chairman; **Professor C. I. Howarth** and **Professor R. R. Porter** have been appointed. *Clinical Research Board,* **Professor Charlotte M. Anderson, Professor Richard Doll** and **Professor O. L. Wade** have been appointed. *Tropical Medicine Research Board,* **Dr L. G. Goodwin** and **Professor J. N. Morris** have been appointed.

Miscellaneous

The **Alfred Egerton gold medal** for 1970 has been awarded to **Professor A. R. Ubbelohde** for his contributions in the field of combustion.

The **George E. Davis medal** of the Institution of Chemical Engineers has been awarded to **Mr F. E. Ireland,** chief alkali inspector.

A prize of about 500,000 Belgian francs will be awarded in 1973 by the **Fondation de Physiopathologie Professeur Lucien Dautrebande,** for outstanding work in human or animal physiopathology. Further information can be obtained from the office of the Foundation, 35 Chaussée de Liege, 5200 Huy, Belgium.

Entries are invited for the **1971 Maccabaean prize** of 25 guineas and a bronze medal. Candidates, who must be less than 30 years old, should submit an essay of 4,000 to 6,000 words on some aspect of the history of medicine or pharmacy. Further information can be obtained from Dr F. N. L. Poynter, Wellcome Institute of the History of Medicine, 183 Euston Road, London NW1.

Nominations are invited by the **Society of Biological Psychiatry** for three awards to be given in 1971. The gold medal award will be made to a senior scientist in the field of biological psychiatry; the two other awards, of $750, will be made to workers under the age of 35, one for clinical research and one for basic research in biological psychiatry. Further information can be obtained from Dr George Winokur, Committee on Research Awards, Society of Biological Psychiatry, Department of Psychiatry, Washington University School of Medicine, Renard Hospital, 4940 Audubon Avenue, St Louis, Missouri 63110, USA.

A new fellowship programme, established through an endowment from John L. Loeb, has been inaugurated at **Harvard University** and is designed to give persons with exceptional promise for leadership in the fields of urban affairs and design an opportunity for independent study at Harvard, either on a part-time or full-time basis. The **Loeb fellows for 1970–71** are: Alexander Rodriguez, director of planning and development for Suffolk County and Brookline United Community Services; Michael A. Levett, assistant to the Secretary of the Interior; M. Daniel Richardson, jun., director, Boston Area Office, US Department of Housing and Urban Development; John Zeisel, social science editor, *Design and Environment*. Further information about the fellowships can be obtained from Professor William A. Doebele, Robinson Hall, Harvard University, Cambridge, Massachusetts 02138, USA.

ERRATUM. In the article "Regulatory Factor for the Transcription of the Ribosomal Genes in Amphibian Oocytes" by Marco Crippa (*Nature*, **227**, 1138; 1970), the end of the first sentence should read "synthesis changes greatly during oogenesis".

ERRATUM. In the article "Numerical Earth" (*Nature*, **227**, 1090; 1970), reference was made to a paper on the form of radiation from earthquake faults. This represented the joint work of R. Burridge and G. S. Halliday. R. Burridge is a research fellow of the UKAEA Blacknest.

ERRATUM. In the review "Introduction to Life" by John Paul (*Nature*, **228**, 187; 1970), line 18 in the first paragraph should read "little more than a list of known hormones. Those on . . .".

International Meetings

October 22–24, **Thermal Pollution,** Chicago (Institute of Environmental Sciences, 940 East Northwest Highway, Mt Prospect, Illinois 60056, USA).

October 26–30, **Industrial Applications of Small Computers,** Glasgow (Birniehill Institute, East Kilbride, Glasgow, Scotland).

October 29–30, **Environmental Influences on Reproduction,** Edinburgh (Kay Crombie, Inveresk Research International, Inveresk Gate, near Edinburgh, Scotland).

November 2–4, **Caring for the Environment in the Seventies,** Llandudno (Dr T. Pritchard, Countryside in 1970 Committee for Wales, Graig Lwyd, 134 Penrhos Road, Bangor, Caernarvonshire, Wales).

November 3–6, **Technological Advances in Britain,** Rio de Janeiro (Board of Trade, 1 Victoria Street, London SW1).

November 6–8, **The Pollution of our Environment,** London (Conference on Pollution, c/o Mrs S. Robson, 31 Chester Terrace, London NW1).

November 9–13, **Technological Advances in Britain,** Buenos Aires (Board of Trade, 1 Victoria Street, London SW1).

November 16–18, **Powder Metallurgy Joint Group Annual Meeting,** Eastbourne (The Secretary, Powder Metallurgy Joint Group, 17 Belgrave Square, London SW1).

November 16–19, **Computing Techniques for Industrial Applications,** Hendon (The Registrar, Hendon College of Technology, The Burroughs, London NW4).

November 17, **The Use of Organometallic Compounds in Synthetic Organic Chemistry,** London (The Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1).

November 25, **Chromatography and Toxicology,** London (Society for Analytical Chemistry, 9–10 Savile Row, London W1X 1AF).

November 26, **Painting and Protection of Structural Steelwork,** London (Mr G. F. Goody, Construction Industry Research and Information Association, 6 Storey's Gate, London SW1).

November 27–29, **Starting One's Own Business,** Slough (Dr W. E. Duckworth, Fulmer Research Institute, Stoke Poges, Buckinghamshire).

December 1–3, **Production Engineering Research Association of Great Britain Conference and Exhibition,** Melton Mowbray (Conference Department, PERA, Melton Mowbray, Leicestershire LE13 OPB).

December 2–15, **Synoptic Analysis and Forecasting in the Tropics of Asia and the South-West Pacific,** Singapore (World Meteorological Association Secretariat, Geneva, Switzerland).

December 7–12, **Plant Growth Substances,** Canberra (Dr D. J. Carr, Research School of Biological Sciences, Australian National University, PO Box 475, Canberra City ACT 2601, Australia).

December 14–16, **Structural History of the Bay of Biscay,** Rueil-Malmaison (Mr Montadert, Division Géologie, Institut Français du Petrole, 1 et 4 Avenue de Bois-Préau, 92 Rueil-Malmaison, France).

January 4–6, 1971, **The Teaching of Mathematics in Universities and Polytechnics,** London (Registrar, Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea SS1 2JY).

January 4–7, 1971, **Fluid Power,** Guildford (H. Stephens, 2 IFPS, British Hydromechanics Research Association, Cranfield, Bedford).

British Diary

Monday, October 19

Development of Immersed Tunnelling in Europe (5.30 p.m.) Mr Alex Christiani, Institution of Civil Engineers, at Great George Street, London SW1.

Karl Marx and the Withering Away of Social Science (5.30 p.m.) Mr G. Cohen, British Society for the Philosophy of Science, in the Joint Staff Common Room, University College London, Gower Street, London WC1.

The Work of the ALRC (2.15 p.m.) Dr P. T. Haskell and Mr R. D. MacCuaig, Society of Chemical Industry, Pesticides Group, at the Anti-Locust Research Centre, College House, Wrights Lane, London W8.

Physiological and Clinical Results from Cardiac Pacing (4 p.m.) Dr Edgar Sowton, University of London, at the Royal Postgraduate Medical School, DuCane Road, London W12.

Tuesday, October 20

Acoustics as a Diagnostic Tool (conference) Institution of Mechanical Engineers, Nuclear Energy Group, at 1 Birdcage Walk, London SW1.

Electricity Distribution (two-day conference) Institution of Electrical Engineers, in the Assembly Rooms, Edinburgh.

Energy (5.30 p.m.) Professor R. King, Royal Institution, at 21 Albemarle Street, London W1. (Lecture for Sixth Form pupils from Schools in London and the Home Counties. To be repeated on October 21, 27 and 28.)

Soil Survey in Agriculture (10 a.m.) Society of Chemical Industry, Agriculture Group, at 14 Belgrave Square, London SW1.

The Control of Ageing in Man (1.20 p.m.) Dr A. Comfort, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Wednesday, October 21

A Return to William Smith (5 p.m.) Professor W. S. Fyfe, FRS, Geological Society, at the Royal Society, 6 Carlton House Terrace, London SW1 (25th William Smith Lecture).

Analysis of Materials for Paint and Ink (7.30 p.m.) speaker from ICI, Oil and Colour Chemists' Association, at the Carlton Hotel, North Bridge, Edinburgh.

Changes in Pharmaceutical Analysis (6.30 p.m.) Dr E. F. Hersant, Society for Analytical Chemistry, in the Renold Building, University of Manchester Institute of Science and Technology, Sackville Street, Manchester.

OR Man Looks at IR: Operational Research and Information Retrieval (5.30 p.m.) Mr W. T. Bane, Aslib and the Institute of Information Scientists, at Imperial College, Exhibition Road, London SW7.

The Role and Control of Human Adrenal Androgens (2 p.m.) Professor I. H. Mills, University of London, at the Royal Postgraduate Medical School, DuCane Road, London W12.

To Cover the Country with Good Schools—a Century's Effort (5.30 p.m.) Professor H. C. Dent, University of London, at King's College, Strand, London WC2

Thursday, October 22

Clinical Research (5.30 p.m.) Sir Harold Himsworth, University of London in the Beveridge Hall, Senate House, London WC1. (First of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Conservation and Productivity of Natural Waters (two-day symposium) Zoological Society of London, in association with the British Ecological Society, in the Meeting Room of the Zoological Society of London, Regent's Park, London NW1

Corrosion (7 p.m.) Dr T. P. Hoar, Oil and Colour Chemists' Association, at the Beech Tree Hotel, Beaconsfield, Bucks.

Organic Microchemistry and Its Applications (two-day meeting) Society for Analytical Chemistry, Microchemical Methods Group, at the College of Further Education, Salisbury, Wilts.

Perturbed Angular Correlations of Gamma Radiation in Biological Macromolecules (2.15 p.m.) Professor J. A. Baldeschwieler, University of London, at Queen Mary College, Mile End Road, London E1.

The Basis of Current Fertilizer Recommendations (2.30 p.m.) Mr L. J. Hooper, Fertilizer Society, at the Geological Society, Burlington House, Piccadilly, London W1

The Impact of Process Requirements on Future Trends for Pressure Parts (conference) Institution of Mechanical Engineers, Pressure Vessels Section of the Applied Mechanics Group, at 1 Birdcage Walk, London SW1.

Friday, October 23

Ion Cyclotron Double Resonance (2.30 p.m.) Dr J. A. Baldeschwieler, University of London, at Queen Mary College, Mile End Road, London E1.

Recent Developments in Dye Lasers and Ultra-Short Light Pulses (1 p.m.) Dr T. Morrow, Royal Institution, Photochemistry Discussion Group, at 21 Albermarle Street, London W1.

The Universe and the Arrow of Time (9 p.m.) Dr J. V. Narlikar, Royal Institution, at 21 Albemarle Street, London W1.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Scientific Proceedings of the Royal Dublin Society. Series A. Vol. 3, No. 24. Some Observations on the Assimilation of Basic Xenoliths by Acid Magma in the Galway Granite, Co. Galway, Eire. By C. W. Claxton. Pp. 247–256. 7*s*. Vol. 3, No. 25: Geology of the Duvillaun Islands. By M. D. Max, W. E. A. Phillips and P. M. Brück. Pp. 257–268+plate 10. 8*s*. Vol. 3, No. 26: Some Mineralization in Part of Northwest County Mayo, Ireland. By Michael D. Max. Pp. 269–274. 6*s*. Vol. 3, No. 27: Mainland Gneisses Southwest of Bangor in Erris, Northwest County Mayo, Ireland. By Michael D. Max. Pp. 275–292+plate 11. 10*s*. Vol. 3, No. 28: The Termon Granite and Associated Minot Intrusives, Co. Mayo, Ireland. By J. S. Sutton. Pp. 293–302. 9*s*. Series B. Vol. 2, No. 22: Aneuploids of *Brassica oleracea*. By P. L. Curran. Pp. 217–220+plate 13. 4*s*. Vol. 2, No. 23: Comparative Trials on the Effects of (2-Chloroethyl) Trimethylammonium Chloride (Chlorocholine Chloride; CCC) and N-Dimethylamino Succinamic Acid (B-Nine; Alar) on Vegetative Characteristics of Apple. By P. F. McDonnell and J. P. Edgerton. Pp. 221–232+plates 14–16. 9*s*. Vol. 2, No. 24: Donegal Herring Investigations 1967/68. By J. J. Bracken and D. R. Phillips. Pp. 233–250. 6*s*. Vol. 2, No. 25: A Study of the Utilization of (2-Chloroethyl) Trimethylammonium Chloride (Chlorocholine Chloride; CCC) Under Irish Conditions. 1: The Effect of (2-Chloroethyl) Trimethylammonium Chloride and Nitrogen on the Yield of Spring Wheat. By E. J. Gallagher. Pp. 251–262. 6*s*. (Dublin: Royal Dublin Society, 1970.) [159

Bulletin of the British Museum (Natural History). Geology. Vol. 19, No. 2: A Revision of the Brachiopod Subfamily Kingeninae Elliott. By Ellis Frederic Owen. Pp. 27–82+14 plates. 105*s*. Botany. Vol. 4, No. 6: New and Little Known Himalayan Species of *Swertia* and *Veratrilla* (Gentianaceae). By H. Smith. Pp. 237–258+plates. 22–37. 31*s*. (London: British Museum (Natural History), 1970). [159

Water Pollution Control Engineering. Pp. 137. (London: HMSO, 1970.) 14*s* (70*p*) net. [159

Other Countries

International Atomic Energy Agency, Vienna. Technical Reports Series No. 106: The Volume Reduction of Low-Activity Solid Wastes. Report of a Panel prepared by J. Pradel, in collaboration with P. J. Parsons and E. Malasek. Pp. 44. 52 schillings; 16*s* 8*d*; $2. Technical Report Series No. 112: Neutron Moisture Gauges: a Guide-Book on Theory and Practice. Pp. 95. 78 schillings; 25*s*; $3. Technical Reports Series No. 114: Training Manual on Food Irradiation Technology and Techniques. (A joint undertaking by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency.) Pp. 134. 104 schillings; 33*s* 4*d*; $4. Technical Reports Series No. 116: Bituminization of Radioactive Wastes: Review of the Present State of the Development and Industrial Application. Pp. 135. 104 schillings; 33*s* 4*d*; $4. Technical Reports Series No. 117: Environmental Isotope Data No. 2—World Survey of Isotope Concentration in Precipitation (1964–1965). Pp. xvii+402. 207 schillings; 66*s* 8*d*; $8. (Vienna: International Atomic Energy Agency; London: HMSO, 1970.) [169

National Institute of Child Health and Human Development, Bethesda, Md. Population Research. Pp. 28. (Bethesda, Md.: National Institute of Child Health and Human Development, 1970. For sale by US Government Printing Office.) $0.45. [179

Australia: Commonwealth Scientific and Industrial Research Organization. Land Research Series, No. 27: Lands of the Goroka—Mount Hagen Area, Territory of Papua and New Guinea. Comprising papers by H. A. Haantjens, J. R. McAlpine, E. Reiner, R. G. Robbins, and J. C. Saunders. Compiled by H. A. Haantjens. Pp. 159. (Melbourne: CSIRO, 1970.) [189

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NATURE Volume 228
OCTOBER 24, 1970

Science is a Trojan Horse

THE problem of South Africa touches everything and everybody. If artificial activities such as playing cricket should become entangled with *apartheid*, how can the development of science be unaffected? The survey of the present state of research and scientific education in South Africa which appears on page 310 must therefore be regarded as incomplete without some assessment of the way in which the life of a professional scientist and the character of his work must be affected by the regulations by which the present government of South Africa seeks to guide the development of its population, one sixth white, in the decades ahead. To pretend otherwise is to ask for the Moon even if it must also be acknowledged that, in a nation that has galloped a long way in the sixty years of its existence but still has a long way to go, social harmony must be peculiarly difficult to attain. So far as scientific research is concerned, however, the conduct of South Africa's affairs is by no means irrelevant. First, *apartheid*, even translated as "separate development", is bound in due course to affect the character of academic life in South Africa. Second, it will affect the efficacy of the efforts spent on scientific research. Third, it will limit, as it has already limited, the freedom with which intellectual people can conduct intellectual business. That is a sufficient reason for this commentary.

Charity requires, however, an acknowledgement of the difficulties with which the government of South Africa must contend. One of the most serious is the vivid memory of recent struggles with which a large part of the population is imbued. The Boer War was, after all, only seventy years ago, and the bitterness with which it was fought still leaves its mark on that part of the population of South Africa which is of European descent. If there are Irish brooding over Cromwell still, there is at least some reason why some Afrikaaners should still resent those who do not naturally speak their language. It must also be acknowledged that most modern governments would shrink from the difficulty of persuading a vociferous and divided European minority that the time has come to endow the much larger native population with elementary political rights. Although the government of South Africa has frequently been able to justify the policy of separate development on the almost theological grounds that people of one "nation", whatever that may mean, have a natural hankering for separate development, the sad truth is that the doctrine may well appeal to South African voters principally because it perpetuates their present dominance. In circumstances like these, it is only proper that questions should be asked not merely about the propriety of what is happening in South Africa but also about the feasibility of what is planned.

Honesty requires a plain statement that outsiders find the present conditions in South Africa as offensive as many residents think them natural. At a time when Western society is overwhelmed by the discovery that underprivilege breeds underprivilege—children from underprivileged homes do less well at school, for example, while the infant mortality rate is inversely correlated with family income—it is an assault on modern sensibility to pretend that communities already underprivileged can be sent out into the bush, sometimes literally, and there invited to take a chance on separate development. The theorists are no doubt sincere in their belief that by this chastening route, the national identities of the native communities of South Africa would be characteristically expressed, but in reality this course of action is more certain to ensure that the gap, cultural and economic, between Europeans and Africans is permanently huge. To be sure, for those who believe in miracles, there may be some way out of the simple truth that deprivation is the origin of underprivilege, but miracles are not part of the fabric of modern government.

Modern sensibility is also offended that separate development is cruel. Black men in South African urban townships are now most of all alarmed that, if they die, their wives and children will be sent to some distant and often unknown homeland, as the saying goes. Another anxiety is that the regulations of separate development, with their implicit definition of several distinctive African peoples, often impede normal personal relationships between black people who have been foolish enough, in marriage or their choice of parents, to fail to anticipate the definitions now prevalent. For the time being, luckily for everybody, native Africans seem to be less acutely conscious than they might be of the ways in which they and their families are damaged by isolation in distant suburban townships, by wage packets that are smaller than those of Europeans doing comparable work, by a school system that requires even poor families to pay up, and by the indignity of the supposition that, for the sake of a proposed urge towards separate development that flies in the face of the known flair for urbanization that most Africans possess, social intercourse between blacks and whites should be more restricted now than at any time in the past century. The question is not whether *apartheid* is moral but whether it can be indefinitely stomached.

Where does science fit in? Two things must be said. First, to the extent that the present arrangements in South Africa may limit the efficacy of scientific research, the whole world and not merely small sections of the South African population will be deprived. There is a danger that the arrangements made for non-

white education may limit the population from which bright ideas will in the long run be derived. Elsewhere than in South Africa, experience has now shown that a diversity of universities is a blessing, not a curse, at least so long as there is intercourse between them. The policy for higher education in South Africa seems paradoxically to be seeking opposing objectives—supposedly identical universities but with hardly any communication between them. Most probably the system is unworkable; otherwise, it is unwise.

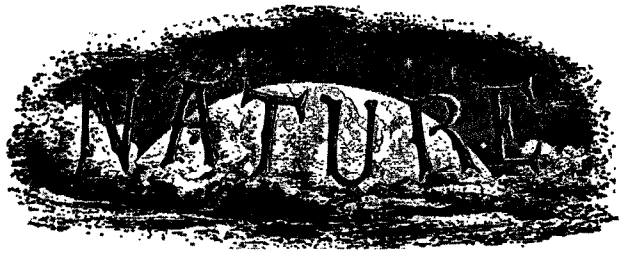
The other side of this coin is the extent to which science can especially illuminate the problems with which South Africa is confronted (and which nobody should ignore). First, it is by now apparent that many of the steps in the arguments which demonstrate the virtue of separate developments are fallacious. There is no objective basis for the assertion that native Africans seek a national identity in a community with people of closely similar geographical origins, but, rather, every sign that they will embrace urbanization as willingly as other people have done if only given half a chance. (The question whether economic development for native populations may not permanently rob them of the chance to reach some quite different social organization is an unreal fear—that should be for the natives to decide.) Equally, there is no evidence that they differ in ways that are significant from those parts of the South African population now registered to vote, even though they may differ in ways that are culturally important and valuable. Moreover, there is no weight of experience to support the rigorous puritanism of the belief that self-improvement is the only improvement that is worthwhile—rather the opposite. This is why one component in the long-term solution of South Africa's problems is an academic and scientific community vigorous enough to do battle for these simple truths.

What other conditions must be met? It would be absurd for anyone to hope that a political solution to the internal problems of South Africa could be engineered overnight. Decades must go by before everything can be settled. In the meantime, the best hope is that there will be no violent breakdown of the social fabric, externally or, more dangerously, internally. The immediate goal—and distant it must seem to many people—is that the two communities in South Africa should be brought so closely into articulation with each other that there is a chance for the natural processes of social evolution to do their work. Luckily, there are now signs that pressures in the right direction are beginning to work. The white population of South Africa seems increasingly aware of what is going on. There is growing recognition that the policy of separate development is an economic nonsense.

In circumstances like these, the universities and the scientific community which they embody are essential bridgeheads to the outside world and to the future. Whatever people may do about cricket matches, the present circumstance requires that links between South Africa and the outside world in science and academic life should be strengthened, not weakened. The universities, even those commonly supposed to be reactionary, are enlightened and even sometimes liberal. Nobody expects that they will ever lead a social revolution, and with the weight of prejudice in the voting population there is little that they can do to lead voters by the hand. Yet politics in South Africa are so explicit that there is much that the universities can do (and should have been doing all these years) to argue the detailed as well as the general case against the present arrangements. This does not imply endless declarations by university senates but, rather, more vigorous argument by individuals who will need as much time as they can be given to change the present climate. The greatest danger is that time is running out.

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100 Years Ago



BRITISH EDIBLE FUNGI

MUSHROOMS and their congeners seem never to have been in good repute since Agrippina employed one of the tribe to poison her husband, and Nero with villanous pleasantry called it the "food of the gods." With proverbial tenacity the bad name thus incurred has clung to the whole family of agarics, and what within certain limits might be called a wholesome dread has become a deep-rooted and irrational prejudice, excluding from popular use a really valuable class of vegetable esculents. We cannot altogether go along with those enthusiastic mycophagists who recognise a substitute for meat in every edible fungus, and dilate on the ozmazome and other nutritious properties of the tribe; but we readily acknowledge that their merits as secondary sources of food-supply have hitherto been unduly neglected. The great difficulty always felt in advocating the claims of the class to more extensive use has arisen from the want of some definite rules, some formula at once simple in expression and universal in application, by which to distinguish the noxious from the innocent members. Pliny, in his Natural History, goes so far as to say that the first place amongst those things which are eaten with peril must be assigned to agarics, and he expresses his surprise at the pleasure which men take "in so doubtful and dangerous a meat." But his observations show that fungi of all sorts, including even such growths as the *Fistulina hepatica*, were known to his countrymen and eaten by them without scruple. Indeed, in one particular the wisdom of the ancient Romans seems to have been superior to that of their descendants, for, while Horace lays down the rule—

Pratensibus optima fungi
Natura est; aliis male creditur—

the modern Ædiles of the Roman market condemn to instant destruction every specimen of the meadow mushroom (*A. campestris*) which comes within their reach. Although, however, it is not always easy to distinguish the wholesome from the unwholesome fungus, and the organs of sight and smell require some training before they can be wholly trusted in the matter, yet the dangers have been greatly exaggerated, and, as a matter of fact, hogweed is more often mistaken for parsnip and aconite for horse-radish than are *Boletus sátanas* and *Amanita verna* for their innocent brethren.

***From* Nature, 2, 518, *October* 27, 1870.**

OLD WORLD

ATOMIC ENERGY AUTHORITY

Quiet Before the Storm

THE annual report of the UK Atomic Energy Authority, published last week (HMSO, 17*s*), has cheerful things to say about fusion. At the Culham laboratory, for example, joint Anglo-Russian experiments on the Russian Tokamak device have raised hopes that a fusion reactor may be more than a fantasy, and progress has been encouraging on several other aspects of closed line systems at Culham. Yet funds for Culham continue to be cut in accordance with decisions made several years ago, even though, as the report puts it, "progress fully justifies the maintenance of a strong and viable programme in the United Kingdom".

The chief preoccupation of the AEA remains the development of new reactor systems, on which it spent £43·3 million last year, compared with £41·3 million in 1968–9. Of this, 62 per cent was spent on the fast reactor programme and 20 per cent on water cooled systems. Sir John Hill, chairman of the AEA, emphasized in London last week that the AEA was keen that the first commercial fast reactor should be large enough to be replicated without major modifications. He thought that construction work on the first fast reactor power station would probably start in 1974.

On water cooled systems, the report recalls that the AEA provided the nuclear design and construction consortia with plans for a commercial 450 MW steam generating heavy water reactor. But there is no mention of the gloomy commercial forecasts for the SGHWR, which is beginning to look more like the AEA's answer to Concorde with every month that passes. The Radiochemical Centre at Amersham increased its profit last year by 22 per cent, of which about 60 per cent was from exports. This may encourage the new government to take up the threads of the fuel bill prepared by Mr Wedgwood Benn, former Minister of Technology, in which the Radiochemical Centre was seen as the backbone of one company and the plants at Springfields, Capenhurst and Windscale were to be the focus of a second company, British Nuclear Fuels.

One point which is hardly clarified in the report is the extent to which the Atomic Weapons Research Establishment at Aldermaston has kept pace with the changing orientation of the AEA. An over-enthusiasm for classifying projects of very marginal secrecy may have hampered progress in fields such as computer applications in engineering, in which the AWRE has considerable experience. A computer system for the control of machine tools, for example, had to make its way to the open market through the workshops at AERE, Harwell.

Both the total expenditure and number of staff at the AEA fell last year, although for quite different reasons. The drop in expenditure of about £7 million is attributed chiefly to the delay in completion of the prototype fast reactor at Dounreay and to deferment of purchases of plutonium for fabrication of its fuel. The reduction of staff has continued consistently since 1961, standing now at 30,000 or 10,000 less than in 1961. This represents a drop of 1,300 in the past year, partly due to transfers to the design and construction companies.

ACADEMIC FREEDOM

Law of Protest

by our Education Correspondent

A REPORT that defines the legal rights of students within an institution of higher education has been published this week by the National Union of Students and the National Council for Civil Liberties (*Academic Freedom and the Law*, NUS, 10*s*). The chief recommendation is that every student should enter into a legally binding contract with his college or university guaranteeing certain rights and limiting methods of protest. This is the first attempt so far to define the limits to which student protest should be taken; it is remarkable because to some extent it flies in the face of trade union opposition to legally binding contracts.

The National Union of Students clearly intends that contracts will be subject to negotiation between individual student unions and the institutions to which they belong, but the report lays down several guidelines. For example, it recommends that "college regulations should prescribe the following offences, and no others: (i) to engage in conduct which actively disrupts the teaching or study or research of the college; (ii) to damage or deface any property of the college". In return, the student would be guaranteed academic freedom, defined as "(i) the right to study and to have access to facilities for study; (ii) the right of freedom of thought and interpretation; (iii) the right of those who teach within or service an institution to withdraw their labour for industrial reasons". In particular, disciplinary proceedings should never be taken in the event of a boycott of classes or an occupation provided that these actions do not involve any conflict with the rights of others to study.

Although there may not seem anything particularly novel about these recommendations, if the report is adopted by the NUS conference next month, it would mean that protests such as that at the London School of Economics, where gates were removed and college property damaged, and at Warwick, where occupation of the administration block infringed the rights of others to study, would be forbidden. On the other hand, the report suggests that disputes arising in colleges are often a product of bad government, and there is a strong hint that such disputes can be prevented only by changing college government to incorporate representatives of all members of the academic community. The report also comes out strongly against bringing police on to college campuses to settle disputes.

Another recommendation in the report is the setting up of a higher education advisory board. This body is envisaged as a watchdog to safeguard academic freedom, and also as a final appeals body which would hear appeals against a college decision to expel a student or to refuse admission on academic grounds.

The report also protests against confidential files. In particular, it points out that no information about the political activities of a student should be gathered or recorded. Some aspects of this part of the report may be difficult for student activists to swallow, especially its suggestion that students should have access to those parts of their own records which are not confidential. At Warwick, and at many other universities which were engulfed last year by the wave of protest about students' files, however, the students maintained that no information should be filed which they are unable to inspect.

NOBEL PRIZES

Neurophysiologists Honoured

WITH the news from Stockholm last week that the Nobel prize for medicine had been awarded to the neurophysiologists, Professor Ulf von Euler, Dr Julius Axelrod and Sir Bernard Katz, the wheel has, in a sense, turned full circle. Sir Henry Dale and Otto Loewi were awarded the prize in 1936 for showing that nervous impulses travel through the nervous system in two different ways. In 1963, Eccles, Hodgkin and Huxley were similarly honoured for their part in unravelling the first of these two systems—the electrical transmission of the impulse along the nerve fibre. And now von Euler, Axelrod and Katz share this year's prize, worth £32,000, for their separate work on the second puzzle—how nerve impulses are transmitted chemically across the minute insulating gaps, or synapses, which separate nerve from nerve or nerve from muscle.

Professor Ulf Svante von Euler, 65, was born in Stockholm and has spent most of his scientific life at the Royal Karolinska Institute, where he is now professor of physiology. In the early 1930s he worked for a time at University College, London, where a major influence was Sir Henry Dale. Von Euler's chief contribution to neurophysiology is his demonstration in 1947 that the transmitter substance which diffuses from the ends of stimulated sympathetic nerve to bridge the synaptic gap is the catecholamine, noradrenaline.

Von Euler's work cleared the ground for the later studies of Julius Axelrod. Born in New York City in 1912, Axelrod was trained both as a biochemist and as a pharmacologist, and is now chief of the section of pharmacology at the National Institute of Mental Health, Bethesda, Maryland. After investigating the metabolism of drugs and the function of the pineal gland, he became interested in the fate of the noradrenaline molecule after it had been discharged into the synaptic gap. Clearly the molecule would have to be inactivated after passing on its message, or the nervous system would be thrown into chaos, just as would a telephone exchange if all the plugs were left connected after the calls had ended. Axelrod discovered two control mechanisms; an enzyme, catechol-o-methyl transferase which methylates and destroys the noradrenaline molecule, and a process found only at sympathetic nerve endings by which discharged noradrenaline molecules are reabsorbed by the nerve endings.

Whereas von Euler and Axelrod have been concerned with the transmission of nervous impulses mediated by noradrenaline—the adrenergic nervous system—Sir Bernard Katz has devoted his energies to nervous transmission mediated by acetylcholine—the cholinergic nervous system. His work has led to the important conclusion that synaptic gaps are bridged in essentially the same manner in all parts of the nervous system.

Bernard Katz was born in Leipzig, Germany, in 1911. In 1935 he fled from the Nazis to London, beginning his studies on nerve and muscle function under the supervision of Nobel laureate A. V. Hill at University College, London, where he is now head of the biophysics department. Working initially on the cat neuromuscular junction, and later on giant nerve cells from the squid, Sir Bernard has used microelectrodes, fine glass electrodes which can be inserted into single cells, to study the electrical behaviour of nerve endings. He has shown that the transmitter substance, acetylcholine, is contained in small vesicles at the nerve endings. In the resting state a very small number of these vesicles are discharged spontaneously, but when a nervous impulse reaches the nerve ending many millions of acetylcholine molecules are released to bridge the synaptic gap between the nerve ending and the adjacent cell. More recently, Sir Bernard has been working on the role of calcium ions in the release of acetylcholine.

In announcing the awards, the Royal Karolinska Institute, Stockholm, said the three scientists had "greatly stimulated the search for remedies against nervous and mental disturbances". Whilst drugs tailored to cure mental illness may be far in the future, no one can deny that the work of von Euler, Axelrod and Katz has led to a new understanding of the nervous system and its chemistry.

FORESTRY

English Elm Threatened

THE future of the elm tree in Britain looks bleak unless the epidemic of Dutch elm disease, at present ravaging the British population of *Ulmus procera*, is quickly checked. The disease was first recorded in Britain in 1927, but was a comparatively minor problem until two years ago, when, helped perhaps by a series of fine summers, the present epidemic exploded. Dr John Gibbs of the Forestry Commission Research Centre described the current situation as "devastation, with more than sixty per cent of the elm population destroyed in some areas".



Hedgerow elms blighted by Dutch elm disease. On the left, a healthy elm; on the right, a newly killed tree left gaunt and leafless by the disease.

Dutch elm disease is caused by the fungus *Ceratocystis ulmi*, but the infection is carried and spread by the elm bark beetles *Scolytus scolytus* and *S. multistriatus*. The sticky fungal spores become attached to the body of the beetle and are transferred from in-

fected to healthy trees when the beetle feeds on the young shoots. The manner in which the fungus kills the branches or the entire tree is macabre. Fungal spores find their way into the conducting tissue of the tree and are carried along in the vascular stream. The tree reacts to the presence of these foreign particles by manufacturing gums and resins. It is the accumulation of these substances in the wood which form a kind of embolism, eventually blocking the transport system and killing the tree. In addition, the fungal mycelium produces a toxin to which the elm is susceptible.

It is difficult to assess how much the present epidemic has cost, for elm is rarely grown on a commercial scale. Nevertheless it is an important landscape tree, especially in areas which have been badly devastated, such as the Severn valley and the Thames estuary. Furthermore, elm is a native hardwood of some quality, and recently has been assuming considerable importance in the manufacture of furniture.

There is at present no sure way of eradicating the disease, but research has been developing along three main lines. In America, where the native elm is much more susceptible to the disease than the British species, some success has been obtained by attacking the elm beetle directly and destroying its breeding ground. This has been achieved by clearing infected trees, and spraying healthy trees with DDT in the early part of the season before bud break. In the present climate of conservation, however, this latter practice is causing some embarrassment. In Britain, systemic fungicides are currently being tested, but perhaps the greatest chance of control lies in the development, in Holland, of a strain of elm with genetic resistance to the disease. This is a fast growing species called the Commelin elm, but development has not yet advanced to the stage where the quality of the wood can be assessed.

If these measures prove unsuccessful in combating the spread of the disease, it seems all too certain that within a few years the elm in Britain may be but a verdant memory.

WATER QUALITY

Changes in Moskva River

by our Soviet Correspondent

THE recently published data from the spring scouring of the Moskva River in the Moscow city area reveal some interesting changes in the quality of the river water (*Priroda*, No. 9; 1970). Since the building of the Perervinskaya dam below Moscow in 1937, and the later building of dams on the headwaters of the Moskva and its upper tributaries, the water regime of the Moscow reaches of the Moskva has changed considerably. To avoid stagnation, an annual scouring of these reaches is carried out. A massive discharge of water from the reservoirs is timed to coincide with the natural spring floods on the Moskva. The water coming downstream to the "reservoir" of the Moscow reaches during scouring (estimated at 650 million cubic metres this year, with a peak flow of 500 cubic metres per second) considerably exceeds the capacity of the "reservoir" reaches, and so scouring is effected rapidly. Scouring usually takes place in mid-April, and samples of the river water are analysed one calendar month later. The figures for 1970 include transparency of the water (compared with standard alcohol), 30; content of carbon dioxide, 6·6 mg/l.; total mineral salt ions, 245 mg/l. and oxidizing power against bichromate 12·3 mg of oxygen/l. Compared with pre-scouring figures, the transparency increased seven-fold, the carbon dioxide decreased four-fold, and the amount of organic admixtures decreased considerably because of the increase in oxidizing power. On the whole these measurements follow the pattern of previous years; it is interesting, however, that the phosphate content of the water, which was 0·044 mg/l. in 1965 and 0·004 mg/l. in 1966, has now declined to zero.

JOURNALS

Goodbye *Science Journal*

THE proposed merger between *Science Journal* and *New Scientist*, which was announced last week, will not only create a gap in the field of scientific publishing, but it is also likely to leave in its wake considerable ill-feeling among the editorial staff of both magazines. The proposal means that most of the staff of *Science Journal* will be transferred to the weekly *New Scientist* and it seems likely that *New Scientist* will be expanded to include special features articles. This means in effect that when the final issue of *Science Journal* is published next January, there will no longer be a monthly general science journal published in Britain.

The driving force behind the merger is clearly the poor financial position in which *Science Journal* now finds itself. Mr Walter Williams, group director of IPC Science and Technology Publications, which publishes *Science Journal*, said for example that the accumulated losses of the magazine during its five year lifetime indicate that it will never be financially viable. Moreover, Mr Williams pointed out that there has been an absolute decline in the advertising space sold in *Science Journal*, and that its sales have deteriorated during the past year or so.

This, however, has not deterred a few publishing companies from sounding out the possibilities of taking over *Science Journal*, and the *Financial Times* even put in a firm offer to buy the magazine. By rejecting the offer, and deciding to merge the two magazines, IPC is clearly hoping that the new-style science magazine will be able to pick up most of *Science Journal's* advertising revenue and readers—especially foreign readers. An added advantage, from IPC's point of view, is that the company will be able to retain most of the editorial expertise which has been built up in *Science Journal*.

Although *Science Journal's* editorial staff accept that the magazine has lost money, their general feeling is that in the past few months there has been considerable improvement and that the financial prospects are far from gloomy. The magazine's chapel of the National Union of Journalists therefore immediately invoked the disputes clause in the editorial staff contract, because it is not convinced of the viability of the merger plans. The chapel's attitude at the moment is that *Science Journal* should be kept going as an independent publication. Redundancy is not yet a chief point of concern because all the writing staff, with the exception of editor Robin Clarke have been offered appointments on the new magazine.

If the merger goes through as planned, the January 1971 issue of *Science Journal* will be the magazine's final edition, and the first issue of the new-style *New Scientist* will be published on January 21. A measure of IPC's faith in the selling power of the new magazine is that it will have a print order of 100,000—at present, each separate magazine has a circulation of about 50,000.

COMPUTERS

Demand and Supply

ALTHOUGH more than £20 million has been spent in less than four years on computers for research, the second report of the Computer Board for Universities and Research Councils published last week (HMSO, 3*s* 6*d*) reveals that academic research in Britain is still weak in overall computing power.

The Computer Board was established in September 1966 following the publication of the Flowers report on the present and future computing requirements of research (*Working Group on Computers for Research*, HMSO, 1966). There has been considerable progress since then, and most research groups now have access to some kind of computing facility, but the Computer Board's report emphasizes that there is still a long way to go.

The report describes the past two years as a period of consolidation. Since 1966, 33 new computers have been installed at a cost of £9·4 million, and 8 new computers worth £6·6 million have been ordered. These include five British International Computers Ltd (ITL) 1906A systems, and two of the very large American Control Data Corporation (CDC) 6400 series machines have been earmarked for London University. The capacity of computers already installed at 31 universities has been enhanced at a total cost of £2·9 million, and equipment worth a further £2·2 million has been ordered to upgrade other existing hardware.

The board, under its new chairman, J. D. Finney, University of Edinburgh, is understandably anxious to buy British equipment where possible, especially where large capacity units are required. Their plans to use the ICL 1908A computer for regional computer centres were thwarted when ICL cancelled research on this system to concentrate on the development of Project 52, a radically new computer of comparable size to the 1908A. Details of Project 52 are still confidential, but it seems likely that its novelty will lie in vastly improved ease of operation rather than electronic innovation. Project 52 will not mature until 1972 at the earliest, and until then the board will probably have to turn to the United States for supplies of very large computers.

An important new feature of the board's activities will be responsibility for the provision of computers and computing facilities for teaching as well as for research. This was recommended earlier this year by a joint working party set up by the UGC and the Computer Board (*Nature*, **227**, 1079; 1970) and means that universities will no longer have to limit the use of computers supplied by the board for research purposes. The working party estimated that an extra £1 million would be required to finance the extra hardware required for teaching purposes, but so far the government has not been able to provide further funds. Fortunately, the board's original estimates had taken such contingencies into account, and a small surplus is available to support this extra commitment.

URANIUM SUPPLIES

New Reserves Needed

THERE will be an acute shortage in the world's uranium supplies by the mid-seventies unless new reserves are discovered and exploited. This fact emerges from the latest report of the European Nuclear Energy Agency and the International Atomic Energy Agency (*Uranium: Resources, Production and Demand*, OECD, $3, £1). The agencies estimate that the present consumption of 15,000 short tons of uranium oxide ore a year will increase to 38,000 short tons a year by 1974–76—a figure equal to the existing production capacity—and by 1980, the demand will be about 73,000 short tons a year. The estimate assumes an increase in the nuclear generating capacity of the world from 18 to 300 GW in the next ten years, and it is based on information gathered from all countries known to have a nuclear power programme, with the exception of the People's Republic of China, the USSR and the countries of Eastern Europe.

Anticipation of the future demand has been the chief impetus behind the recent boom in prospecting: since 1967, discoveries of uranium in Canada, Australia, the Central African Republic, Gabon, Niger and the US have increased the estimated world reserves from 700,000 to 840,000 short tons. The report points out, however, that if the consumption of uranium increases at the estimated rate, it will be necessary to discover and develop an additional one million short tons of uranium oxide by 1985. In the opinion of the working party responsible for the report, it would be prudent to give greater emphasis now to relatively low cost primary prospecting with the objective of discovering new uranium provinces suitable for subsequent, more detailed exploration. It is difficult to predict where new reserves will be found, however, but of the nine uranium producing countries, Canada and South Africa seem to have the most room for expansion of existing industry.

The future demand should end the lean days of the uranium mines. At present, low prices, fluctuations in the market and an unexpectedly slow movement of the world nuclear power programme have produced a state of over production and hesitancy in the industry. At Elliot Lake in Canada, for example, where once there was a bustle of activity to supply the military needs of the US government, many of the mines lie idle.

But when the promised boom does come, it could produce the familiar pattern of sudden shortage followed by over supply. The report suggests that consumers could improve the market stability either by making advance purchases or by negotiating long term supply contracts; this would encourage suppliers to proceed with plans for new production capacity and to search for the additional reserves needed to satisfy future needs. In this way mismatching of supply and demand could be avoided. Whether or not enough uranium will be found in time, the report seems reluctant to predict.

NEW WORLD

Rashness that may Pay Off

by our Washington Correspondent

JUST as the rubella vaccination campaign in the United States is reaching its full momentum, critics are repeating their earlier fears that the programme is based on a shaky strategy and could even do more harm than good. The root of the controversy is the classical dilemma of whether to act on the basis of incomplete knowledge to try to forestall a certain tragedy. In this instance the decision has been to act now in the hope of preventing the rubella epidemic that is expected to break out this winter. But although no qualitatively new evidence has come to light since the decision to proceed was taken a year ago, the doubts about the basic strategy of the campaign have been accumulating. Meanwhile the Department of Health, Education and Welfare's Center for Disease Control in Atlanta, Georgia, continues undeflected in its aim of vaccinating between 40 and 60 million children before the next epidemic begins.

Rubella, or German measles, is a harmless enough disease in people, but the virus can cause death or malformity in the foetus if contracted by the mother at a certain stage of pregnancy. In the last rubella epidemic in the United States, in 1964, there were some 30,000 foetal deaths and 20,000 children born with congenital abnormalities. So far some $41 million has been allocated to the present vaccination campaign, which has already reached 16 million children between the age of one year and puberty, or about a quarter of the total target population.

The campaign is modelled on the successful vaccination programmes conducted against polio and measles but it differs in the indirectness of its approach; the immediate beneficiaries are not the children who are vaccinated but the susceptible women in the community for whom the children are the commonest source of infection in an epidemic. With the live attenuated virus that is the basis of the present vaccines, the risks of vaccinating susceptible women directly seemed unacceptable—some of the vaccinees might unknowingly be pregnant and the effects of the attenuated virus on the foetus are unknown. Instead, it was decided to try and immunize enough of the children in the population to prevent an epidemic from starting.

This so called "barrier" strategy has been successful with measles and with polio, but several surveys of which the most recent was reported this month by Horstmann and colleagues at the Yale University School of Medicine*, have shown that at least in the special conditions of military camps the virus can still reach all the susceptible victims even though more than 8 men out of 10 have a natural resistance to the disease. Unless the vaccination programme can reach more than the 80 to 85 per cent of the population which is naturally resistant to rubella, some critics argue, the barrier it throws up will not be sufficient to halt the epidemic.

Another criticism is that the present vaccines provide a smaller degree of immunity to rubella than does natural infection, with the result that vaccinees are often reinfected when exposed to the wild virus. (In the survey reported by Horstmann and her colleagues, for example, 80 per cent of the vaccinated soldiers became reinfected during the epidemic.) Even though the infection in these cases is almost always clinically inapparent, being detectable only through a rise in the circulating antibodies, there is the possibility that the reinfected vaccinees may transmit the virus to others. The Public Health Service Advisory Committee on Immunization Practices said in a report in August that the probability of such spread is "exceedingly low". Others are not so sure. The epidemiologists at Yale University, for example, conclude that the question is far from settled although they agree that risk of contagion from reinfected persons is greatly reduced.

Another source of uncertainty is the duration of the immunity conferred by vaccination. Will a girl vaccinated against rubella at the age of 6 still be immune to the virus by the time she starts to bear children? The Advisory Committee on Immunization Practices bases its view that long term protection is likely, on the evidence that antibody levels have declined very little during the four years of observation of the children who were the first to be vaccinated. But others believe that a substantial proportion of vaccinees may lose their immunity after ten years or more.

A different question is whether a mother who has been vaccinated but is reinfected runs the same risk as a susceptible woman of bearing a rubella baby. Malformations are known to occur even when a susceptible mother has suffered from rubella in a clinically inapparent form; the clinically inapparent form of the disease with which vaccinees become reinfected could in theory also be teratogenic. However, although this possibility cannot yet be ruled out, no one has yet detected the virus in the bloodstream of reinfected vaccinees, which suggests that it is unlikely to cross the placenta in these cases.

A more theoretical worry concerns the quality of immunity to rubella in subsequent generations, should the wild virus be eliminated by the vaccination programme. Since the vaccine provokes a smaller degree of immunity than does exposure to the wild virus, it could be that generations which have to rely on vaccination for their immunity will be less strongly protected against the virus.

Defenders of the vaccination programme reply that none of these criticisms is new, and all were fully considered before the programme was started. It is true there are uncertainties in the knowledge of how the vaccine will work, but to wait for more complete knowledge whilst suffering the toll of the rubella babies born in the approaching and later epidemics would be absurd, especially when a viable rubella vaccine is in existence. The two crucial questions are whether a reinfected vaccinee can participate in the chain of transmission and whether, if pregnant, she can transmit the virus to her foetus. While these possibilities cannot be ruled out, they seemed at the time, and still seem, sufficiently small to justify the present use of the vaccine. Defenders of the programme argue that the spread of the virus in a military camp, as reported by

* *New England Journal of Medicine* (**283**, 771; 1970).

Horstmann, should not be construed as evidence that the barrier strategy is mistaken, since for several diseases the progress of transmission in these conditions is known to be quite different from that among the civilian population.

The severer critics of the present campaign believe that the Division of Biologics Standards of the National Institutes of Health, which is responsible for recommending licences for the vaccine, should have allowed more research data to accumulate before giving the programme its blessing. As it happens the attenuated virus strain on which two of the three vaccines are based was developed by two scientists in the Division of Biologics Standards, Drs H. M. Meyer and Dr P. D. Parkman. In deciding to recommend licences for the rubella vaccines the director of the CAP D division, Dr Roderick Murray, took advice from all people concerned, not excluding Drs Meyer and Parkman. But to avoid any conflict of interest Dr Murray specifically requested that no member of the Biologics Standards Division should also have a voting position on the board responsible for developing the rubella vaccines, the Vaccine Development Committee of the National Institute of Allergy and Infectious Diseases. Dr Meyer was one of the non-voting liaison members of this committee but, with his exception, no member of the Division of Biologics Standards sat on this or either of the two other committees involved, the Surgeon General's Advisory Committee on Immunization Practices and the Immunization Committee of the American Academy of Pediatrics. The latter two committees issued a joint statement at the time the rubella vaccines were licensed recommending their routine use on children.

A more moderate view of the vaccination programme is taken by the Nobel laureate Dr John Enders of the Boston Children's Hospital. In a judiciously balanced editorial article in the *New England Journal of Medicine* he said that the "accumulating doubts" about the long term effectiveness of the programme "should not for the present be allowed to interfere with its continuation. But in the meantime they may usefully encourage a reconsideration of other, more direct procedures, such as the vaccination of susceptible parturient women or of adolescent girls".

Though it is far too early to assess how the vaccination is working, those involved with the programme have the impression that in areas where there has been heavy vaccination there is less rubella around than usual. Even should the campaign not succeed, there are many who will say it was worth the gamble.

AIR POLLUTION

Academy to Take on Detroit Oil Men

by our Washington Correspondent

THE National Academy of Sciences is a rarity among learned societies in that it is required by charter to tender practical advice, when requested by government, on the issues of the day. Unlike the British Royal Society, for example, whose only purpose in life is self-perpetuation, the National Academy dares to put the talents of its members to practical use, though with the ever present risk of offending powerful interest groups or agencies of government. The academy now seems likely to be charged with a task as awesome politically as any that it has yet faced, that of monitoring progress in meeting Senator Muskie's clean air bill which states, among other things, that all cars manufactured after January 1, 1975, will emit 90 per cent less pollutants than present models. The academy may thus be brought into conflict with the automobile manufacturers, if it finds them dragging their feet, and maybe with the producers of gasoline as well, or with the Congress should it find the legislated standards technologically impossible to meet.

The standards demanded by the Senate for 1975 model cars have not yet reached the statute book but have every chance of doing so now that they have been agreed to at a meeting this month between Senate and House conferees. What the House and Senate have yet to agree upon is the precise role to be played by the National Academy in seeing that the legislation is fulfilled. It appears that the academy will not be asked, as at one time seemed likely, to say at this moment whether the 1975 deadline for reduced emissions is technologically feasible. At the meeting between Senate and House conferees on October 8, it was agreed that the academy should prepare reports at six-monthly intervals, the first being due next July, on the progress made by the manufacturers towards meeting the deadline. But the academy may also be required to comment in its six-monthly reports on the progress made in light of what is technologically feasible at the time—in other words, on whether the car-makers are trying hard enough. In assessing what is technologically feasible the academy will not be asked to consider price as a factor.

One point at which the academy's advice will clearly be crucial is in deciding the option, allowed for in the bill, of postponing the deadline by one year should the manufacturers be able to prove to a federal court that it is too harsh. The precise description of the Academy's role will be decided when the conferees from the Senate and House meet again next month. The Academy is said to have refused government requests for advice only half a dozen times in its existence and is unlikely to pass up this one, however great the temptation.

ANTARCTICA

NSF declared Sole Heir

by our Washington Correspondent

THE National Science Foundation will become the most puissant power in Antarctica, following President Nixon's decision last week that the foundation should be the sole channel for funding United States work in the continent. The budget of the NSF will be increased by $25 million when the transfer of programmes from the Department of Defense and the Coast Guard is complete. But the changeover will not necessarily mean more money for Antarctic research, since the programmes to be transferred are concerned almost wholly with providing logistical support to existing research efforts.

This year the foundation is spending $7·5 million on all forms of Antarctic research, which at the height of the season, during the austral summer, brings some 200 scientists and technicians to the four year-round bases the United States maintains at Byrd, McMurdo, Palmer and the Amundsen-Scott South Pole station. The eleven major projects being supported this year include a balloon study of cosmic rays under J. Barcus at the University of Denver, continued exploration by

Ohio State University of the rich fossil fields excavated last year, and a study of the plasmapause under J. Katsufrakis at Stanford University.

The US Army Cold Region Research and Engineering Laboratory, which has the distinction of having drilled cores down to bedrock through both the Antarctic and Greenland icecaps, is not venturing forth on the ice this summer. The intriguing record of climatic history that is stored in these cores—the ratio of oxygen isotopes in ice samples at various depths gives a measure of the temperature prevailing when the snow was formed—is unfortunately very expensive to extract. The core drilled at Byrd station in the summer of 1968–69 cost some $250,000 and the determination of the $^{18}O/^{16}O$ ratio costs $50 a sample, although this part of the research, conducted under W. Dansgaard at the University of Copenhagen, is supported by the Danish government.

The ice at the bottom of the Byrd station core is about 70,000 years old, whereas that in the Camp Century core from Greenland is nearer 100,000 years. There are plans to drill another core through the Antarctic ice cap, probably as part of an international glaciological programme in which the Soviet Union, Britain and France would participate. A second core would hopefully extend the climatic record further back into the Pleistocene as well as providing a fuller comparison with the thousand century temperature chart extracted from Greenland.

MALNUTRITION

New York Children lack Vitamin A

by our New York Correspondent

A WIDESPREAD deficiency of vitamin A among children in New York City, particularly those from lower-income homes, was reported last week by Dr Robert Newman, director of the National Nutritional Survey of New York City, at a meeting of the New York Academy of Sciences. Dr Newman noted that there is still no agreement as to what constitutes malnutrition, each scientist defining it according to his own frame of reference. But he hoped that the New York survey, along with those being conducted in ten other states, would enable present definitions and techniques to be re-evaluated.

The New York study figures released last week are based only on laboratory analyses, so that correlation of dietary deficiencies with specific symptoms or illnesses must wait until the clinical examination results have been tabulated. Deficiency of vitamin A is known to contribute to a loss of vision in poor light, but it is not known how great the deficiency must be before sight is affected. There is also great disagreement over what constitutes the "minimal daily requirements" of nutrients. For example, in Britain 20 mg is considered an adequate daily amount of vitamin C, while in the United States the common figure is 70 mg. The National Survey originally defined the requirement as 60 mg but dropped this level in the course of the survey to 30 mg. The distinction must also be made between nutritional levels and "good eating habits". Dr Newman pointed out that the reason why so few people were found with vitamin C deficiencies might well be because it is added to many sodas and soft drinks. "These drinks are still frowned upon and not considered a desirable source for this nutrient," he said.

Forty-six per cent of children under seven years old in lower income families were found to have vitamin A deficiency, compared with 18 per cent of children of that age in upper income families. Between the ages of seven and twelve the figures were 27 per cent for the lower-income and 25 per cent for the upper. Ten per cent of the low-income children between seven and twelve were deficient in vitamin B, riboflavin, compared with six per cent of the upper-income children of that age group. For purposes of the study, the dividing line between lower and upper income families was placed at an annual income of $4,156 for a family of four.

COMMUNICATIONS

Beware the Polluted Spectrum

IT is admirable that the National Academy of Engineering should be aware of the electromagnetic spectrum as a natural resource that is in as much danger of pollution as any river, but the latest report by the panel on spectrum value is a crashing disappointment*. The lengthy report now published could almost stand as a deliberate example of how words can be a pollutant. That itself does not auger well for the spectrum. The aim of course is to devise a sensible way of deciding how to parcel out the finite band of the electromagnetic spectrum that can be used for such activities as communications, radar, aircraft control and so on. Part of the reason for the continuing importance of this topic is that parts of the spectrum at shorter wavelengths than used to be impracticable to use are now being opened up through the development of new equipment and the pressure on the conventional wavebands. What the panel has been trying to do without much luck is to think up some social and economic criteria that can be applied to the judgments that have to be made about the purposes to which unused parts of the spectrum are to be applied. To begin with the report expresses surprise that up to now things have gone as well as they have, in the United States at least, with a system of spectral management which the report criticizes. For example, the panel finds that the Federal Communications Commission is slow at coming to decisions, usually hears evidence only from those directly involved in a contest over a particular band, and has inadequate technical advice. What the panel would like to see is more representation for the general public above that which the officials of the FCC themselves provide, and a better backing staff to provide the technical background and speed up the negotiations. The reason these deficiencies have not made themselves felt more strongly earlier, the report says, is that technical advances have tended to cover up where mistakes have been made. The panel makes no secret that what it would really like is a formula to express the social and economic value of a given application of part of the spectrum as an index. Reluctantly the panel concludes that no satisfactory formula is likely to be devised. All that the panel can suggest then is to strengthen the present methods of allocation. What this boils down to is that the people who have to make the decisions about the use of the spectrum should be backed by better advisory teams, and it is a shame that this lukewarm recommendation is all that the panel can find to say.

* *The Application of Social and Economic Values to Spectrum Management*, available from the Committee on Telecommunications, National Academy of Engineering, Washington DC.

SOUTH AFRICA

Public Support for Science

THE government of South Africa has a creditable record in the support of scientific research, chiefly through the agency of the Council of Scientific and Industrial Research, which has just been celebrating its twenty-fifth birthday. The organization was established by Dr Basil Schonland, who has in his time served as director of the Atomic Energy Research Establishment at Harwell. Over the years, the council has grown until total expenditure is Rd80 million (£1 = Rd1·7). This represents an increase by a factor of 2·5 in the past decade, during which time the council has also produced by fission the Medical Research Council of South Africa, established a year ago and still from all appearances learning how to find its administrative feet. The chances are that the CSIR is directly responsible for roughly one half of expenditure on basic research and applied research in South Africa—substantial sums are also spent by the Atomic Energy Board and by other government departments.

As a vehicle for the public support of scientific research and development, the council is an interesting variant on the theme of a research council. At the beginning, it was modelled on the National Research Council of Canada, but care has been taken to ensure that funds intended for the support of university research cannot dribble away into government laboratories.

The president of the CSIR, Dr S. M. Naudé, explains that the funds intended for university research, Rd1·34 million in 1968–69, are administered entirely separately from the budgets for the several research institutes which make up the CSIR campus at Pretoria and which are also scattered elsewhere about the country. Dr C. E. M. Brink, who administers this money, explains that the task is carried out much in the way now familiar in Western Europe and the United States—a series of seven interlocking advisory committees drawn from the academic world serves at once to provide advice on how to spend the funds available and also as a kind of lightning conductor to assure universities that their rights are well looked after. One innovation is that the academic awards committees are built up by selecting suitable members of the academic staffs of universities in strict rotation. Another, which administrators elsewhere will no doubt envy, is that there is a built-in growth factor of 7·5 per cent applicable to both halves of the budgets.

In the balance between extramural and internal research, there are grounds for thinking that the council has been less than generous to the universities. Excellent though much of the university research may be, there is no doubt that academic research is spread too thin. A part of the trouble is the shortage of research students, itself a function of the rapid attrition of the entering university population with progress towards an honours degree (see page 312). Another seems to be the difficulty of persuading universities which are inordinately jealous of their independence from government direction that sensible concentration on particular fields of activity may be appropriate. The council has nevertheless been able to establish in the past few years research units at universities which are responsible for diverse programmes of research—chromatography (Pretoria), cosmic rays (Potchefstroom), geochemistry (Cape Town), marine research (Durban), natural products (Cape Town), oceanography (Cape Town), palynology (Bloemfontein), polyenes (Stellenbosch) and solid state physics (Witwatersrand). The objective of these units is to concentrate the efforts of a full-time staff on particular problems, and in the process, toughen the fibre of university research. There is some way to go before the influence of the several units will be fully apparent.

Like other research councils, the CSIR is prepared to support students with bursaries for further study. People staying on for an honours degree can hope to pick up Rd600 a year; the stipend increases to between Rd700 and Rd1,000 a year (with a maximum of four years) for postgraduate students, while there is a system of postdoctoral stipends (not particularly effective so far) amounting to Rd 1,400 a year but with a possibility of supplementation by means of fees for university teaching. The council is at present also dickering with a scheme for providing three "prestigious bursaries" of between Rd2,500 and Rd3,000 a year for periods of three years but, for an organization seeking to tempt back emigrés and to attract people from overseas, this looks like small beer. In the battle against the brain drain, however, there is more in the scheme for tempting South African nationals back to the Republic by sharing the cost of a person's salary

South Africa

THE haunting dilemma of the Republic of South Africa is that no question can be thoroughly divorced from the central issue of South African politics—*apartheid*. The accompanying account, based on observations during a visit to South Africa sponsored by the Council for Scientific and Industrial Research, is an attempt to describe the present state of scientific research and of the institutions related to it. For many people in South Africa, that will be sufficient, and indeed there is a great deal to be learned from cold-blooded comparisons. Many South Africans will also be puzzled to know why their present policies should cause such deep resentment eleswhere, which is why this survey should be read in conjunction with the leading article on page 301.

between the CSIR and the government up to a maximum of Rd,6000 a year for a period which may be as long as two years—this device seems already to have brought back several good people.

What are the principles on which the national research council of a small country should operate? From the beginning, the CSIR has made no bones about its interest in the full exploitation of national resources. The result is that the bulk of its expenditure goes on the support of fifteen research institutes, most of them in Pretoria. The directors of the individual establishments echo the views of the president of the council as a whole in his insistence that the operation of a successful research establishment requires that applied research should be leavened with an admixture of more fundamental work, and this is the spirit in which laboratories such as the National Physical Research Laboratory and the National Chemical Research Laboratory, to name two, devote a substantial part of their energy to work that could well be carried out in universities. In practice, the research institutes seem also to have been successful in achieving one of Mr Wedgwood Benn's old ambitions for British government establishments—a measure of success in supporting the work done by means of contracts from outside. On the face of things, no less than Rd5·45 million of the Rd14 million spent on research institutes in 1970–71 came from sources other than the parliamentary grant, but Rd1·85 million consists of a payment by the United States National Aeronautics and Space Administration for the operation of the space tracking station at Hartebeesthoek. Elsewhere in the council's empire, substantial sums are provided by contracts with other government departments—the work carried out by the National Chemical Research Laboratory on the improvement of what is patronisingly known as bantu beer is, for example, financed by means of a levy on the 151 million gallons of this beverage sold each year by municipalities in South Africa. (A part of the justification for this enterprise is that the particular product of a two-stage fomentation of maize which yields the regional mildly alcoholic drink is also an important source of protein for diets that may otherwise be unbalanced.)

The insistence on a modicum of basic research in the research institutes is also a valuable means of keeping the staff concerned in touch with scientific developments elsewhere. In the past few years, the CSIR has been vigorous in supporting research conferences and symposia in South Africa. During the same period, it has helped to let its scientific employees feel at liberty to travel overseas at frequent intervals and has encouraged the same optimism among its pensioners in the universities. Those in charge of these operations say that this policy is necessary to compensate for the geographical isolation of South Africa. Most people insist that there have been no signs so far of political isolation, although there are individual and occasional tales to be coaxed out of unfriendly receptions overseas or of difficulties in attracting scientists of precisely the right kind to South Africa. As in the universities, so in the government establishments of the CSIR, however, there seems to be general agreement that the flow of visitors in both directions has become more free in the past three or four years. The newly created Medical Research Council has an explicit declaration of its willingness to encourage foreign travel.

The ecumenical influence of the CSIR is also seen in the way in which the council has been able to mix up Afrikaans and English speakers. In many other fields, the two communities of separate European origin are almost as carefully separated from each other as white people are from those with black or coloured skins. One of the most serious defects of the educational system in South Africa is, for example, the requirement that primary and secondary public education should be carried out in what is called the mother tongue—the result is that the distance between the English and the Afrikaans population which is in many ways responsible for the political unreality of South Africa is perpetuated and even intensified.

The work at the council's institutes has an interest that often extends beyond the severely practical. Thus there is immense potential interest in a research programme being carried out by the National Institute for Nutritional Diseases, until recently one half of the National Nutrition Research Institute of the CSIR and now a dependant of the Medical Research Council.

The objective is to investigate possible relationships between the occurrence of liver cancer among the native Africans—one of the diseases frequently occurring among migrant workers in South African gold mines. Demography has shown that the highest incidence of liver cancer is to be found in northern Mozambique and the object of the investigation by the National Institute for Nutrititional Diseases (in collaboration with a Portuguese group) is to investigate the possibility that liver cancer is caused by mycotoxins such as, for example, the aflatoxins. So far, the surveys carried out have shown that the incidence of the disease is in many places one hundred times that found elsewhere, but so far there has been no obvious link with the fungi cultured from the local diet. A similar project of potential regional benefit and international interest is an investigation of the aetiology of oesophageal cancer among the black native population of the Transkei—one of the regions of South Africa now set aside as a "homeland" for the native population and already blessed with a measure of independence.

The National Institute for Building Research has made its name by the development of a cheap and easily constructed house for black Africans. One product of this work is the stark uniformity of the African townships which are to be found at distances of between three and twenty miles from the large cities in which large amounts of native labour are employed. The original research was based on the result of a careful survey of the needs and habits of the black population of South Africa in the late fifties. Then, it seems, the needs of the typical black labourer could be most adequately met by a simple four-roomed house, without internal doors and without a ceiling. In the past few years, houses on this pattern have been constructed in several African townships, sometimes by the would-be residents themselves and sometimes on a production line basis by comparatively unskilled labour. Now, however, the National Institute for Building Research has embarked on a new survey of the needs of contemporary Africans. By all accounts, it is almost certain to emerge that urbanization has brought with it increased expectations and increased capacity to pay among black Africans. The institute is also deeply

concerned to find ways of planning African townships so as to make them seem less stark. The work now under way could, in other words, have a powerful influence on African society in the seventies.

Similarly far-reaching work is under way at the National Institute for Personnel Research, just off the campus at Witwatersrand. The institute's reputation stems from its work in the late forties in the development of a simple test for estimating the capacity of would-be mine workers (black) to carry out the tasks expected of them. Over the years, the test has become so well established that rural parts of Africa are now said to provide coaching in the assessment test for those about to seek employment in the mines. Thus the institute is bringing its assessment procedure up to date. In the process, it is seeking evidence to show how, over a span of two decades, urbanization, modestly increased prosperity and an awareness of the modern world may have made would-be mine workers more skilful in western ways.

The institute is beginning to ask questions about the way in which black people and white people are influenced by changes in their environment. The reputation of the institute's people is high enough to ensure that they will tell the truth, however unpalatable it may be, and for whom.

In the past year, arrangements have been made to extend the scope of the South African Government's influence on scientific research and development, chiefly by the creation of a Scientific Advisory Council, providing advice both for the Prime Minister, the Minister of Planning (formally responsible for the CSIR) and the government departments with an interest in scientific research. It remains to be seen how deep the influence of this body will be.

In general, however, there is a sharp contrast between the success with which the CSIR and other bodies have been able to develop their research programmes and the apparent lack of objective criteria for deciding how the balance should be struck between competing claims. In such a situation, there is at least a danger that the work that gets done is the work most easily done. And why should the funds available for university research be such a modest proportion of the government's total expenditure. Elsewhere, the money spent in this way has been linked with the output or the desired output of trained scientists. South Africa is more conscious than most countries of this need and thus would seem a natural place for the application of the doctrine that university research expenditure is a necessary component of higher education.

Universities Between Millstones

THE problems of higher education in South Africa seem to be determined largely by the quality of secondary school education, of which most university teachers complain, by the heavy teaching load which most of them carry and by the problems occasioned by the government's policy of separate development. In the past few years, there seems to have been a generous increase in the funds available for university development as well as for research, and everybody agrees that the government has been fair in its allocation of funds to universities considered loyal, chiefly those that teach in Afrikaans, and those which make no secret of their opposition to separate development, chiefly the University of Witwatersrand and the University of Cape Town. Research funds, at least when they are provided by the CSIR, are said to be administered efficiently and fairly; the chief complaint is that time for research has to be squeezed out of a timetable overcrowded with teaching.

The growth of the universities whose existence is justified by the doctrine of separate development is perhaps of the most immediate interest. By now, there are four universities for the Bantu (blacks), one for coloured people (in western Cape Province) and one for Indians (soon to be constituted in Natal). Until July last year, the first four of these universities were constituent colleges of the University of South Africa which still has 25,000 students on its books, most of them following correspondence courses. The philosophy underlying the setting up of the non-white universities is that each "nation" in the Republic of South Africa should have its own university to act as a focus for its own characteristic cultural development. Quite apart from the question whether such a policy is meaningful, there are practical questions of whether the universities are doing the job for which they are intended and which their students expect of them.

In the ten years of its existence, the University of the North at Terfloop, 200 miles north of Johannesburg, has grown to a total of 800 students. As with the white universities in South Africa, the undergraduate course consists of three years of study, with the option of a fourth year for those who wish to obtain an honours degree. The assistant registrar at Terfloop, Mr L. Coetsee, explained some days ago that the university is planning for between two and three thousand students by 1980. Many of the students at Terfloop, all of whom are black, come from as far away as South-West Africa, Rhodesia and even Malawi. Mr Coetsee expects that it will be possible to accept between 200 and 250 of those who have applied for admission for next year (beginning in January). The average age of the students is 24 and more than a third of those now in residence are thought to pay their own way, although others survive on grants which may range up to Rd600 a year (compared with fees of about Rd250 a year), usually—as in white universities—provided in the form of loans by government departments or private companies wishing to stake a claim in advance on a student's services.

At Terfloop, the standard language of instruction is Afrikaans and the staff for the time being is also largely white. Mr Coetsee estimates that some thirty of the 100 people on the staff at present are black. One of the complications of the situation, stemming not so much from the doctrine of separate development as from the older prejudice that white men do not take orders from black men, is that the black members of staff are either

junior people or nominally at least the heads of separate departments such as "history of education". The assistant registrar, Mr Coetsee, says that there is no difficulty in recruiting staff to the university, which is after all "a part of the university system", and which, in spite of the deserted plain in which it is sited, "is only 200 miles from Johannesburg".

Professor D. J. Fourie, dean of the faculty of mathematics and natural sciences, says, however, that one of his chief difficulties is that of recruiting staff, many of whom are prepared to come for short periods while finishing off a thesis. One result is that a splendid array of equipment for the investigation of surfaces and physical structure of solid materials remains under-used because the two junior members of the physics department this year are nuclear physicists and not solid state physicists. Professor Fourie is also more sceptical than Mr Coetsee about the speed with which it will be possible to recruit a teaching staff which is entirely black.

Everybody seems to agree that there are serious problems in preparing students in mathematics, but those who blame poor teaching in the schools are matched in numbers by those who say that there may be more fundamental causes. As a practical expedient to help students along with mathematics, two parallel courses are being provided which are expected to carry a student to the point of school-leaving mathematics after two years at the university, and this is only one of the reasons for thinking that, in spite of its name, the University of the North is still very much a high school. The most fierce critics of the system will of course agree that ten years from start to finish is a short spell in the lifetime of a university, and there is every chance that growth will bring simplification—at Terfloop as at the Western Cape University for coloured students, people complain that in small classes the chance is small of finding a handful of stimulating students. Professor E. P. Lekhela, from the department of history of education, agrees with Mr Coetsee that separate development applied to universities is worthwhile—"you work better for your own kind". The Western Cape University does, however, have a dash of the mission about it. The principal, Professor N. Sieberhagen, says that "we would not be here if we were not missionaries".

In the year just finishing, more than 900 students have been enrolled and the university is expected to grow to between 2,500 and 3,000 by 1980. Eventually there may be a second university for coloured students, but only when the population of the Western Cape University has grown to 6,000 or more, possibly by 1990. At present, the university is not residential but, rather, dependent on the nearby coloured communities of Cape Town for lodgings. As at Terfloop, there is a great deal of headshaking about the quality of the schools from which the students come but everybody insists that academic standards must not be allowed to drop. One result is that there are only two coloured members of the staff of seventy and—given the insistence on standards of academic achievement and the likelihood that the university will have to train most of its own staff—there is very little prospect that this ratio will rapidly increase.

Another consequence of the insistence on high academic standards and the poor preparation that the schools provide for higher education is that the drop-out rate is often extremely high. In chemistry, for example, more than half the students are expected to fail their first year examinations even after many of them have been prevented from competing by advance testing. Among the students at Western Cape, science seems to be a popular option—of the sixty-one degrees awarded in 1969, twenty were BScs and seventeen were BAs. (In the same year, there were three honours degrees in science and four in arts, while the university as a whole awarded fifty-six diplomas indicating training in teaching and social work.) The dilemma of the non-white universities is evidently that of knowing how best to satisfy the needs of students without so markedly adjusting the curriculum to suit them that students may complain of being given an inferior education. Another is that of striking a balance on the staff—many teachers at the non-white universities are gifted people with the highest ideals, but others are plainly much less well endowed. The problems of how to recruit a teaching staff which is racially identical with the students is still more daunting. So too is that of improving standards of teaching in the schools. One of the problems peculiar to the Western Cape University is that many of the brighter students never find their way there because of the temptation to register for medicine or engineering at one of the white universities willing to take in non-white students—a procedure which, with government permission, can still be followed when non-white universities have no suitable courses available. (One calculation at Western Cape University is that there are 450 coloured students registered at Cape Town and Witwatersrand and 500 at the University of South Africa. The staff seems much more constant than at Terfloop.)

The problems of inadequate preparation at school, particularly in scientific subjects and their ancillaries, seems constantly to plague the white universities as well as those set up to provide non-whites with higher education. Entrance to South African universities is by exemption from matriculation. At Stellenbosch, for example, more than 60 per cent of the matriculation examination provides more or less automatic admission. As elsewhere, the attrition through the years is rapid—one estimate is that 200 first year students intending to specialize in chemistry will be whittled down to 120 in the second year and will produce sixty graduates at the end of their third year. The number of students staying on for an honours degree is usually about 20 per cent of the first degree class. Witwatersrand and Cape Town, both English-speaking, seem to be able to be a little more selective, as is the new Afrikaans University of the Rand (established three years ago at Johannesburg).

There is a good deal of talk about curriculum development in the schools but very little interest among the university establishments in reforms of the higher education system itself, except that there is now something of a tussle under way at Stellenbosch, with younger members of the faculty urging that the diversity of its students predicates a need for a broader education than the classical pattern based—especially at the Afrikaans-speaking universities—on what is called in South Africa the continental pattern. (The word European is of course preempted.) The Afrikaans University of the Rand, with only a few hundred students to boast of, is also hoping to break new ground by relying on open-ended teaching patterns and on a modified tutorial system.

Research, when there is time for it, is as imaginative

and excellent as anybody could ask. Pretoria has made a name for itself, for example, in chromatography and other methods of chemical separation; Stellenbosch has a strong line in the ecology of desert animals; Western Cape is pursuing the study of indigenous snake venoms with distinction; Cape Town has geochemistry and marine ecology to boast of, as well as the study of insect pheromones, and is also the possessor of Moon rock samples; Witwatersrand has a phytotron and a splendid programme in geophysics. One difficulty in estimating how much research work is carried out in universities stems from the way in which South African universities are encouraged by the government to buy new equipment by raising loans from outside sources repayable over forty years. Even the most casual inspection shows, however, that university departments are almost lavishly equipped (even if the oceanographers at Cape Town now wish they had a larger boat in which to go to sea). The most acute shortage is that of time in which to pursue research. Research grants as such usually come from the CSIR, and tend to be at once rather small in amount and uncoordinated—a pattern which is as much a consequence of the jealous independence of the universities as of niggardliness by the CSIR. Dr C. E. M. Brink, the deputy director of the CSIR and the man responsible for administering university research, has made a reputation for himself by his diligence in interviewing potential grant recipients.

People in the universities say that it is comparatively easy to keep in touch with scientific developments elsewhere. In the past three years at least, universities seem to have made conscious attempts to provide members of staff with opportunities to travel abroad. Good research students are encouraged to seek research experience overseas before settling down to academic life in South Africa—a practice begun by the English-speaking universities and now being copied by the others.

There seems some confusion about the rate at which people going abroad for study are lost to South Africa—in the early sixties, it seems that 70 per cent of those going abroad failed to come back again but now the ratio is more like 20 per cent. Academics and administrators agree that in the past few years there has been a tendency for people from abroad, especially Western Europe and the United States, to seek openings at South African universities. Part of the explanation seems to be that academic salaries (in contrast with those to be earned in school teaching) are comparatively high, that South Africa abounds with interesting problems to be solved and that, in any case, positions in the United States are now much harder to find than in the past.

University Politics

FROM outside South Africa, it is hard to appreciate how strongly some of the universities, but especially Witwatersrand and Cape Town, dissent from government policy on *apartheid* or, as it is now called, separate development. Both universities have commemorated their protests in 1958 against the decision by the government that non-white students should not register at white universities except by government consent by plaques spelling out their devotion to the cause of academic freedom. Two weeks ago, the students at Witwatersrand surprised themselves and the authorities with the orderliness of a march through Johannesburg to protest against the banning (i.e., isolation) of seventeen alleged terrorists acquitted in the previous week of the government's allegations against them. At both universities, members of staff and students alike—dissenters, if any, go undeclared—talk of their universities as "liberal", but it may be significant that this attitude is not usually accompanied by a willingness to embrace modern causes such as student representation.

At other universities, members of the teaching staff are usually less outspoken. The key question is whether, if there were no laws, the admission of non-white students to white universities would be welcomed. Even before the formal declaration of *apartheid*, only the Universities of the Witwatersrand, Cape Town and Rhodes (Grahamstown) chose to be "open". It is therefore remarkable how many members of the academic staffs at Afrikaans-speaking universities will now admit that the admission of non-white students would be desirable—at one university, it was estimated that 80 per cent of the staff was so minded—and one distinguished dean surprised himself and his small audience in conversation two weeks ago when, under pressure, he said that the introduction of the formal regulations of *apartheid* had probably been mistaken.

In the event, both Cape Town and Witwatersrand have quite large numbers of coloured (but not black) students on the books, usually in schools of medicine and engineering where there are at present no corresponding courses in the non-white universities. One estimate is that nearly a third of the first year class in the medical school at Cape Town consists of coloured students, and that the corresponding percentage at Witwatersrand is 25 per cent.

There is of course no way of knowing how representative university opinions may be of those of the rest of the population, but the chances are that in Africa as elsewhere they are more enlightened. In the sciences, moreover, there is no evidence at all that the politics of a university help to determine the scale on which government support is provided. To be sure, in more contentious subjects such as the social sciences, there is some disquiet over cases such as that in which a sociologist at the University of the Witwatersrand has this year been prosecuted for alleged indecorum in a study of the comparative incidence of the application of the death penalty to the black and white communities—the judgment (an acquittal) implied that the author of this research would have been guilty of contempt of court if he had been made aware in advance of the derogatory implications of his work. To scholars of all persuasions, this is a cloud much larger than a man's hand.

Should Palaeoanthropology be Big Science ?

THE natural flora and the ancient rocks of southern Africa have given South Africa a compelling interest, but the attractions of the terrain for palaeoanthropologists are even more striking. Indeed, the valley to the west of Johannesburg, in which the two caves of Swartkrans and Sterkfontein confront each other from the sides of two low hills a mere mile apart, suggests that so far palaeontologists have scratched only the surface of southern Africa. And even so, there are signs that the materials being excavated from these sites may soon outstrip the efforts being made to record and interpret them.

In the past year or so, the activities at Swartkrans directed by Dr C. K. Brain, the director of the Transvaal Museum at Pretoria, have been the most spectacular (see *Nature*, **225**, 1112; 1970). Although the cave has been known since 1948, when it was first investigated by Dr Robert Broom and Dr J. T. Robinson, work in the past few years has helped to resolve several important questions about the development of hominid creatures in South Africa and their relationship to similar processes of evolution in East Africa.

The interest of the excavations at Swartkrans is that they have thrown new light on the coexistence of two types of hominids—the robust type (*Paranthropus robustus*) and the gracile creatures now thought to lie more directly on the line of descent to modern man. But Swartkrans has also yielded evidence of the use of tools of a primitive character by these early creatures and a rich store of mammalian fossils by means of which it is possible to relate the ages of the South African fossils to those being found in East Africa, where dating by radioactive methods is possible. Dr Brain has also extracted from Swartkrans a vast collection of fossils of small rodents, presumably killed by predatory birds—one half of a plastic bucket in the Transvaal Museum is filled with such remains and may yet yield a sensitive understanding of the ecology of Swartkrans in the late Pliocene. Across the valley at Sterkfontein, the pace of excavation has also been increased in recent years. At both sites, a dolomite cave has been filled with breccia. Swartkrans seems to have been connected with the surface of the hillside by a vertical chimney channelling remains to the underground site. Sterkfontein was once a cave inhabited by Pliocene creatures and excavations by Professor P. V. Tobias have now shown that the cave is perhaps three times as great in horizontal extent as originally thought when it was first excavated by Professor Dart. Here, too, there are vast stocks of tiny rodent bones as well as the remains of larger mammals and Australopithecines. A large galvanized hut is used as a storeroom for fossils recovered from the breccia, and systematic attempts are being made to catalogue and to classify hominid and mammalian fossils—baboons, for example. Professor Tobias says that the work would be faster if more people were available.

At the other end of the South African Republic, in Cape Town, Mr Q. B. Hendey seems to be confronted by an even more daunting collection of fossils—the marine deposits at Langebaanweg on the coast in the south-western Cape Province. Although there have so far been no traces of hominid remains, the site has yielded one of the richest collections of vertebrate remains in the whole of Africa. As well as more than sixty distinct types of mammals belonging to eleven different orders, fish, amphibians, reptiles and birds are also represented as fossils. One of the hazards of the work is that the fossils are recovered from a mixture of sand and phosphates in which they are embedded only with the connivance of the mining company extracting phosphates from the region. The result is that much of the vast collection of material at the South African Museum at Cape Town consists of broken pieces of bones. Nevertheless, there seems no doubt that in due course the site at Langebaanweg will eventually be made to yield a good understanding of the fauna of South Africa in the early Pleistocene and before. One interesting find, for example, is that of the first late Pliocene bear to have been found south of the Sahara. Outside observers cannot fail to be impressed with the disparity between the richness of the fossil store and the resources of the people concerned with it. Everybody speaks highly of the willingness of the CSIR to help with money, although the amounts involved are comparatively small—Rd20,000 is considered handsome. Although the work at Swartkrans and Sterkfontein is going ahead quickly, moreover, the shortage of trained people does limit the extent to which other sites can be explored. Yet a comparison between Sterkfontein and Swartkrans has already yielded an understanding of human evolution which could not have been obtained by the detailed exploration of one of them alone—this comparison, for example, is one of the reasons for excluding the hypothesis that the difference between the heavily built and the lighter forms of hominid creatures from two million years ago is an example of sexual dimorphism—why should one site yield females only and the other predominantly males ? From this point of view, there is probably a great deal to be said for a more thorough search for cave sites in the dolo-



Lateral view of the facial skeleton of the Swartkrans hominid articulated with a robust australopithecine jaw bone.

mites of South Africa, but everybody seems to shudder a little at the prospect of how to describe and interpret what material might be found. Is it possible that the unique character of the palaeoanthropological sites in South Africa—five of them have so far produced material of great importance—is a sign that something should be done to conduct this enterprise on a scale that would not be justified elsewhere ?

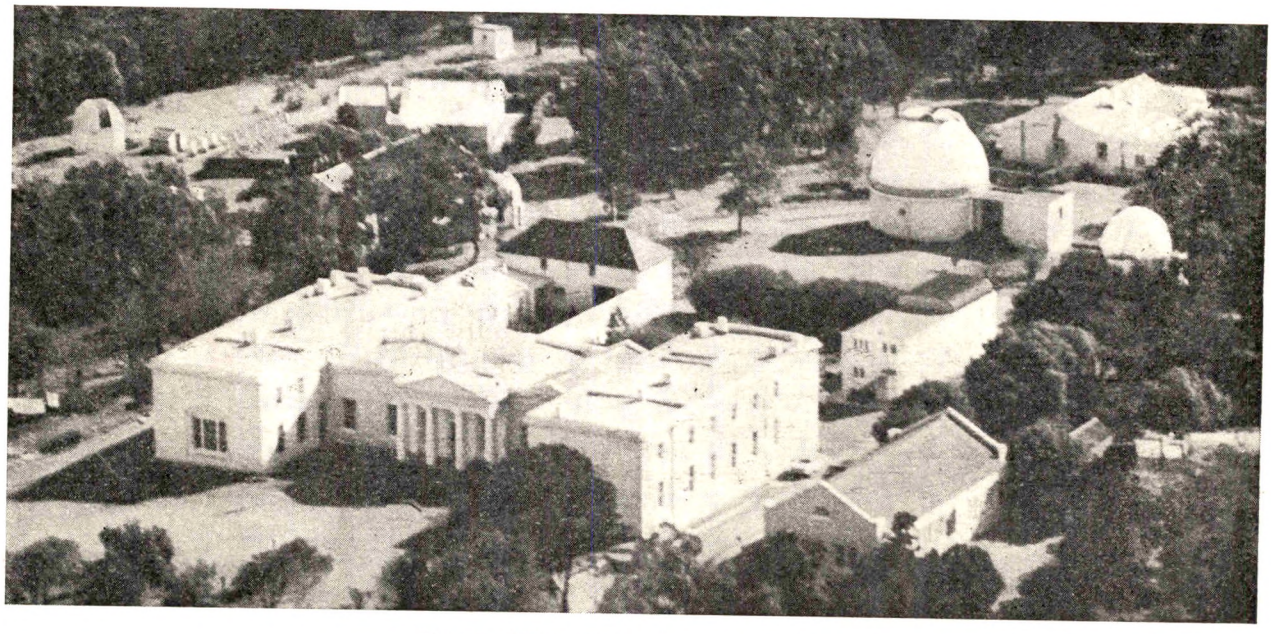
Good Cheer for Astronomers

THE reorganization of the Royal Observatory at the Cape and the Union Observatory of the South African Council for Scientific and Industrial Research into a single operating agency is likely to be a great boon for South African astronomers as well as for those in Britain and elsewhere in the northern hemisphere for whom South Africa is a convenient vantage point south of the equator. After two years of planning, the point seems now to have been reached at which there is a clear plan for the next few years. To begin with, the 40-inch reflector at the Royal Observatory will be moved at the beginning of 1972 to a site at Sutherland, 200 miles north of Cape Town and sufficiently far on the leeside of the western escarpment to be comparatively free from cloud. Work has already begun on the roadworks around the site, which seems to have been chosen both for its accessibility and for its good seeing. Although there will eventually be a landing strip for light aircraft, astronomers at the Cape will be able to reach the site in four hours of reasonable driving on a good clean road, but those who will have to commute from Johannesburg will have farther to travel and are less keen on the choice of Sutherland. They are also somewhat discontented with what they consider the loss of their identity in the joint venture.

In the development of the joint observatory, which will be financed by the CSIR in South Africa and the Science Research Council in Britain, the willingness of Sir Richard van der Riet Woolley to become the director of the new organization seems to have played a crucial part. "A name to conjure with" is what one administrator said, and it is not every decade that the retiring Astronomer Royal has a passion for building telescopes and an origin in South Africa as well (Sir Richard graduated from Cape Town in 1929). The first phase of the work at Sutherland, consisting of site development and the building of a housing for the 40-inch telescope, is intended to be completed by the end of 1971. By that time, plans will have been completed for dismantling the instrument and for moving it, in pieces as large as possible, to the new site.

The 40-inch telescope is intended only as the first instalment of the project, which will be completed later in the seventies by the addition of a larger telescope. Approaches have apparently been made to the trustees of the Radcliffe Observatory in Pretoria, where the 74-inch reflector which has been operating since 1948 and which has contributed valuably to the study of the southern skies in the past two decades, is supported by a contract with the British Science Research Council which expires in 1974.

The Radcliffe instrument has suffered less than the Cape Town instrument from the problems of city lighting systems, but the suburbs of Pretoria have now crept a long way up the hill on which the Radcliffe Observatory stands. In the past few years, it has been necessary to restrict the use of the telescope for photometric work, but spectroscopic observations are still entirely feasible. In the past few weeks, the telescope has been fitted with one of the image intensifiers developed by Professor J. D. McGee at Imperial College, London, and the director of the observatory, Dr A. D. Thackeray, hopes that the new equipment will provide not merely greater sensitivity but also a



The Royal Observatory, Cape of Good Hope.

measure of convenience. No doubt the fate of the telescope will ultimately be determined not merely by the desirability of moving it to Sutherland but by the feasibility of such an undertaking, and much will depend on whether it is considered that the driving mechanism would need to be replaced.

Observing conditions at the Cape have for several years required that observation should be confined to the brighter objects in the sky. The Cape Observatory, built in a way that shows quite clearly its Victorian naval origins, will remain in use as a base for observers working at Sutherland as well as an experimental station for the department of astronomy on which the University of Cape Town has set its heart. The university is clearly anxious to exploit in every way it can the opportunities which the new arrangements will provide, and it has already begun to scour the world for just the right man to fill the chair.

The Republic Observatory in Johannesburg has also fallen a victim to city light, but Johannesburg is more than three times as far away as is the Cape from Sutherland. Moreover, it must have been plain for several years to those in charge that the future for an observatory in or near Johannesburg would always be restricted and indeed the observatory has been without a permanent director for some time.

The joint observatory at Sutherland is only one of several smaller observatories maintained by other organizations from outside South Africa. The site of the Republic Observatory's annexe 40 miles north of Johannesburg also houses a southern station maintained by the Leiden Observatory, there is a 60-inch reflector at the Boyden Observatory maintained by the Harvard College Observatory near Bloemfontein as well as a 36-inch Baker–Schmidt telescope and a 27-inch refractor maintained by the University of Michigan at another site in Bloemfontein. In the past few years there has also been some useful development in radio astronomy, partly stimulated by the construction of the NASA tracking station at Hartebeesthoek (which is used in its spare time for radio astronomy), while a number of universities such as Rhodes University, Grahamstown, have established useful experimental groups in this field. South Africa nevertheless must still regret the decision of the consortium of European nations operating the European Southern Observatory to settle not in South Africa but in Chile.

NUCLEAR FUEL

Enrichment Up the Sleeve

THE announcement in June this year by the Prime Minister of South Africa that the Atomic Energy Board is developing a novel technique for the enrichment of uranium has left a great many scientists in South Africa as puzzled as their colleagues abroad about the potential of the claim. Some are openly sceptical. What follows is an account, culled from authoritative sources, of what has been happening.

First, the enrichment process, whatever it is, is based on the use of gaseous UF6. The project has been under way since 1959, when the Atomic Energy Board first set out to investigate what seemed a potentially promising alternative to the then standard method of enriching uranium—the building of a diffusion plant. By their own accounts, the people concerned seem at first to have been unwilling to believe their good luck in having found such a promising route to enriched uranium, and now regret the wasted years from 1963 to 1966 when it would have been possible to launch a more intensive study of the process. Since then, however, experimental studies have been carried out which have justified the decision to build a pilot plant.

The year 1966 is held to be significant, for then the Atomic Energy Board announced the abandonment of work on the reactor concept which had until then been its chief public pride and joy—a reactor operating with natural uranium (of which South Africa has plenty) and liquid sodium as coolant. In retrospect, this decision is said to be a necessary redeployment of skilled labour. But as things are, however, the scale of activity at the main research establishment at Pelindaba has grown slowly over the years (and cost Rd 5·28 million in 1969). The study of sodium loops continues, and there are some who hold that the new enrichment process will make it economical to return once more to the PELINDUNA reactor concept, for the inconveniently large power output of the original concept may now be avoidable.

The development of the enrichment process, whatever it is, is rigorously separated both geographically and financially from the public work of the Atomic Energy Board. One of the reasons given for the decision to make public the existence of the pilot plant is the fear that the growing scale of activity associated with it would soon inevitably leak out. Although the Prime Minister said that "under South African conditions, a large-scale plant can be competitive with existing plants in the West", it does also appear that the expected saving of cost is likely to be a comparatively small fraction and certainly less than 50 per cent of the current American price for enrichment—at present rather less than $30 per unit of separated work. In short, it looks very much as if there is a good idea on which the builders of the pilot plant can chew, but that development has not yet reached the point at which the production costs can be accurately predicted, which in turn implies that the operation may yet be frustrated by economics if not by technology.

One of the obvious advantages of a process for enriching uranium indigenously is that South African uranium suppliers would enjoy a larger slice of the profits to be obtained from nuclear power. The political leverage provided by a supply of enriched uranium is also evident. One of the difficulties with which the government of South Africa is now faced, however, is that of knowing how to exploit the process without also making it available to potential competitors. By all accounts, the process, whatever it is, could be replicated in a comparatively short time by any comparable organization worth its salt.

But what is the process? The statement that the South African process "is unique in its concept" is held to exclude the use of centrifuges. For what it is worth, Dr A. J. A. Roux, the chairman of the board, accepted a bet of £5 that chromatography is somehow involved with such alacrity that attempts are now being made to hedge the bet with an investment in one other frequently mentioned process—a combination of a cyclone and gas diffusion.

NEWS AND VIEWS

Accelerating Somatic Cell Genetics

THE standard technique for detecting linkage between human genes and assigning them to particular chromosomes involves correlating the chromosomal make-up with the biochemical properties of interspecific hybrid cells. This approach to somatic cell genetics stems, of course, from the pioneering work of Watkins and Harris. They found not only that inactivated Sendai virus mediates cell fusion and, in Harris's words, converted "cell hybridization from an exercise in the exploitation of chance events to a method of general applicability", but also that when mouse and human cells are fused the hybrids preferentially lose most of their human chromosomes. If a normal human cell is fused with a mouse cell carrying some mutant biochemical marker and the hybrids are grown in conditions which do not support the growth of either parental cell, the hybrid should retain the human chromosome which carries the human gene that compensates for the defective murine function. A series of independently isolated clones of such hybrids should always have one human chromosome in common, that which carries the compensating gene. Once this has been established the hybrid cells can be analysed for a wide range of other human enzyme activities to see if any of these are always present and therefore carried on the same chromosome as the compensating gene. Using this basic approach Bodmer and his colleagues and Ruddle's group, for example, have established that the human genes specifying lactate dehydrogenase B and peptidase B are linked (*Nature*, **227**, 248; 1970), while Weiss, Green and Basilico have established that the thymidine kinase gene is probably located on a human chromosome of group *E*.

This mouse–human system, however, has its disadvantages. The hybrids emerge only slowly, and so experiments are protracted; the number of available genetic markers for the mouse cells is limited and the mouse cells in culture have as many as sixty chromosomes, which makes the identification of individual chromosomes in the hybrids extremely tedious. It will be no surprise therefore if in future the use of Chinese hamster cells, as described on page 329 by Kao and Puck, becomes widespread. Kao and Puck find that when these cells, which have only twenty chromosomes, are fused with normal human cells, the loss of human chromosomes is very rapid. Only a week after the fusion many of the hybrid cells have about twenty chromosomes and, moreover, eleven different auxotrophic markers of Chinese hamsters cell have already been isolated.

Kao and Puck have used three of these in an experiment which failed to establish any new linkage groups but proves beyond doubt the feasibility of their approach. They took Chinese hamster cells which were dependent for growth on the supply of either proline and glycine or proline and inositol, and fused them with normal human fibroblasts. The hybrid cells were grown in calf serum which does not support the growth of the human parental cells and in the presence of proline, but in the absence of either glycine or inositol. This condition selects, of course, for the retention of the human chromosomes which carry genes for the biosynthesis of glycine and inositol respectively. Once the hybrids had been isolated they were tested for the capacity to grow in the absence of proline. If the gene specifying the ability to make proline is on the same chromosome as either the glycine or inositol genes the hybrids should invariably grow in a medium lacking proline. In fact Kao and Puck found the proline requirement of their hybrids to be very variable. Out of fifteen hybrid clones able to grow in the absence of glycine only five could also grow in the absence of proline, while only seven of the forty-two clones able to grow without inositol could also grow without proline. Clearly there is no linkage between these genes in human cells, moreover the human lactate and malate dehydrogenase genes do not seem to be linked to the inositol gene.

It is disappointing that no new linkage groups have been detected but the technique of using doubly marked Chinese hamster cells and normal human cells holds great promise for the future. It means that possible linkage between two genes can be tested for directly, and there are eleven genes corresponding to the eleven auxotrophic markers of Chinese hamster cells that can immediately be studied by this approach. Kao and Puck's experiments have also thrown some light on the process of chromosome shedding. It seems that when cells with such disparate generation times as those of Chinese hamster cells, which divide every twelve hours, and human cells, which divide every twenty-two hours, are fused the condensation of the two sets of chromosomes becomes asynchronous. The faster growing partner seems to dominate the heterokaryon, and this leads by one or more mechanisms to the loss of the chromosomes of the laggard. From observations of individual cells Kao and Puck suggest that chromosome pulverization and the clumping of human chromosomes is related to the mechanisms of shedding and it seems likely that many of the human chromosomes are lost as groups rather than individually.

No Support for Gravitational Waves

MUCH can be learnt about the methods of science from situations in which discoveries of a rather contentious nature are made by one scientist or group, and are then searched for by other interested workers, often without success. During the past year or two there seems to have been a rash of examples. In 1968, cosmic ray physicists at the University of Utah announced that a rather well established behavioural pattern of the intensity of cosmic ray muons with zenith angle had been found to break down at energies above several hundred GeV (*Phys. Rev. Lett.*, **19**, 1487; 1967). Their results seemed entirely convincing, yet nobody else has been able to reproduce them, either with similar experiments or by approaching the problem from a different standpoint. Professor C. B. McCusker's triumphal announcement to the last international cosmic ray conference that he had found the elusive quark hiding in the cores of extensive air showers has likewise received no corroboration (*Phys. Rev. Lett.*, **23**, 658; 1969). The latest candidate for addition to the list of unsubstantiated discoveries is gravitational radiation—but the problems entailed in proving or disproving it are immense.

In 1969 Professor J. Weber reported that he had detected gravitational waves (*Phys. Rev. Lett.*, **22**, 1320; 1969). This was the culmination of years of patient work on two rather carefully designed detectors situated 1,000 km apart at the Argonne National Laboratory and the University of Maryland. The vibrations of two large metal cylinders with natural frequencies about 1,660 Hz are measured by piezoelectric instruments and coincidences between the two signals are taken to indicate the existence of gravitational waves. But where is the supporting evidence? Weber continues to find coincidences between his detectors and has even recently suggested that the greater part of the gravitational radiation he detects comes from the galactic centre.

In this issue (page 346), thirteen workers from five laboratories in Great Britain report attempts to confirm Weber's findings by searching for the small amount of radio frequency energy almost certainly accompanying gravitational waves. At each of five sites (Cambridge, Dublin, Glasgow, Harwell and Jodrell Bank), a pair of dipole aerials was set up to detect radiation arriving within a frequency band of 1 MHz about 151 MHz from almost anywhere in the sky. A number of coincidences between two of the stations were found, but tests have shown that it is unlikely that they are attributable to gravitational waves. Five coincidences were observed between three of the stations, but none of these was consistent with emission from a distant source. No coincidences, however, have been observed between four or all five of the stations. The outputs of the stations have also been examined. Since Weber's suggestion that the galactic centre may be a source of gravitational waves, the collaborators in the radio waves have put a directional detector in Malta and have replaced four of the original five detectors with directional types. It is possible with this array to observe the galactic centre and look for radio pulses which could be compared in time with the coincidences observed by Weber with his own apparatus. This could, however, pose difficulties because of the separation of the two experiments on the Earth's surface and the small probability of the two sets of apparatus observing the galactic centre at the same time.

Negative results from their experiment do not by any means invalidate Weber's original claims. There could be many reasons why radio pulses associated with gravitational waves may not be observable. The radio waves could be absorbed between their source and the Earth or, indeed, there may be no radio emission associated with gravitational waves. This, however, seems unlikely, for the violent conditions necessary to emit the enormous amounts of energy in the form of gravitational waves are almost certain to provide also the ideal source for radio signals.

LIQUIDS

Electrical Switching in Alloys

by our Solid State Physics Correspondent

THE disclosure by G. Busch, H. J. Guentherodt, H. U. Kuenzi and A. Schweiger (*Phys. Lett.*, **33**, 64; 1970) that a liquid alloy of tellurium and selenium has the electrical properties of a switch comes very much as a bonus in the search for a non-crystalline version of this important circuit element. So far little headway has been made in explaining the switching behaviour in amorphous solids discovered by Ovshinsky two years ago, and liquids are always one degree harder to understand. But the common features in the disordered structures of liquids and amorphous solids offer the chance to pick out some fresh clues as to why the electrical resistance of these materials should dive so sharply on a small change of voltage, and may help to point to the type of amorphous substance which can perform this function most efficiently.

Busch *et al.* found a sharp transition between resistive and conductive state in various alloys of both tellurium and selenium and tellurium and sulphur. They observed switching behaviour up to 200° C above the melting point with the samples held between graphite or platinum electrodes. The property was apparent from the sharp breaks in the current voltage characteristic.

In the simplest form of switch the resistance changes reversibly as the voltage is altered. It is, however, possible to obtain bistable switching action in which the device remains indefinitely in the desired "on" or "off" state until specifically switched out of it. The understanding of solid state switching is still too insecure for anybody to be sure whether the latter type of memory switch is related to the former variety or if it arises from an essentially different electronic process.

Busch *et al.* believe that they have witnessed a memory or threshold type of action, but they confess to being eager to publish their first results of switching in a liquid alloy before embarking on what must necessarily be a highly speculative assay on the theory.

METAL VAPOURS

New Way of Synthesis

from our Inorganic Chemistry Correspondent

SINCE Skell and Westcott (*J. Amer. Chem. Soc.*, **85**, 1023; 1963) first described the production and reactions of carbon atoms in the gas phase, the use of high temperatures and low pressures to produce unstable gaseous atoms and molecules, which can be rapidly reacted with stable molecules to produce new compounds has been of increasing importance.

Recently P. L. Timms (*Chemical Communications*, 1033, 1969; *J. Chem. Soc.*, A, 2526; 1970) has extended this technique to the production of vapours of transition metal atoms at very low pressures ($\sim 10^{-5}$ Torr) and has shown that these vapours can be reacted with gaseous compounds such as trifluorophosphine, PF_3. The reactions proceed cleanly and give good yields in gram quantities of several unusual compounds which are either unknown or difficult to prepare by other methods.

For example, when chromium vapour is reacted with PF_3 the compound $Cr(PF_3)_6$ is produced in 60 per cent yield on the metal. If benzene vapour is used instead of PF_3, chromium dibenzene, $(C_6H_6)_2Cr$, is produced.

When iron vapour and trifluorophosphine are used, two volatile products are isolated; the first is the already known compound $Fe(PF_3)_5$, and the second has the molecular formula $Fe_2P_8F_{22}$, and seems to have the structure $(PF_3)_3Fe(PF_2)_2Fe(PF_3)_3$. With cyclopentadiene iron vapour gives ferrocene, $(C_5H_5)_2Fe$. With benzene, however, iron vapour forms a compound which explodes on warming to $-50°$ C; this may be $(C_6H_6)_2Fe$.

Cobalt, nickel and palladium vapours give with trifluorophosphine respectively $[Co(PF_3)_4]_x$, $Ni(PF_3)_4$ and $Pd(PF_3)_4$. The palladium compound is fairly unstable and decomposes back to metallic palladium and trifluorophosphine. Oddly enough no compounds are formed with manganese or copper vapours.

Timms has also shown that the transition metal vapours can be reacted with mixtures of volatile compounds to give compounds containing mixed ligands. Thus when vapour is reacted with a mixture of phosphine, PH_3, and trifluorophosphine, PF_3, both $Ni(PF_3)_4$ and $Ni(PF_3)_3PH_3$ are formed.

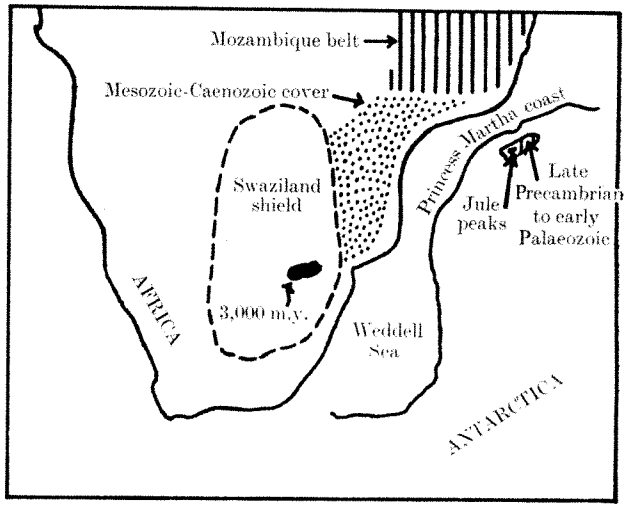
It is very likely that transition metal vapour reactions will prove useful in the synthesis of a wide range of new transition metal compounds of low oxidation state, and may in many cases provide an easier synthetic route to already known compounds.

CONTINENTAL DRIFT

Dating the Fit

from our Geomagnetism Correspondent

THE matching of discrete geochronological zones across the boundaries of continents thought to be adjacent before the onset of continental drift does not, in itself, prove that drift has occurred, but it is strong supporting evidence. The correlations across the boundary between South America and Africa are particularly impressive because they are based on many dated rocks. Other pre-drift configurations have not been so well documented geochronologically, however; and the fit of Antarctica with southern Africa has hardly been examined at all in this way. But Halpern (*Science*, **169**, 9771; 1970) has obtained a rubidium–strontium age of 3,060 ± 80 million years from a single sample of biotite granite from Jule Peaks, Antarctica. This is comparable with the age of the granites of the Swaziland shield (see map). He suggests that this supports the continental reconstruction of Dietz and Sproll (*Science*, **167**, 1612; 1970) in which the Princess Martha coast of Antarctica fits against the coast of southern Africa—as long as the Swaziland shield extends north-eastwards below the observed Mesozoic–Caenozoic cover.



One swallow does not, of course, make a summer; but to support his claim, Halpern has also collected information on the stratigraphy of the relevant coasts and finds some general correlations. Thus the early Palaeozoic to late Precambrian granitic rocks of the Mozambique belt (about 440 to 800 million years) correspond in age to sedimentary and crystalline rocks in Antarctica. Precambrian sedimentary rocks in the age range 1,400 to 1,800 million years are also found in both regions, as are late Palaeozoic to Jurassic volcanics. To these must be added the new Precambrian correlation, although many more ages are required to make it really convincing. Incidentally, Halpern's biotite granite is the oldest Antarctic rock reported so far.

RAMAN SPECTROSCOPY

Impact of the Laser

from our Inorganic Chemistry Correspondent

LASERS have wrought a transformation in Raman spectroscopy through their use as sources of illumination. The recent rapid growth of interest was evident at the international conference on Raman spectroscopy, held in Oxford from September 13 to 17. Whereas a few years ago it was difficult and sometimes impossible to

obtain a Raman spectrum from a powdered sample, even when several grams were available, spectra are now routinely obtained on a few milligrams of powdered sample. Raman spectra can also now be obtained from single crystals of quite modest size because of the small cross-sectional area of the illuminating laser beam, and some results of work with small single crystals were reported by Dr D. M. Adams and co-workers (University of Leicester). The Raman spectra of matrix isolated species, previously only studied by infrared spectroscopy, have also been obtained; both from alkali halide matrices by Drs D. B. Fitchen, A. R. Evans and C. Sawicki (Cornell University), and from noble gas matrices by Drs G. E. Levi, D. O'Hare and D. Hatzenbulher (Michigan State University).

The high coherence and narrow band width of laser light mean that it is possible to obtain Raman spectra of higher resolution than before, and also that very low frequency Raman shifts can readily be measured. The improved resolution possible in laser Raman spectroscopy is beginning to approach that possible with the most highly developed infrared techniques. One of the most interesting developments reported at the conference was the use of interferometric techniques for studying pure-rotation and rotation-vibration spectra of gases by Raman spectroscopy, reported by Drs J. J. Barrett (Perkin–Elmer Corporation) and W. J. Jones (University of Cambridge).

Another feature of laser illumination, if a pulsed laser is used, is the great intensity of the oscillating electric field that is incident on the sample. The induced polarization, P, is related to this electric field, E, by the equation

$$P = \alpha E + \beta E^2 + \text{etc.}$$

The αs are the well known coefficients of the polarizability tensor and their magnitudes are responsible for the intensities of Raman lines. The βs are so small that normally the term in E^2 can be ignored, but when the light intensity is sufficiently high, as it is in a powerful pulsed laser, this term becomes important and hyper-Raman lines due to it can be seen. The selection rules for hyper-Raman lines are quite different from those for conventional Raman activity. For example, the torsional oscillation of the ammonium, NH_4^+, ion in a crystal lattice is hyper-Raman active. Successful detection of the hyper-Raman effect in various systems, using a pulsed laser and a multichannel photon counting detector, was reported by Dr P. D. Maker (Ford Motor Company, Dearborn, USA).

PROTEINS

Gelatin to Collagen

from our Molecular Biology Correspondent

THERE are, of course, people who place collagen in the outer darkness of molecular biology—a dreary protein with no function to fire the imagination. This is a pity, for collagen has engaged the attention of some of the most accomplished practitioners of the day in the fields of sequencing, X-ray analysis and physical chemistry. Apart from the potential utility of deeper structural and chemical knowledge about collagen, lessons of the broadest relevance to protein chemistry are often to be drawn. An example is in the nature of the refolding process, when the randomly coiled gelatin chains revert to ordered collagen forms. Earlier studies of the process left an uncomfortable residuum of unexplained and often paradoxical observations, and many of these have now been assimilated into a generalized scheme by Harrington and his co-workers.

In the first of five articles of intimidating thoroughness, Rao and Harrington (*Biochemistry*, **9**, 3714; 1970) re-examine the renaturation kinetics of homogeneous chains—that is to say, separated α_1 or α_2 chains, which differ from each other in sequence. Optical rotation was used to follow the change. The first objective was to resolve conflicting reports on the order of the reaction, which in the event was found to be a function of the concentration. Only at very low protein concentrations was the reaction first-order; with increasing concentration, the order of reaction rose rapidly. The simplest explanation is that the triple-helical native structure can be generated intramolecularly by antiparallel folding—which according to model-building studies is a feasible way of achieving a collagen fold. This conclusion was borne out by molecular weight measurements. The temperature-dependence of the renaturation reflects the slow formation of longer helices near the melting temperature, for only these are stable in this range. This mechanism leads to a high degree of order, whereas by contrast at low temperatures short helices are rapidly produced. Regeneration of three-chain structures, as opposed to the intramolecular triple helix, is thus expected at high concentrations (where the order of reaction approaches three), and at the highest workable temperatures. The intramolecular folding rate for a series of collagens is also apparently related to the sizes of proline clusters in the chain, which most probably act as the primary nuclei for helix initiation.

To analyse the temperature effect further, Harrington and Karr (*ibid.*, 3725) have extended the working range downwards below 0° C, by adding glycol to the aqueous solutions. Working with *Ascaris* (invertebrate) collagen, in which the disulphide cross-links peculiar to this species have been broken, they find that the refolding rate has a maximum at a temperature about 46° C below that of melting. Two or more processes differing in their temperature dependence are therefore at work, and become rate-determining in different temperature ranges. In the upper region the kinetics are as expected for a reaction limited by a nucleation rate, the growth of the helix from the nuclei being rapid. At low temperatures, on the other hand, it is the growth of the helix, by incorporation of residues one at a time, that evidently becomes rate-determining. This kind of situation, in which the two types of mechanism become dominant in turn, could, one might suppose, be widely relevant in refolding and assembly processes.

When the cross-links in *Ascaris* collagen are preserved, or when cross-links are chemically introduced into uncross-linked collagen species, refolding is rapid and essentially complete, for the three chains are then always in register. The remaining three articles, by Hauschka and Harrington, are concerned with the characterization of the refolding mechanism, especially in relation to the cross-linked molecules. They relate *p*H effects on renaturation rate (*ibid.*, 3734) to evidence about the distribution of charged groups in the two types of collagen, and they consider (*ibid.*, 3745) the cooperativity of the refolding reaction, which is acutely temperature-dependent, and they also derived

the lengths of helical nuclei which seem to be common to all collagens. They show (*ibid.*, 3754) that the kinetics of renaturation of cross-linked chains bear out all the predictions of theory for three independent first-order processes—nucleation, helix growth and a slow annealing process. In *Ascaris* collagen, the native state is a disulphide-linked lattice of single chains, each folded on itself into a triple-helix.

FLUORESCENT LABELLING

Getting a Grip on tRNA

from a Correspondent

ONE of the most favoured techniques for disentangling the structure of transfer RNA is to label certain of its bases. But it is difficult to know just what goes where in the molecule. Labelling the bases by chemical modification, for example, is far from straightforward because several bases may be affected by any particular chemical agent. A more elegant handle to approach this problem is provided by a built-in label in the phenylalanine tRNAs of yeast, wheat and rat liver, all of which have a fluorescent base, "Y", adjacent to the anticodon loop.

Earlier this year, by measuring the energy transfer between the Y base and a chromophore attached to the 3′ end, Beardsley and Cantor (*Proc. US Nat. Acad. Sci.*, **65**, 39; 1970) showed that the distance from the anticodon to the 3′ end, where the amino-acid would be attached, is 24–30 Å. Two recent reports from Cantor's laboratory (*Biochemistry*, **9**, 3514, 3524; 1970) continue the exploration of the structure of yeast phenylalanyl tRNA through various measurements based on the fluorescence properties of Y and a bound dye, ethidium bromide, which also fluoresces.

They report that the fluorescence intensity of the phenylalanyl tRNA is enhanced two and a half times when magnesium is added to a solution containing the tRNA at 320 nm. This change is accompanied by a 5 per cent drop in the extinction coefficient of the tRNA at 260 nm, simultaneously with a similar change in the magnitude of the principal positive circular dichroism band. The fluorescence intensity of Y, as in the case of many fluorescent molecules, increases in nonpolar solvents. Thus the enhanced intensity as a function of the magnesium concentration is interpreted as a conformational change that tucks Y into a hydrophobic region of the tRNA molecule. That such a large change occurs in the fluorescence intensity compared with absorption or circular dichroism, makes the Y base a very sensitive probe for studying the conformation around the anticodon loop of this tRNA. Beardsley and Cantor have shown that this change is not due to the interaction of the magnesium ions with Y itself, for there are no such changes in its fluorescence when the tRNA molecule is digested with nuclease. There is a further decrease in fluorescence as a consequence of the increased contact of Y with water molecules.

This natural fluorescent probe has also been used to demonstrate directly that there is a specific interaction with polyuridylic acid in a solution free of ribosomes (Eisinger *et al.*, *Proc. US Nat. Acad. Sci.*, **65**, 638; 1970). They found a small blueshift in the fluorescence spectrum as well as a drop in the fluorescence intensity in the presence of U_5 as well as poly U, but not in the presence of poly C.

Further information can be obtained by observation of the nanosecond depolarization in the fluorescence of Y. Because the molecule is tumbling freely in solution, the rotational diffusion coefficients can be estimated by measuring the randomization of the fluorescence induced by polarized light at the excitation frequency. The interesting result is that Y is rotating faster than the whole tRNA molecule. It is not possible to deduce whether this is due to free rotation of the fluorescent group itself or to a relatively flexible attachment of the anticodon loop to the rest of the tRNA. There is, however, greater fluorescence anisotropy than expected for a completely freely rotating chromophore, indicating some hindrance in the motion of Y.

In their second report in *Biochemistry*, Cantor *et al.* report that the dye, ethidium bromide, which intercalates into DNA, binds to tRNA chiefly at one site in the presence of a low concentration of magnesium. From a consideration of the quenching effect that this dye molecule has on Y, they conclude that it is probably bound in the double helical region adjacent to the anticodon loop. From nanosecond depolarization measurements, they conclude that the data are consistent with a tRNA molecule that changes from a prolate ellipsoid with an axial ratio of 2–3 in the presence of magnesium to one with an axial ratio of 4–5 when magnesium is removed. An interesting incidental observation is that the tRNA can still be activated by the activating enzyme with the bound dye present.

NUCLEOTIDES

Fragments and Factors

from our Nucleic Acids Correspondent

SOMETIMES experiments are elegant and interesting even if they do not lead to entirely unambiguous conclusions. Two pieces of work recently reported in the *Journal of Biological Chemistry* display these symptoms markedly. Siddiqui and Ofengand (*J. Biol. Chem.*, **245**, 4409; 1970) have attempted to study the function of the modified nucleotide pseudouridylic acid (Ψ) in tRNA. They chose to examine the formylmethionine tRNA from *E. coli*, because it only contains a single Ψ residue (occurring in the common sequence GTΨC). Unlike most other nucleotides, Ψ can be cyanoethylated with the aid of the reagent acrylonitrile, affording a way to probe its function in the tRNA. Unluckily, this tRNA also contains the modified nucleotide 4-thiouridylic acid, which also reacts with acrylonitrile to yield the S-cyanoethyl derivative. Siddiqui and Ofengand circumvented this problem using large fragments of the tRNA obtained by digesting it with T_1 ribonuclease. The fragments were fractionated by chromatography on columns of DEAE-cellulose. In this way they partially purified a piece encompassing about a quarter of the tRNA molecule (from the 5′ end) containing the 4-thioU residue, and also a fragment—the remaining three-quarters of the tRNA—containing the Ψ. The two pieces could "stick" together, and subsequently accepted methionine in the normal way.

It was therefore possible to effect the cyanoethylation of each of the fragments separately, and assess the amino-acid-accepting activity of such modified fragments when recombined with the complementary unaltered pieces. When 4-thioU alone was cyano-

ethylated, accepting activity was virtually unchanged. Cyanoethylation of the Ψ residue, however, decreased accepting activity considerably. Regrettably, the elusive conclusion slips from their grasp at this point. It cannot definitely be said that Ψ is specifically involved in forming a complex with the activating enzyme and that this complex cannot form when the Ψ is modified, for the effect of the cyanoethyl group can be to distort the tertiary structure of the tRNA molecule. This might be the true reason for the lack of activity. The second reason is likely to be correct because others have shown that the excision of the TΨCG or the replacement of Ψ by 5-fluorouridine does not lead to loss of acceptor activity.

Bernardi and Leder (*J. Biol. Chem.*, **245**, 4263; 1970) describe the purification and characterization of a mutant G (elongation) factor from *E. coli*. This protein φ is involved in the translocation of the peptidyl-tRNA from the recognition site to the holding site on the ribosome, and surprisingly comprises more than 2 per cent of the soluble protein of the cell. A steroid antibiotic, fusidic acid, inhibits the activity of G factor, as well as the equivalent factor in mammalian cells.

Bernardi and Leder examined five strains of *E. coli* which were resistant to fusidic acid, but found that the G factor of only one of them resisted the effect of the acid on *in vitro* protein synthesis. They purified the mutant factor and also the sensitive factor from the parent strain to apparent homogeneity by chromatography on DEAE-'Sephadex' and DEAE-cellulose. The factors could not be distinguished by chromatography or electrophoresis on polyacrylamide gel or even by immunological reaction. There must be a definite difference between them, however, presumably as a result of a single mutational event, for their properties are indubitably distinct. The specific activity of the G factor from the sensitive strain was roughly twice that of the resistant factor throughout purification. Furthermore, the Michaelis constant for the hydrolysis of GTP by the resistant factor together with ribosomes was twice that of the sensitive factor. Normally GTP hydrolysis is concomitant with peptide chain elongation and is also inhibited by fusidic acid.

These findings could be explained in two ways, and it is not possible to distinguish between them. On the one hand, the mutation causing fusidic acid resistance might directly alter the binding site or catalytic site of the G factor for GTP, if the G factor contains such a site. On the other hand, the GTP might be hydrolysed in a site on the ribosome with the required presence of the factor, and the mutation would alter the interaction of the factor with the ribosome, and indirectly the ribosomal GTPase site. A long term aim of these studies will be to exploit this mutation in an important component of the protein-synthesizing system in genetic studies, now that a firm biochemical basis has been established. In a note added in proof, the authors claim that the fusidic acid resistance mutation lies close to the streptomycin resistance marker.

FISH

Exclusive Diet of Mullet

from our Marine Vertebrate Correspondent

THREE species of grey mullet are found in English waters but none are so abundant as to be of great importance to the fishing industry. There is, however, a small fishery for them chiefly in the south-west, which has recently produced about twenty tons of fish per year. Probably because they have only a marginal economic value the biology of grey mullet has never been investigated by fisheries workers in Britain, and knowledge of these fish is sketchy. They do have some value to anglers and, largely because of this, such recent work as has been undertaken has been in the capable hands of the Irish Inland Fishery Trust's staff (see for example, M. Kennedy and P. Fitzmaurice, *J. Mar. Biol. Assoc. UK*, **49**, 683; 1969).

A recent study of the natural history of the English grey mullets by C. F. Hickling (*J. Mar. Biol. Assoc. UK*, **50**, 609; 1970) goes a long way towards redressing the past neglect of these fish. Hickling has paid particular attention to the food and feeding of the mullets, especially the thick-lipped mullet (*Crenimugil labrosus*), although also dealing with aspects of the growth rate, maturity, spawning and fecundity in this and the other species.

The mullet has three ways of obtaining its food. In the first, and probably the least important, large edible items are ingested by a sudden extension of the protrusible mouth into a circular sucker, and simultaneously a powerful pumping of water through the gills creates a suction current whisking food with a cloud of mud into the mouth. Another feeding process involves scraping, with the hard-edged lower lip used as a scoop to skim off fine algal growth or bottom living diatoms. Grey mullet can often be seen browsing in this way up and down vertical surfaces or over rocks. Finally, they can stir up a cloud of mud and swim into it filtering the mud vigorously through the gill complex.

Mullet have long been notable among fishes for their gizzard-like pyloric stomach and for the extreme length of their gut, which has usually been attributed to their presumed herbivorous diet. Hickling confirms that in large *Crenimugil labrosus* the gut may be five times the length of the body, but he suggests that the long gut is not a result of herbivorous diet but is correlated with the large quantities of indigestible inorganic matter in their diet. Combustion of the cardiac stomach contents of fifty-four fish revealed that only two had more than 30 per cent organic matter, and prior examination showed that much of this was woody matter and chitin. It seems, however, that grey mullet are selective feeders and do not simply suck in mud and food fortuitously, because the stomach contents contain a notably higher percentage of organic matter than the sediment in which they are feeding.

Evidently these fish use their gill rakers to strain off the minute animals such as harpacticoid copepods and free-living nematodes which form their principal animal diet. Mullet fed in an aquarium on cod roe (which contained only 8 per cent inorganic matter) were later found to have 82 per cent of inorganic matter in their stomachs, and this tends to confirm earlier suggestions that the mineral matter in the muscular pyloric stomach is an essential grinding paste, and that this organ is in effect a gizzard.

Grey mullet are thus of singular interest, both for their adaptation to this diet and because few other fish can live on such poor food material. They seem to have evolved a feeding regimen in which they suffer no competition from other fishes.

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British High Flux Beam Reactor

by

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The neutron scattering technique has become an accepted method for the study of condensed matter. Because of the great scientific and technical value of neutron experiments and the growing body of users, several proposals have been made during the past decade for a nuclear reactor devoted primarily to this technique. This article reviews the reasons for and history behind these proposals.

SLOW neutrons have been used widely in condensed matter physics, for many problems in chemistry and for industrial experiments. They represent a powerful tool of considerable versatility for several reasons. First, neutron wavelengths are about equal to the spacing between atoms in condensed matter while the corresponding energies are about equal to the spacing of the energy levels that determine chemical and physical properties of the material. The scattering by an isolated nucleus is isotropic and elastic so that there are no complications due to form factors, selection rules or competing cross-sections. Diffraction effects from neutrons scattered by nearby nuclei will show up atomic structure, and energy exchange between the neutron and matter gives a new determination of energy levels; simultaneous analysis provides new information.

Second, neutrons penetrate matter in bulk, having mean free paths of about 1 cm, so that surface effects can be neglected and a sample can be measured inside a furnace, cryostat or pressure container. Third, because neutron cross-sections are distributed virtually at random, both throughout the periodic table and between isotopes, light elements can be studied in the presence of heavy elements and the scattering properties of a sample can be changed by changing the isotopes without altering the chemistry of the sample. In addition the scattering of differently oriented but otherwise identical nuclei is different: for example, hydrogen with its two possible spin orientations scatters with a weak mean scattering amplitude and a strong random value. The incoherent scattering arising from such random effects contains no structural information, but makes possible the study of essentially "one-atom" dynamical effects.

Fourth, neutrons possess a magnetic moment so that magnetic atoms scatter neutrons through an effect which allows the structure and the dynamics of the electron spins, and hence of certain chemical bonds, to be studied. Finally, the range of wavelengths available extends from 0·3 to 10 Å and (by varying scattering angles) spacings or particle sizes between 0·1 Å and 20,000 Å can be measured.

This range of useful properties led to a wide usage of the technique once satisfactory neutron spectrometers became available. In many experiments it is possible to measure the strength, position and width of some peak in the dependence of scattering intensity on angle of scatter or on energy change of the neutron. Where such a well defined peak exists the interpretation is usually unique and quantitative in an absolute way. Experiments of this type are listed here as i–v. Some of the most exciting new applications, such as vi–x, however, arise where the scattering is not organized into well defined peaks, for example, the study of liquids, amorphous substances and disordered crystals. It is possible often to prepare samples that differ only in one feature, and a comparison of these samples allows the scattering by that feature to be studied, as in experiments xi and xii. Clearly neutron scattering experiments have an unparalleled richness, variety and depth, and touch on most of the problems connected with the several forms of condensed matter. Although neutron fluxes are low by comparison with, for example, X-ray or light fluxes the experiments are powerful. It is to extend the range and power of these experiments that the building of a high flux beam reactor (HFBR) is being considered. Extensive descriptions of the results of neutron experiments have been published[1,2], and I shall describe some of them briefly.

EXPERIMENTS IN WHICH NEUTRON SCATTERING HAS PROVED USEFUL

(i) Atomic structure of crystals deduced from Bragg reflexions in a way analogous to X-ray work.

(ii) Magnetization distribution in crystals deduced similarly; no alternative technique is available.

(iii) Frequency, wavelength, amplitude and polarization of crystal thermal vibrations, deduced from energy and momentum changes of neutrons; no alternative technique is available.

(iv) Thermally excited oscillations of magnetization in magnetic crystals, deduced similarly; no alternative technique is available.

(v) Energy of thermally excited molecular oscillations and rotations in crystals, liquids or gases—analogous to infrared spectroscopy but frequently specific to proton motions and applicable to symmetrical molecules which are otherwise inaccessible.

(vi) Static pair and triplet distributions of atoms in a liquid (including the partial distribution functions in a binary system).

(vii) Time-dependent pair distribution in a liquid, which is related to the cooperative modes of motion.

(viii) Self diffusion of atoms on atomic distance and time-scales (related to small angle incoherent scattering).

(ix) Time-dependent correlations of magnetization near and above the magnetic critical point.

(x) States of electrons in conducting substances (related to the magnetic scattering).

(xi) Distribution of atomic or magnetic distortion around atomic scale defects in crystals has been measured.

(xii) Dynamics of different molecular groups in one molecule has been measured by the proton/deuteron substitution technique.

Atomic Distributions in Crystals

The determination of the positions of light atoms in structures and the differentiation of atoms of similar atomic number are possible with neutrons. This has removed serious restrictions on X-ray work which have held up studies of hydrogen bonding, hindered rotations of ions, hydride structures, heavy element oxides, carbides and nitrides, and studies of ordering in many interesting metallic systems.

Samples with linear dimensions of about 1 mm are used and through the use of automatic diffractometers the reciprocal lattice can be explored in three dimensions in a reasonable time. Recent work has not only resulted in a greatly increased accuracy of parameter determination (for example, a standard deviation of ± 0·008 Å is claimed for the location of the hydrogen atoms in hexamethylene

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tetramine) but has provided general conclusions on groups and series of compounds, simply because of the greatly increased output of information. For example, the high accuracy of hydrogen location which neutron beams permit has made it possible to examine accurately the problem of random and rigid body motion in relatively simple molecules which can be studied theoretically by quantum chemists. Thus naphthalene, resorcinol, salicylic acid and hexamethylene tetramine have been studied in detail by university groups at Harwell. In the case of hexamethylene tetramine it has also been demonstrated that a concurrent refinement of X-ray and neutron data produces a more satisfactory result than the use of either set of data separately.

Magnetization Distributions and Liquid Structure

Although the close connexion between strong magnetic properties and the anti-symmetry condition for electron wave functions was recognized by Heisenberg in 1926, it is still impossible to predict the magnetic structures of matter. Even for technologically important materials, such as ferrites and garnets, there is only a skeleton empirical knowledge of the facts, provided mostly by neutron techniques. The magnetic electrons are the outer electrons and are interesting for chemical as well as physical reasons.

Scattering of polarized neutrons from unsymmetrical antiferromagnets, for example, MnF_2, shows the transfer or sharing of spin between "magnetic" ions (M_n^{++}) and "non-magnetic" ions (F^-). This is the most direct evidence of covalency available and is in a form suitable for direct comparison with theory.

The detailed understanding of the liquid state remains one of the outstanding problems of condensed matter physics. Measurements of the liquid structure factor for monatomic liquids can be made to higher accuracy than with X-rays and good data can be obtained with the samples inside a cryostat, furnace or pressure containers. This makes it possible to measure pressure and temperature derivatives which, in addition to providing data on the pair distribution function, allows the triplet and higher order distributions to be studied. Thus a new insight into liquid structure is being achieved.

Of special interest are studies on binary (A–B) systems (for example, alloys or fused salts) where by isotopic substitution three or four separate diffraction patterns can be obtained for a single system. From these data the three pair distribution functions (AA, BB and AB) can be obtained and which transform treatment of binary systems from "primitive guesswork" into a "scientific subject". Examples are provided by the resistivity of a binary alloy (CuSu) and the interaction potential in a fused salt (CuCl).

Crystal Lattice Vibrations

Neutrons are unique in that their energies are thermal when their wavelengths are atomic. This makes possible precisely defined experiments on phonons and spin waves, which have led to a new understanding of interatomic forces.

The first vibrational studies to be completed in detail (at Chalk River in Canada) showed that although some of the forces between atoms in a metal were best thought of as comparatively local, some are of very long range, and probably represent forces derived from the electron gas in some way. In ionic and covalent crystals the phonon frequencies showed some unpredicted effects, now regarded as evidence for polarization by deformation. A study of lead has shown anomalies which are due to the interaction between the electron Fermi surface and the phonons—the interaction which is the basis of the current explanation of superconductivity. Solid helium has been investigated in a range of temperature and pressure and the phonon dispersion relations for argon at 4 K have been determined.

Inelastic scattering of neutrons also provides a means of studying directly fluctuations in the magnetization of crystals (spin waves), for example, in ferromagnets, antiferromagnets and ferrimagnets. Again this is the only available method for work on bulk substances (there is a resonance technique available for thin films of ferromagnets). In metallic systems the inadequacies in the original theoretical formulation of the problem are now clearly exhibited for the first time.

Liquid Dynamics

Inelastic scattering from liquids facilitates study of the dynamics of atoms or molecules. In the study of diffusion it is possible to examine the details of a single diffusive step on a time scale of about 10^{-12} s. The scattered neutron spectrum can be compared with the predictions of diffusion theories, and it can be shown, for example, whether the diffusion process consists of atoms jumping from site to site, as in a solid, or of combined movements of groups of atoms, analogous to Brownian motion, or, as actually occurs, the process is a combination of several mechanisms. The significance of this knowledge in the study of chemical reactions has been realized, but the accuracy of individual experiments, and the throughput of different compounds, is severely limited by present fluxes and facilities.

The discussion of the short wavelength collective motion in liquids is complicated because these modes are heavily damped due to their strong interaction with one another. Their behaviour is described by current–current correlation functions which can be measured only by the neutron scattering technique. This area is stimulating theoretical work on many body systems, and this in turn leads to more exacting neutron inelastic scattering studies of simple liquids. Such work is relevant also to the study of the dynamics of amorphous materials. Here the problems are usually greater, for molecular materials are being examined, and work so far (on SiO_2, for example) has produced results on a limited number of modes only.

Molecular Dynamics

A major growth point has been the application of neutron techniques to the study of molecular spectroscopy in gases, liquids and solids of interest to chemists. The studies include work on small molecules, on synthetic and natural macromolecules and on molecules absorbed on surfaces, and they complement infrared and Raman spectroscopic techniques. The special features of the neutron technique include the removal of the restrictions imposed by optical selection rules and very different (often simpler) intensity relationships. The intensity of a vibrational band is simply related to the amplitudes of nuclear displacement in the normal mode. Particular use is made of the abnormally high scattering cross-section of the proton; substitution of ^2H for ^1H greatly reduces the

scattering cross-section and provides an important diagnostic tool. Recent applications include studies of (i) torsional modes in $CH_3{\cdot}CH_3$, $CH_3{\cdot}CF_3$ and $CH_3{\cdot}CCl_3$, (ii) the dynamics of polymer main-chains, side-chains and crystal lattices to obtain information relevant to structure–property relations in polymers, and (iii) direct observation of hydrogen bonds.

Defect and Domain Scattering

Long wavelength neutrons can be scattered from crystals only if deviations from the crystal perfection are present, so that the scattering of neutrons of 4–10 Å gives a powerful method of studying defects on a size scale of 2–20 Å. This is just below the resolution of the electron microscope and there is considerable promise in this method for the study of crystal damage caused by, for example, radiation.

An important recent development is the investigation of diffuse scattering by non-stoichiometric compounds and solid solutions. It is already clear that the technique has great potential for providing information about clustering and short range ordering of atoms in various solids.

High Flux Reactor Proposal

The justification of a large neutron beam facility lies in the overall impact made by neutron experiments on the study of condensed matter. Generally a neutron experiment is so definitive that it is used as the basic experiment in a field, and the interpretation of the data obtained by other methods is greatly improved through the use of reliable molecular knowledge. Thus neutron scattering has a central role, well illustrated by the examples of magnetic structure and crystal lattice dynamics given here. In these cases the entire field rests on the neutron data, which are used by theoreticians and experimentalists for further interpretations and extensions of knowledge. Thus the important ideas in a discussion of neutron beam facilities are those involving the broader implications of neutron work, the strengthening or weakening of entire fields of study and the basic significance of the study of condensed matter in the life of mankind.

The gradual development of neutron scattering techniques through the 1950s had led by 1960 to a relatively sophisticated programme for the study of condensed matter. This was recognized by the International Atomic Energy Agency (IAEA) which started in 1960 a series of conferences on the inelastic scattering of neutrons in solids and liquids. Four such conferences have been held. In the United Kingdom the main team doing this work was concentrated at Harwell, but other groups (chiefly at Cambridge) were involved. Thus in 1960 the future heralded the expansion and development of a new and very powerful technique. Many very important problems, concerned with the study of condensed matter and with significant practical applications, were waiting to be investigated, and many scientists both within the Atomic Energy Authority (AEA) and in universities were anxious to help.

But the reactors used until this time had been designed for other purposes—primarily the irradiation of materials of significance to the reactor power programme: the Harwell reactors DIDO and PLUTO are called materials testing reactors for this reason. As a result they had three basic disadvantages for thermal neutron scattering experiments. These concerned the shield, the building and the flux. First, the shield of a materials testing reactor is arranged to be just thick enough to reduce the radiation to a safe biological level. But this level produces a very high background in thermal neutron scattering experiments and a substantially thicker and more efficient shield is required in a purpose built reactor. Second, the building which houses DIDO and PLUTO is too small to contain much of the sophisticated neutron scattering equipment. When a reactor is used to irradiate materials there is little need for horizontal space: most of the specimens are loaded in vertical rigs and headroom above the reactor is the major requirement. Horizontal space is needed only for coffins to accept active materials from the horizontal holes. For this reason the containment buildings of the DIDO and PLUTO reactors are very small, and when the neutron scattering apparatus was installed both the design and operation of the spectrometers were restricted. Third, the flux available from these reactors is not optimized for neutron beam experiments. The fuel elements are spaced at distances which allow samples to be placed between them, and the thermal neutron flux then peaks at the irradiated specimens. Unfortunately, neutron beam tubes are placed at the ouside of the core and are therefore not in the region of maximum flux. In the design of a thermal neutron beam reactor, the fuel elements are placed as close together as possible so that the core has a very high flux of fast neutrons. These fast neutrons enter the heavy water around the core and are thermalized, producing a peak in the thermal flux at approximately the position of the neutron beam tubes. This design advantage together with a higher power level will give the HFBR a factor of 15 advantage in flux over DIDO and PLUTO.

Table 1. RESEARCH REACTOR COMPARISONS

Country	Reactor	Date	Relative flux for typical beam user
UK	DIDO	1956	1
UK	PLUTO	1957	1·5
UK	HERALD	1960	0·3
USA	HFBR	1966	10
USA	HFIR	1967	15
Germany/France	HFBR	1971	20
Holland	HFR	1962	1·5
Belgium	BR2	1961	1·5
Sweden	R2	1960	1·5

During the 1960s, the horizontal holes of DIDO and PLUTO at Harwell and HERALD at Aldermaston were progressively taken over by the neutron beam programme until now they are almost all used for neutron spectrometers. Because of the low neutron fluxes, very substantial efforts were made to maximize the efficiency of the neutron spectrometers and of the detecting and data handling systems. In addition the number of university users increased during this period from six to approximately 100: thus the development anticipated in 1960 took place in spite of the restrictions in the use of the existing reactors. This, of course, demonstrates the attraction of the neutron scattering technique for the study of condensed matter.

A comparison of research reactors available or under construction in various countries is shown in Table 1 (the Germany/France figure is an estimate). It shows that the DIDO and PLUTO reactors are old compared with the reactors in other countries and the newer reactors in the USA and Europe have a significant flux advantage

(as well as more modern experimental facilities). Until 1960 the reactors at Harwell had kept in step with developments in other countries, but we have now fallen materially behind.

It was in anticipation of these developments that a group of reactor designers and neutron beam experimentalists proposed in 1960 that a new HFBR be built at Harwell. A design study was made of this project and in 1961 a proposal was ready.

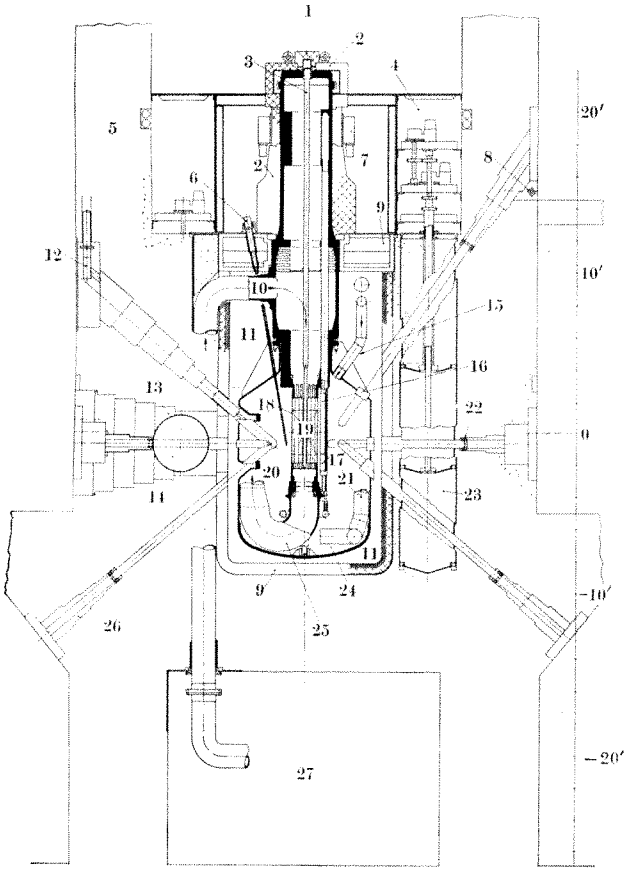


Fig. 1. Cross-section of the proposed high flux beam reactor. 1, Fuel element handling room; 2, shielding; 3, in-core rig; 4, beam shutter drives; 5, high density concrete biological shielding; 6, irradiation facility; 7, control rod drives; 8, removable shield block; 9, thermal shield; 10, core D_2O inlet; 11, CO_2 void; 12, cold source services; 13, removable shield; 14, cartwheel beam shutter; 15, reflector inlet; 16, upper control rods; 17, lower control rods; 18, zirc II pressure barrel; 19, core; 20, core D_2O outlet; 21, reflector D_2O outlet; 22, aluminium windows; 23, split rotating beam-shutter; 24, reflector tank-D_2O at atmosphere pressure; 25, core circuit-D_2O at 400 pounds/inch^2; 26, low angled beam hole; 27, sub-pile D_2O plant room.

This design was discussed later as the possible basis for a European international project, but no agreement was reached on various aspects of siting and finance, so that the project was shelved. In 1967 the French and German Governments set up a bilateral agreement to construct such a reactor at Grenoble, and this is now being built. The general outline design for a reactor suitable for the range of experiments appropriate to the United Kingdom has been completed, and there is strong user support for its construction. The proper channels of funding such a project of both technological and scientific promise involve more than one government department. The considerable expenditure involved (the Franco-German project budget is £11 million), naturally comes under close scrutiny when the national scientific budget is under great pressure, and the matter is still *sub judice*.

Matters singled out for careful study include the relative merits of a continuous reactor and a pulsed neutron source. The comparison is complicated because the continuous source techniques are better developed than the pulsed source techniques, and the two techniques are not equally applicable to all classes of experiments. The continuous beam reactor is clearly superior in experiments which require a continuous neutron beam and experiments based on long wavelength neutrons (greater than 4 Å). On the other hand, for some neutron time of flight experiments (for example, with relaxed collimation and for short neutron wavelengths) the pulsed neutron source will be superior. In the former category are most of the experiments with crystals, including the study of structure and dynamics using both the nuclear and the magnetic scattering processes. The long wavelength field includes studies of defects and many transient phenomena in liquids and polymers on a long time scale. On the other hand, in the category of time of flight experiments (with $\lambda < 3$ Å), concern is chiefly with inelastic scattering processes on a short time scale, particularly for the study of liquids, polymers and molecular spectroscopy. Experiments of this type are less detailed in interpretation at present, though they include significant work in a rapidly developing scientific area. Thus the decision between these sources rested on the balance of user interest. Because time of flight experiments of high quality can be done on the reactor, the larger number of users in the former category persuaded the minority in the latter category to accept the HFBR as the preferred source. This choice also satisfies the requirement that it is possible to prosecute a balanced programme on any major neutron source.

The HFBR project is ten years old and the present design is not far removed from the design of 1960, so that it might be asked whether the reactor is obsolete ? There are several reasons why this is not so. First, the reactor field is reaching a plateau in its development determined by the heat removal rates from the fuel elements (which in the HFBR are approximately 1 kW/5 cm of fuel element or 100 MW in 0·16 m^3). It is difficult to increase the heat rating and hence the flux without making major, and very expensive, changes in the design amounting to the development of a new reactor system. These changes might include very high flow rates of cooling water through the use of very high pressures or might involve the use of a liquid fuel rather than a standard metallic fuel element, but do not offer obvious advantage at a reasonable cost. Nevertheless, the 1970 design does include improvements in the design of the reactor components which have occurred during the intervening ten years. Second, a more significant factor is the balance between the different types of user or, in other words, the overall scientific programme. Special Science Research Council (SRC) committees have confirmed that the outcome of the discussions on the type of source to be preferred, held during the 1960s, is still valid, and therefore the basic decision on the type of source is still valid.

SRC Neutron Beam Programme

The SRC makes use of neutron beam time on three reactors owned by the AEA: these are the HERALD reactor at Aldermaston and the DIDO and PLUTO

reactors at Harwell. The flux available at the beam holes of HERALD is about one-third of that available at the DIDO and PLUTO reactors, but because many users cannot be accommodated on the Harwell reactors it is necessary to use this reactor as well. This programme is managed by the SRC Neutron Beam Committee which reports annually on progress. Its scientific reports cover the fields in which predictions of neutron beam interest have been made during the past few years and university scientists are translating into successful experiments many of the approaches which have been claimed to be practicable.

The value of neutrons to chemists as well as physicists is clearly evident in the scope of the reports on chemistry submitted by the Neutron Beam Committee. Approximately half of the scientists using the neutron beam facilities are from university chemistry departments. Some of the areas they cover were listed earlier in this article. Some proposals have been made and initial experiments conducted on biological problems with neutrons, but this field is in its infancy. It is anticipated, however, that it would expand rapidly through the improvements provided by the HFBR. An analysis of the present programme into categories of use is given in Table 2.

Table 2. DISTRIBUTION OF UNIVERSITY USERS OF AEA REACTORS

Field	No. of users
Chemical crystallography	30
Magnetic crystallography	18
Inelastic excitation of solids	21
Liquids	11
Defects in solids	13
Properties of neutrons	2
Total	95

Each physical technique seems to follow a general pattern of development. First, it is developed by the physicists concerned; in this case the nuclear physicists. It is then taken over by other physicists for use in basic research; in this case the condensed matter physicists. Subsequently other scientists become interested (in this case chemists and biologists) and use the technique. If as a result the technique is shown to have a wide scientific application it is used increasingly for applied problems in many industries. This pattern of development can be seen, for example, in X-rays, in infrared absorption, nuclear magnetic resonance and so on.

It seems likely that neutron scattering is following this general pattern, and is entering the final phase of its application to applied and industrial problems. The number of industrial scientists using the neutron spectrometers at Harwell and Aldermaston is small but increasing: their scientific and technical interest covers the same general field as the SRC scientists, though the motivation of their companies is industrial development. Although their work includes the structure and the dynamics of condensed matter, in many cases the samples are complex. This is partly because they are a mixture of many species and partly because large molecules are involved. Thus as the neutron scattering technique is developed for chemical and biological purposes we may expect parallel development in its use for applied and industrial applications. In some areas this is aided by the high efficiency of modern neutron spectrometers and the fact that they operate continuously and on a routine basis.

Present Proposal

Fig. 1 shows the cross-section of the proposed HFBR. Twelve horizontal beam holes are fed by different neutron sources. Several observe the ambient neutron spectrum in the heavy water while others are connected to the cold neutron source for long wavelength experiments and a group is connected to the hot neutron source for short wavelength experiments. In addition to these horizontal holes several inclined holes (both upwards and downwards) have been included. The flux viewed by these holes is lower than that seen by the horizontal holes but is still higher than that available on existing reactors. Also shown in the figure are the multi-hole arrangements for the cold and hot neutron sources. A limited number of irradiation facilities are provided also. It is proposed to operate the reactor at 100 MW and the maximum flux available would be $1{\cdot}5 \times 10^{15}$ neutrons/cm^2 s. The fuel is an Al/U alloy cooled by heavy water under a pressure of $28{\cdot}2 \times 10^4$ kg/m^2.

It is anticipated that after the project has been approved it will take five years to reactor criticality, and all twenty or so experiments should be in full swing after two more years. Because the neutron spectrometers could be constructed while the reactor is being built the experimental programme could commence as soon as the reactor is operating continuously at full power. At present it is proposed that the new spectrometers should include all the major types in use at Harwell: but the position will be reviewed at stages so that new techniques can be included in the instruments for the HFBR. Present proposals include the provision of several time of flight spectrometers, several three-axis neutron spectrometers as well as diffractometers for single crystal analysis. Other specialized instruments for long wavelength work and defect studies are proposed, making in all twenty-seven different spectrometers.

The higher flux may be used in various ways, for example; smaller specimens may be examined, higher resolution in energy and/or momentum may be used, the wavelength range may be extended to higher or lower limits, the statistical accuracy of the data may be improved, the study of weak scattering processes in the presence of strong ones may be improved and so on. The choice between these alternatives is open to the scientist concerned, for neutron spectrometers can be designed with flexibility in mind. Once again the neutron beam work is characterized by its breadth, and when transferring to a new reactor the neutron beam scientist reconsiders his experimental programme and makes use of the higher flux in one (or more) of the six or so ways open to him.

With a reactor of the type proposed the university teams would be able to consolidate their neutron beam programmes and to maintain the lead the United Kingdom has given in the field of condensed matter science.

I thank Dr W. M. Lomer for helpful suggestions and Professor E. W. J. Mitchell for permission to use some material from his SRC report on the scientific case for the HFBR.

[1] *Thermal Neutron Diffraction* (edit. by Willis, B. T. M.) (Oxford University Press, 1970).
[2] *Inelastic Scattering of Neutrons* (IAEA, Vienna, 1969).

Genetics of Somatic Mammalian Cells: Linkage Studies with Human–Chinese Hamster Cell Hybrids

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Linkage relationships between human genes have been determined by following chromosome losses from human–Chinese hamster hybrid cells.

GENES can be located on the chromosomes of mammalian cells by taking advantage of the extensive chromosome loss which occurs in certain inter-specific hybrids after cell fusion *in vitro*. Normal human cells have been fused with mouse cells which contain a specific genetic defect affecting growth, and hybrids selected in conditions which fail to support the growth of either parental cell alone. These are cultured until there is maximum loss of their human chromosomes. Any human chromosome which is uniquely common to all the surviving clones is presumed to carry the normal gene which compensates for the defect in the mouse cell.

This system has shown that the thymidine kinase gene is probably located on a human chromosome or group E[1–3]. But experimental difficulties exist: it may be difficult to identify unequivocally the human chromosomes in the hybrid karyotype; there may be chromosomal breaks and rearrangements; the number of human chromosomes is not always reduced sufficiently, and only a limited number of usable genetic markers are available in the mouse culture. Moreover, because cell growth and loss of chromosomes occur slowly, these experiments take a long time.

We have described previously[4–6] a Chinese hamster cell system which offers advantages for this purpose. First, its generation time (T_g) in defined media is 12 h or less. This represents a greater difference from that of the human cell (T_g about 22 h) than does that of the mouse (T_g about 16–20 h). Cell hybrids presumably grow slowly initially[3], but if extensive loss of chromosomes of the slower parent occurs, they approach the growth rate of the faster parent. An automatic selection advantage should therefore be established for rapid isolation of human–Chinese hamster hybrids which have lost the maximum number of human chromosomes. Second, this standard Chinese hamster cell has only 20 chromosomes instead of approximately 60 as does the mouse L cell. Third, we have already isolated eleven different auxotrophic markers, so that simple linkage experiments can be performed in this system.

Thus a Chinese hamster cell with two auxotrophic deficiencies can be fused with a normal human cell and chromosome loss of the hybrids allowed to take place in the presence of only one of the critical metabolites. If the resultant clones are always simultaneously relieved of both deficiencies, both genes must be carried on the same chromosome (provided that the different clones contain only one human chromosome in common). But if there is random loss of the unselected marker, the two genes must be unlinked, and in this case, detailed chromosome analysis is not necessary. We report here the dynamics of chromosome loss from human–Chinese hamster hybrid cells and linkage determinations between selected genes.

The auxotrophic mutants of the CHO-K1 Chinese hamster cells have been described in previous publications[4–7]. These cells are cultivated in medium F12 (ref. 8) supplemented with 10 per cent foetal calf serum. Single cell plating was carried out in F12 from which specific omissions were made as required, and which was supplemented by the macromolecular fraction of foetal calf serum in an amount sufficient to support maximum growth of the cells[4]. The human fibroblasts originated in a skin biopsy from a newborn, normal male and were routinely cultured in F12 supplemented with 13 per cent human cord serum and 5 per cent foetal calf serum. The generation time of the CHO-K1 cells is approximately 12 h whereas that of the human fibroblasts is approximately 22 h in the present conditions. We used human cells which had previously undergone 20–25 generations of growth in culture.

Cell fusion was carried out as described[9] by mixing 2×10^5 cells of each type with 200 units of ultraviolet irradiated Sendai virus and incubating for 15 min at 4° C and then for another 15 min at 37° C. The cells were then placed in Petri dishes containing the respective selection media. These were prepared to permit growth of colonies only from hybrid cells formed by fusion of the Chinese hamster mutants with the normal human fibroblasts.

Chinese hamster cell mutants doubly deficient for inositol and proline (ino⁻pro⁻) and for glycine and proline (gly⁻pro⁻) were used. Different gly⁻ mutants, A, B, C, and D (ref. 6), were used in various experiments. Each mutant grows with a plating efficiency nearly 100 per cent in complete F12 supplemented with the macromolecular fraction of foetal calf serum and with zero plating efficiency if its specific auxotrophic requirement is omitted[5]. Single cells of normal human fibroblasts do not grow in F12 supplemented with the macromolecular fraction of foetal calf serum but do produce colonies if the corresponding fraction of human cord serum is supplied (Table 1); thus, by plating fused cells in a medium containing the macromolecular fraction from foetal calf

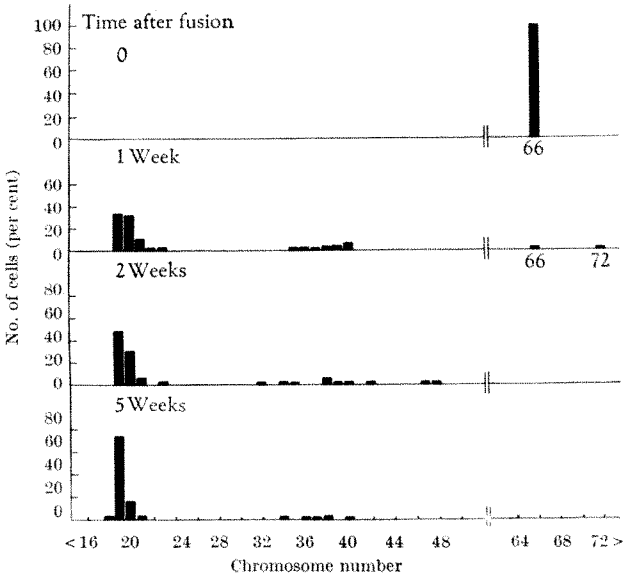


Fig. 1. Distribution of chromosome numbers in uncloned cultures of human–Chinese hamster cell hybrids, at various periods of incubation following fusion of the cells. Mitotic cells were collected after treatment with velban for 3 h.

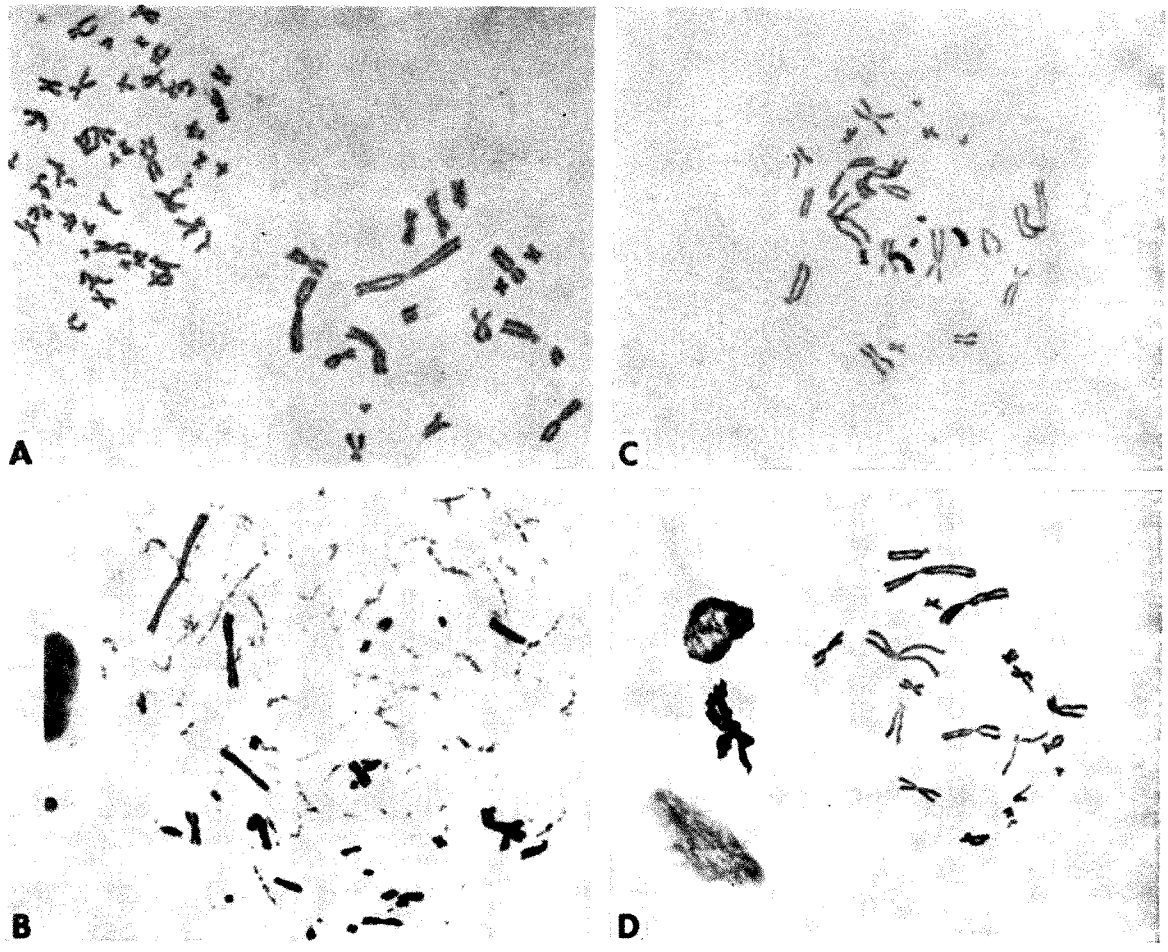


Fig. 2. Mitotic chromosomal patterns observed in a random culture of human–Chinese hamster cell hybrids sampled after 2 days of growth following fusion. *A*, A complete set of mitotic chromosomes from each parental cell is present in synchrony. *B*, All or most of the Chinese hamster and approximately ten presumed human chromosomes are mitotic. The remaining chromosomes display a "pulverized" appearance reminiscent of that obtained when a mitotic cell is fused with a cell in S. *C*, All or most of the Chinese hamster chromosomes but only about five of the human chromosomes are present. The two kinds of chromosomes have achieved synchrony, for all are in mitosis, although the presumed human ones seem more highly condensed and therefore more deeply staining. *D*, This cell is like *C*, except that the presumed human chromosomes are clustered together in a compact mass.

serum only, and lacking the specific auxotrophic requirements of the parental Chinese hamster mutant, colonies result only from hybridization of cells from the two animal species.

Chromosomal examination of mitotic cells from a fused cell mixture in a medium supporting growth only of the interspecific hybrids was carried out by standard methods[10] at various periods after fusion, and the distribution of chromosomal numbers was determined. Hybrid clones were grown from single cells plated two weeks after fusion and grown up in a selection medium containing proline, but not the other nutrient (glycine or inositol respectively). These hybrid clones were then tested by single cell plating to see whether they had retained their requirement for proline.

Examination for the presence of isozymes of lactate and malate dehydrogenases was carried out by acrylamide gel disc electrophoresis as described in Fig. 4.

Table 1. DEMONSTRATION THAT SINGLE HUMAN CELLS GROW WELL IN F12 SUPPLEMENTED WITH THE MACROMOLECULAR FRACTION OF HUMAN CORD SERUM, BUT NOT AT ALL IF SUPPLEMENTED ONLY WITH THE MACROMOLECULAR FRACTION OF FOETAL CALF SERUM

Concentration of macro human cord serum (per cent)	Concentration of macro foetal calf serum (per cent)	Plating efficiency (per cent)	Generation time (h)
10	0	13·0	25·0±1·7
15	0	14·5	22·7±1·1
10	5	16·5	22·2±1·1
0	10	0	—
0	15	0	—

Concentrations are indicated relative to the normal serum content of the respective macromolecular component.

Chromosomal Loss in Human–Chinese Hamster Cell Hybrids

Extensive loss of human chromosomes begins almost immediately. Fig. 1 shows the results of chromosomal counts made on a human–ino⁻ hybrid culture at 1, 2 and 5 weeks after fusion. Within 1 week, the chromosome number has fallen to a point close to and sometimes even less than that of the original CHO-K1 cell. Similar results were obtained in the fusion of normal human fibroblasts with the following CHO-K1 mutants: gly⁻A pro⁻, gly⁻B pro⁻, gly⁻C pro⁻, and gly⁻D pro⁻.

In the first several days of growth after fusion, the cell size is highly heterogeneous, presumably reflecting the heterogeneity of chromosome numbers during the early period of chromosome loss. The stabilized hybrids are much more uniform. The generation time of the stable hybrids is approximately 15–17 h, a value intermediate between the two parental cells. The hybrid which no longer requires inositol for colony formation grows with the same rate regardless of the presence or absence of inositol in the medium.

During the early growth period of the hybrid cell, the human and Chinese hamster chromosomes often are morphologically distinct because of a slight difference in the degree of condensation, a phenomenon also noted in other inter-specific hybridizations[11,12]. In addition we have observed that groups of human chromosomes cluster together. In Fig. 2 are shown three different patterns occurring in a random culture sampled two

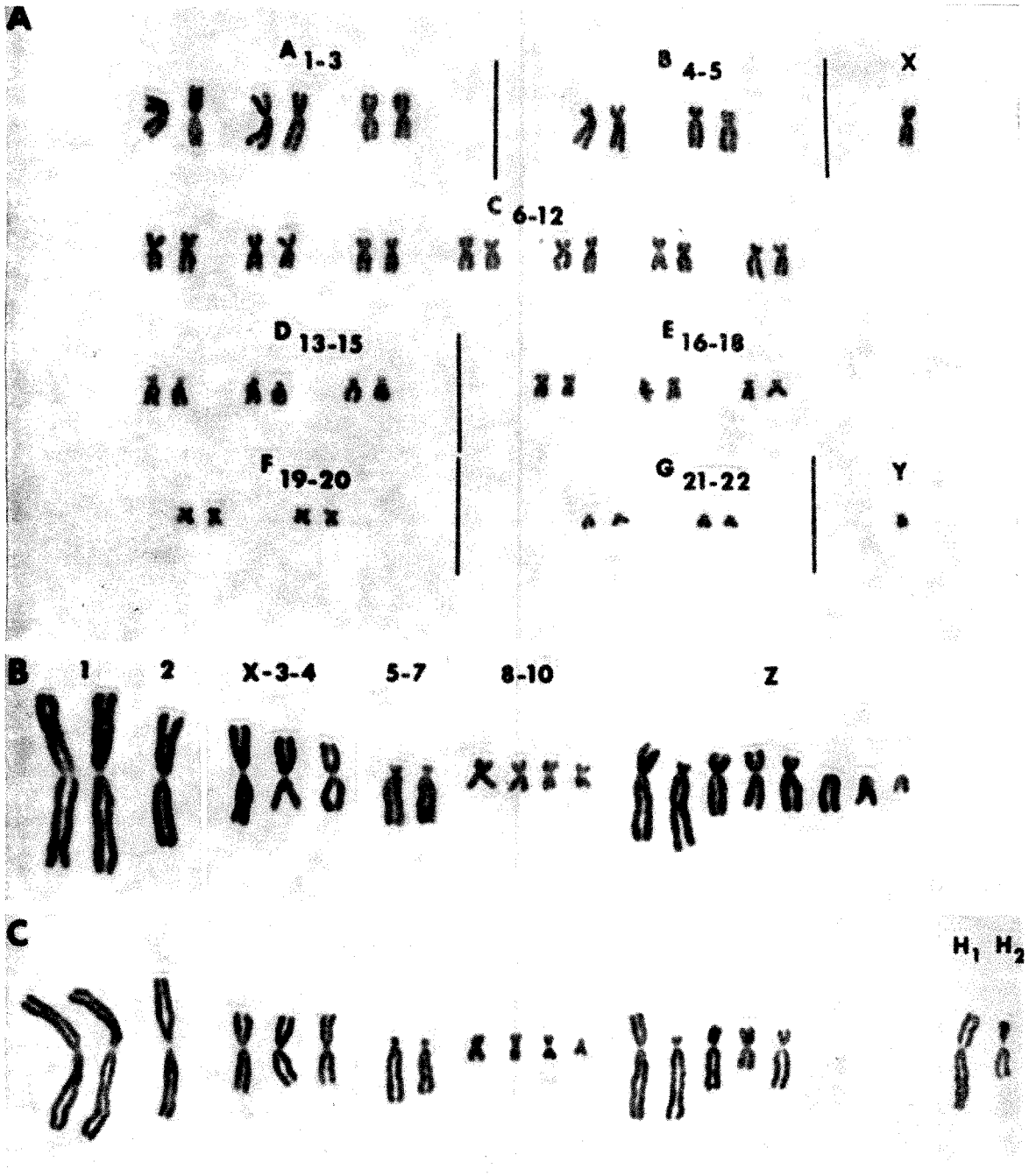


Fig. 3. *A*, The karyotype of the human fibroblast used in these fusion experiments. *B*, The karyotype of the CHO-K1 cell. *C*, Karyotype of the ino⁻pro⁻ hybrid produced by fusion of the cells of *A* and *B* and subsequent growth in medium lacking inositol but containing proline.

days after fusion. Fig. 2*A* presents a cell in which a complete set of chromosomes of each type is present and in which synchrony has been achieved so that both sets are in mitosis. Fig. 2*B* demonstrates a cell in which all or most of the Chinese hamster and about nine or ten presumed human chromosomes are mitotic. The remaining chromosomes have a "pulverized" appearance, and would probably be lost from the cell as a group. In Fig. 2*C* and *D* are displayed mitotic figures of cells which have retained all of their CHO-K1 chromosomes but only a few of the human ones. Synchrony seems to be achieved although it may not be perfect, because in Fig. 2*C* the several presumed human chromosomes appear more highly condensed and therefore more densely stained than the others. In Fig. 2*D* the chromosomes which seem to be clustered together are presumed to be of human origin and might also tend to be lost as a group by some non-disjunctive process. These figures illustrate that one or more processes may operate to cause simultaneous and extensive loss of chromosomes of one species.

The chromosomes lost from the human–ino⁻ hybrids are not always exclusively human. A few Chinese hamster chromosomes are also lost occasionally, and such losses are most frequent among the telocentric members of group Z of CHO-K1 (ref. 7).

Gene Linkages

Fusions of normal human fibroblasts and the doubly deficient CHO-K1 mutants, ino⁻pro⁻ and gly⁻A pro⁻, respectively, were carried out. In each case the hybrid cells were grown up in the presence of proline but in the absence of the other nutrient, inositol or glycine respec-

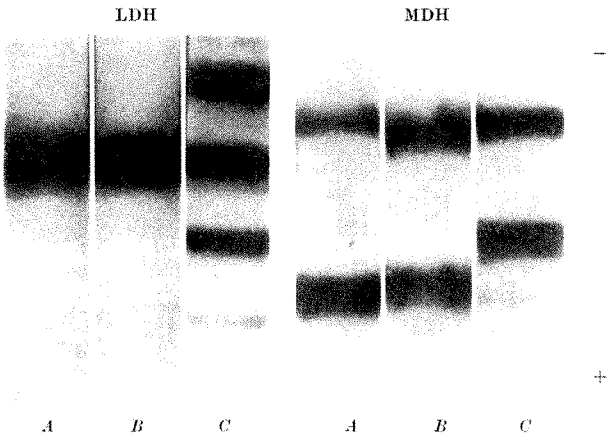


Fig. 4. Gel electrophoretograms of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) obtained from extracts of (*A*) the CHO-K1 cell; (*B*) the ino$^+$ pro$^-$ hybrid; and (*C*) the normal human fibroblast. In each case, 10 μl. of an extract from approximately 2×10^5 cells for LDH or 10^6 cells for MDH, to which was added an equal volume of 40 per cent sucrose, was layered over a 5·5 per cent polymerized acrylamide gel without use of a stacking gel. Electrophoresis was carried out for 45 min with a current of 2·5 mA per tube at 4° C in a Tris-glycine buffer (*p*H 8·3). Thereafter the gels were stained for LDH and MDH activities using appropriate substrates. In each case the pattern of the hybrid is indistinguishable from that of the CHO-K1 cell.

tively. After 2 weeks of growth in the partially deficient media, at which time maximum chromosome loss had occurred and the hybrids had achieved stable karyotypes, hybrid cells were plated, the resulting clones selected, and their nutritional requirements determined. As expected, all the clones could grow in the absence of inositol in the one case or glycine in the other. But, as shown in Table 2, the proline requirement of the original Chinese hamster cells was found to be variable. Thus absence of linkage between the pro$^-$ locus and either of the ino$^-$ and gly$^-$A loci is demonstrated.

Confirmation of the absence of linkage between the inositol and proline genes is afforded by a separate fusion experiment in which the converse selection medium was used. Thus the human–ino$^-$pro$^-$ hybrid cells were grown up in the presence of proline, but not inositol. Four clones were picked and tested: of these, three were ino$^+$pro$^-$ and one was ino$^+$pro$^+$.

Preliminary Chromosomal Analysis

Although karyotype analysis of these stabilized hybrid clones requires more than morphological methods of identification of the chromosomes particularly if errors from visually undetectable rearrangement are to be avoided, some preliminary conclusions are possible. Sixteen different human–CHO-K1 hybrid clones of the ino$^+$pro$^-$ type were picked from single cells grown in inositol-free F12 for 2 weeks after fusion. All had similar karyotypes. Fig. 3 presents a comparison of the karyotypes of (*A*) the normal human cell; (*B*) the original Chinese hamster cell (ino$^-$pro$^-$); and (*C*) a typical stabilized hybrid clone (ino$^+$pro$^-$), containing two chromosomes designated H_1 and H_2, which seem to be human. The stabilized hybrid cell contains only nineteen chromosomes, one less than the number in the original Chinese hamster cell. Seventeen of these seem to be chromosomes of the original CHO-K1. These include the twelve —presumably normal Chinese hamster chromosomes of the CHO-K1—and five of the eight abnormal ones which we previously designated as the *Z* group[7]. The three missing chromosomes of this group are the small telocentric ones.

The two sub-metacentric chromosomes which are present (designated H_1, H_2) presumably are of human origin. Measurement from more than twenty good metaphase spreads, of the arm lengths of these two presumed human chromosomes and their ratios (1·82 and 1·72 for the larger and smaller one respectively) suggests that the larger of the foreign chromosomes could be No. 2 and the smaller one could be either No. 6 or *X* of the human karyotype.

Table 2. TEST FOR LINKAGE BETWEEN THE INOSITOL AND PROLINE AND BETWEEN THE GLYCINE AND PROLINE GENES IN THE HUMAN CHROMOSOMES OF STABLE HYBRIDS

Parental genotypes		Growth media for hybrids	No. of hybrid clones isolated with the indicated genotypes
Human fibroblast	CHO		
ino$^+$pro$^+$	ino$^-$pro$^-$	Inositol-free proline present	ino$^+$pro$^-$ 35 ino$^+$pro$^+$ 7
gly$^+$pro$^+$	gly$^-$pro$^-$	Glycine-free proline present	gly$^+$pro$^-$ 10 gly$^+$pro$^+$ 5

Linkage of Dehydrogenase Genes with the Inositol Gene

Acrylamide gel electrophoresis of extracts of cells from CHO-K1, normal human fibroblasts, and the ino$^+$pro$^-$ hybrid with two presumed human chromosomes as shown in Fig. 3, was performed, and the resulting gels were stained to reveal the distribution of the lactate and malate dehydrogenase enzymes. The isozyme patterns of the Chinese hamster and human cells are distinct, and the hybrid patterns are indistinguishable from that of the CHO-K1 cell. The simplest interpretation of these results is that in the human genome the genes for these two enzymes are not linked to the inositol gene.

Our experiments show that human–Chinese hamster cell hybrids lose human chromosomes rapidly and extensively, so expediting genetic analysis. The rapidity of this process may in large part be determined by the disparity in growth rate of the two parental cells which would operate in two ways. First, because the two cells have widely different rates of traverse of their respective life cycles, asynchrony in the two chromosomal complements is to be expected in a large fraction of the initial fused population. Johnson and Rao[13] have shown that a set of mitotic chromosomes in a hybrid cell can induce premature condensation in a set of interphase chromosomes. When the latter are in G1 or S their condensation results in abnormal chromosomal structures, some of which resemble the pulverized chromosomes of Fig. 2*B*. Such chromosomes might be lost in relatively large groups. Second, because the Chinese hamster cells grow more rapidly than the human ones, a selective advantage is granted to the hybrid cells which lose the maximum number of human chromosomes. These considerations suggest that for optimal results, cells with the largest possible difference in growth rate be used, and that the slowly growing cell be the one from which chromosome loss is desired. Studies analysing the life cycles of such fused cells should illuminate this process.

The rapidity with which unnecessary chromosomes seem to be lost from the human–Chinese hamster hybrid cell commends it as a particularly useful system for gene linkage studies. By the use of appropriate auxotrophic mutants of the Chinese hamster cells and of accumulating knowledge of the chromosomal location of various human genes it should become possible to design conditions from which stable hybrids of Chinese hamster cells with particular human chromosomes will result.

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Electron Microscopic Evidence for Chloroplast Fusion in Zygotes of *Chlamydomonas reinhardii*

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In *Chlamydomonas reinhardii* zygotes the two chloroplasts contributed by the two gametes fuse, as do nuclei, to form a single zygotic chloroplast; this would enable non-Mendelian genes residing in the chloroplast to undergo recombination.

CHLOROPLASTS are currently believed to be formed only by the division of existing plastids[1], and there is much evidence that certain chloroplast defects are inherited as if controlled by non-Mendelian genes residing in the chloroplast itself[1]. Because chloroplasts have been shown to contain DNA[1,2], several authors[1,3-5] have suggested that these non-Mendelian genes are in fact chloroplast DNA. The non-Mendelian genes of the single-celled alga *Chlamydomonas reinhardii* have received intensive study by Sager[4] and by Gillham[5] who have shown that they are "particulate" and that linkage and recombination can occur between certain of them. Recently Margulis[6] has questioned the belief[3-5] that this recombination is the result of recombination within organelles (which she calls "intraorganellar recombination") and proposes instead that it simply results from the assortment of whole but phenotypically distinguishable organelles.

If genuine "intraorganellar recombination" does occur, as recent genetic evidence strongly indicates[4], one would expect it to be preceded by the fusion of the organelles derived from the two parents. So far as I am aware, such organelle fusion has not previously been convincingly demonstrated in any organism either in the case of chloroplasts or of mitochondria.

In this article I present electron microscopic evidence that in *Chlamydomonas* the two chloroplasts contributed by the two parents actually do fuse together to form a single "zygotic" chloroplast, in a similar way and at approximately the same time as the two parent nuclei fuse to form a diploid zygotic nucleus (see Figs. 1 and 2). I also outline a model for the behaviour of the non-Mendelian genes.

In most organisms it is difficult to observe the fate of chloroplasts in the zygote by electron microscopy because of the large number of indistinguishable chloroplasts and the difficulty of studying every stage. *Chlamydomonas*, however, has only one chloroplast per cell, which contains one distinct pyrenoid and one eyespot; these features make it possible to show unambiguously that chloroplast fusion does occur in the zygotes.

Gametes of both mating types (32C and 32D, Cambridge culture collection) of *C. reinhardii* were prepared[3,7] and mixed together to allow mating to occur. The resulting cell suspension was observed by light microscopy and fixed for electron microscopy at various intervals of time after mixing. Fixation was in 1 per cent glutaraldehyde in 0·02 M sodium phosphate buffer (*p*H 7·4) followed by a wash in phosphate buffer and post fixation in 1 per cent OsO_4 in phosphate buffer. After araldite embedding the sections were stained in uranyl acetate followed by lead citrate.

Observations of Chloroplast Fusion

One can obtain some information on the behaviour of the chloroplasts in the zygote by observing living cells by phase contrast microscopy. Newly formed zygotes (Fig. 1; 2 min) have four flagella and contain two nuclei and two discrete green chloroplasts; each chloroplast contains one orange birefringent eyespot and one small (1 μm) spherical pyrenoid. The two chloroplasts are cup-shaped and lie side by side (separated by a thin layer of colourless cytoplasm) at the base of the cell with the open ends of the cups towards the flagella. Three to four hours later the chloroplast bases, containing the pyrenoids, have moved so close together as to appear to be touching. Those inner parts of the two chloroplasts' "rims" which were earlier side by side have disappeared, so that each chloroplast is no longer cup-shaped, but the two together form one large green cup. One eyespot persists in each of the two remaining outer chloroplast rims and there are still two discrete pyrenoids. An hour or two later still there seems to be only one large chloroplast with two eyespots and two pyrenoids. By 10·5 h after mixing the gametes most zygotes contain only one pyrenoid. Zygotes at 12 h still contain two eyespots, but these are only about one-quarter the size of those of vegetative cells or gametes. Thereafter eyespots can generally not be seen in zygotes 1–6 days old; occasionally one very small one is present at the limits of visibility. The single chloroplast and its single pyrenoid at 12 h have both much more than twice the volume of those of gametes. By 24 h the chloroplast fills most of the cell which has also grown larger. In one mating, quadriflagellate zygotes were spherical and about

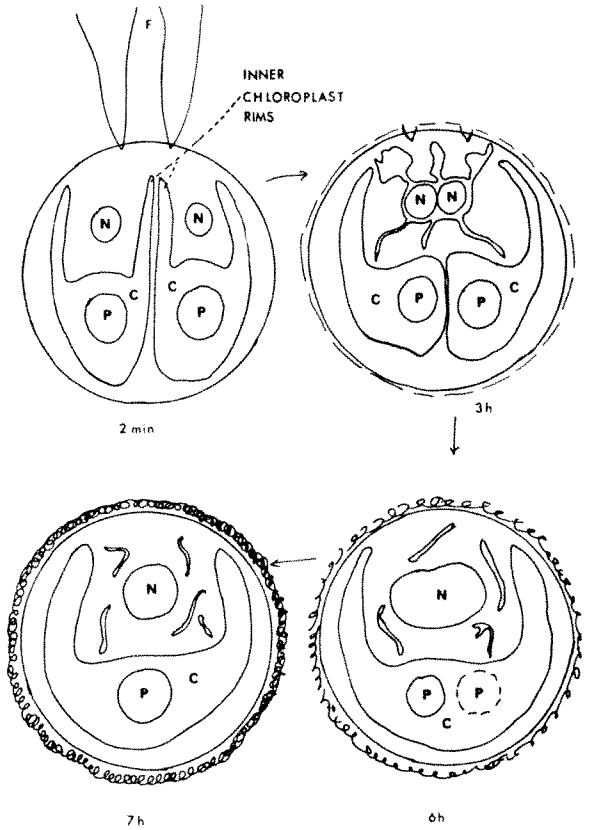


Fig. 1. Diagrammatic summary of the major events (approximate times indicated) during the first 7 h after cell fusion in *Chlamydomonas*, showing fusion of nuclei (N), chloroplast (C) fusion, regression of flagella (F) and basal bodies, loss of one pyrenoid (P), proliferation of the endoplasmic reticulum from the outer membrane of the nuclear envelope, and formation of part of the fibrous layer of the zygospore wall.



Fig. 2. 5·5 h old zygote in which the two nuclei (N) and the two chloroplasts (each containing a distinct pyrenoid, P) are in the early stages of fusion. In both cases both the inner and outer membranes of the envelopes have already fused. Note the large, interconnected, inflated rough endoplasmic reticulum cisternae connected to the outer membranes of the nuclear envelope. The outer fibrous layer of the zygospore wall is partly laid down. ×10,500.

8·8 μm across. Twenty four hours later zygotes were 15·8 μm across: this represents an 8·8-fold increase in volume. In the same time the remaining pyrenoids increased from 1 μm to 4·4 μm in diameter. The pyrenoid can usually be seen using both phase and bright field microscopy. Using the latter and iodine staining they are conspicuous, at all stages up to 12 h after mixing, as deep brown spheres standing out from the pale yellow-brown cytoplasm.

These observations with the light microscope suggest that chloroplast fusion may occur, but they cannot be regarded as conclusive, for the resolution is insufficient to distinguish two closely adjacent chloroplasts. Nonetheless they clearly show that, within 12 h of mixing the gametes, the zygotes contain only one greatly enlarged pyrenoid instead of the two small ones contributed by the gametes. This could come about in one of three ways: (1) the two chloroplasts fuse, in which case the two pyrenoids might fuse, one might degenerate or both might do so and one new one be formed; (2) the chloroplast from one mating type degenerates, the other persists; (3) both degenerate, and one new one is formed.

The changes in shape of the two chloroplast cups before the formation of the "single" cup suggest that fusion is the most likely explanation, but the resolution of the light microscope is insufficient to establish this. Because sections through a single branched chloroplast may reveal an interconnected system or several apparently distinct "chloroplasts", depending on their orientation, electron microscopy would also be inadequate without very extensive serial sectioning but for the fact that the pyrenoid (and also the eyespot) may be used as a chloroplast label or marker. Chloroplast fusion could be established by showing that two pyrenoids become enclosed by a common chloroplast envelope, provided one makes sure that one is observing zygotes and not gametes or vegetative cells, a small proportion of which might conceivably be in the predivision state when chloroplasts normally contain two pyrenoids[3]. This is easy to do, for at the critical times (2 h 15 min to 5 h 30 min after mixing) the zygote wall is beginning to be laid down—which distinguishes them from naked gametes—but is still thinner than and distinguishable from that of vegetative cells (see Fig. 3)[3]. At 4 h 40 min and 5 h 30 min I have often seen two pyrenoids enclosed in a common chloroplast envelope, which establishes chloroplast fusion as a fact (Figs. 2–4).

Chloroplast fusion begins by the formation of slender bridges between the basal regions of the two chloroplasts, often in more than one place (Fig. 4). Before this happens inner parts of the two chloroplast rims have usually regressed as also seen by light microscopy (see above) and the basal, pyrenoid-containing regions of the two chloroplasts have moved very close together. Most of the bridges are traversed by disk membranes (Figs. 2–4) which suggests that this happens soon after their formation. During fusion the remaining chloroplast rims are often elongated and sometimes branched, but by 5 h 30 min many cells have a single chloroplast with a fairly normal morphology which contains two pyrenoids in its basal region. Sometimes one of these shows signs of degeneration; its dense core may be almost absent and the tubular membranes swollen and disorganized. By 6 h 30 min sections of chloroplasts showing two pyrenoids are rare: most contain only one (Fig. 1; 7 h) and possibly the remains of a second. From 9 h onwards only one chloroplast with one pyrenoid (and no trace of a second) is normally present in each zygote. These observations suggest that the core material of one pyrenoid is degraded or dispersed, but do not establish whether the tubule membranes are broken down or reorganized, possibly as disk membranes or through incorporation into the remaining pyrenoid. My observations do not entirely rule out fusion of the two pyrenoid cores or some combination of lysis and fusion: sometimes, indeed, pyrenoids are seen partly intact and partly apparently degraded; occasionally a lobed pyrenoid is found which might conceivably be interpreted as a stage in fusion. These observations are summarized in Fig. 1.

Chloroplast Fusion and Non-Mendelian Heredity

There is no firm evidence as to the chemical nature or the location in the cell of the various non-Mendelian genes of *Chlamydomonas*. It is a reasonable working hypothesis that the DNA present in both mitochondria and chloroplasts of *C. reinhardii* has a genetic role and that some non-Mendelian genes will prove to be borne on chloroplast DNA and some on mitochondrial DNA. Mutations in the acetate genes presumably cause defects in photosynthesis, so it seems likely that these genes and those linked to them[4] reside in the chloroplast. The fusion of the two chloroplasts as described here brings together the chloroplast DNA from the mating type plus and minus parents into a common chloroplast matrix and therefore makes the recombination of non-Mendelian genes by the

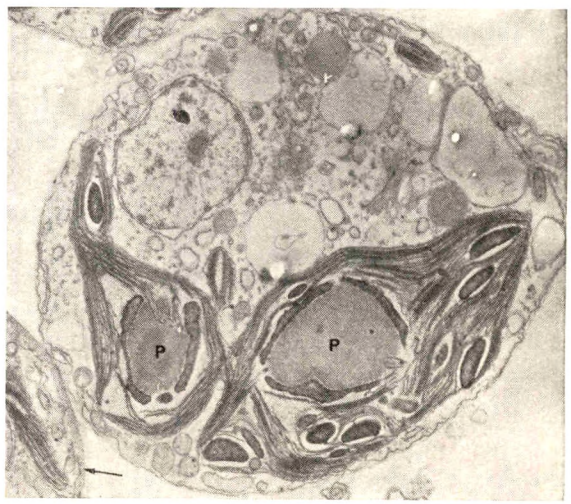


Fig. 3. Fusing chloroplasts in 4 h 40 min zygote, showing two pyrenoids (P) within a single chloroplast envelope. The chromatin clumps in the single nucleus are characteristic of a diploid zygotic nucleus. The fibrous layer of the zygospore wall is still very tenuous and clearly distinct from that of the vegetative cell in the lower left corner (arrow). ×6,000.

physical exchange of pieces of DNA a very attractive hypothesis. I have suggested[3] that chloroplast DNA is the bearer of at least some of the non-Mendelian genes studied by Sager and Gillham, and have put forward the following model to account for some of their detailed results.

First, during the early part of maturation the DNA from the minus parent is entirely broken down but that of the plus parent remains. This explains the normal uniparental inheritance of non-Mendelian genes.

Second, in a certain proportion of cases the degradation mechanism fails and both plus and minus DNA persist. Cells in which this happens would be the "exceptional zygotes".

Third, in exceptional zygotes both plus and minus DNA are replicated during and after maturation and are segregated at subsequent chloroplast divisions during and following germination. The time at which this occurred would depend on the number of copies of each DNA molecule present at each chloroplast division. If no process comparable with meiotic pairing between homologous chromosomes occurs, this segregation would be largely postmeiotic, even if each gamete originally contained only one copy of each molecule and only two replications[8] occurred before the first chloroplast division during germination.

Fourth, recombination[4,5] would result from the physical exchange of parts of the plus and minus DNA molecules after chloroplast fusion.

I suggested[3] that the most direct test of the proposed model would be to mate gametes of one sex, produced on a ^{15}N medium, with those of the opposite sex from a ^{14}N medium and to determine the fate of heavy and light chloroplast DNA during zygote maturation.

Preliminary results by Sager and Lane[9] using this method indicate that the minus DNA is either lost, as predicted by this hypothesis, or at least not replicated. Chiang's evidence[10], using ^{14}C and ^{3}H-adenine as labels for the two mating types, indicates that minus chloroplast DNA is conserved. If alternative explanations for his results (for example, the very efficient re-use of nucleotides derived from degraded minus chloroplast DNA) can be ruled out by future work, then a different explanation for uniparental transmission would have to be considered, for example, the restricted replication or transcription of minus chloroplast DNA in the normal maternal zygotes. The full publication of Sager and Lane's results ought to clarify this issue.

Whatever the exact mechanism of uniparental transmission, four features of this model would remain: (1) the equivalence of chloroplast DNA and certain non-Mendelian genes; (2) the selective "inactivation" of minus non-Mendelian genes in normal maternal zygotes and the failure of this inactivation process in exceptional biparental zygotes; (3) the segregation of non-Mendelian genes as a result of the segregation of chloroplast DNA; (4) the recombination of non-Mendelian genes as a result of physical exchange between plus and minus chloroplast DNA in the single zygotic chloroplast.

Detailed investigation of the behaviour of plus and minus chloroplast DNA, using both density and radioactive labels, not only in normal maternal zygotes but also in populations consisting predominantly of biparental or of paternal zygotes should confirm or disprove this model and also reveal the mechanism of inactivation.

My observations on chloroplast fusion clearly do not prove that recombination of non-Mendelian genes occurs within the single zygotic chloroplast, but they do refute the postulate of Margulis that in *Chlamydomonas reinhardii* uniparental inheritance can be mediated by the transmission of only the female chloroplasts through the zygotes. In addition, her simple model seems unable to explain satisfactorily the various degrees of linkage between the different non-Mendelian genes[4].

In flowering plants the frequent (but not universal) uniparental inheritance of plastid characters has often led to the belief that this is the result of uniparental transmission of entire plastids[1], as Margulis has suggested for *Chlamydomonas*. Because this is clearly not the case in *Chlamydomonas*, the idea must therefore be open to question in other plants. There are, however, a number of classic instances[1,11] where light microscopists have observed the apparent breakdown of male chloroplasts in the zygotes of algae such as *Spirogyra* and *Zygnema*, and also indications in some other species (for example, the liverwort *Anthoceros*) that the male gamete may possess no plastid at all. If these observations are confirmed by electron microscopy it seems that uniparental inheritance of plastid characters can in some species result from the uniparental transmission of whole plastids. It would be very interesting to know how widespread the phenomenon of plastid fusion is in plant zygotes. Even in zygotes of other unicellular algae the fate of the chloroplasts is generally unknown[12]; a few light microscopists, notably Stein[13–15], have believed that chloroplast fusion occurs, but electron microscope studies are needed to confirm this.

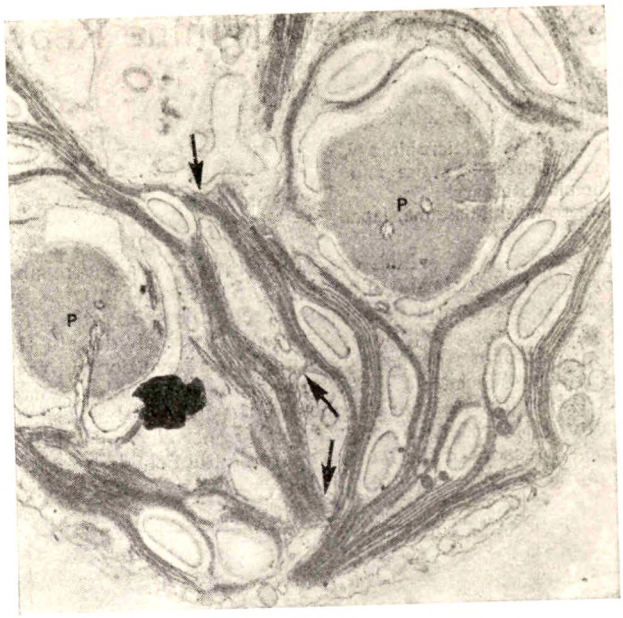


Fig. 4. 5·5 h zygote showing two chloroplasts whose envelopes have fused in three places (arrows). The pyrenoids (P) of both chloroplasts are intact. ×14,400.

It would clearly be of great interest to determine whether mitochondria, as well as chloroplasts, fuse in zygotes of *Chlamydomonas*, or indeed in any organism. Unfortunately the large number of indistinguishable mitochondria in each cell makes this difficult.

I thank Sir John Randall for support and encouragement.

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Isolation of the Arginine Repressor in *Escherichia coli*

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The repressor produced by the *argR* gene of *E. coli* K-12 has been isolated. This protein seems to repress several separate operators.

THE eight genes which code for the enzymes responsible for the biosynthesis of arginine in *E. coli* map in five separate groups on the chromosome. Their expression is controlled by a regulator gene, *argR*, which directs the synthesis of a repressor exerting a negative control over enzyme synthesis[1,2]. This repressor must somehow control all the eight structural genes of the system because, in spite of their separation, they are controlled in parallel. The repressors of the lactose operon and of the lysogenized lambda phage are proteins which seem to block transcription by binding directly to DNA[4,5]. That the arginine repressor is also a protein is suggested by *in vivo* experiment[6] and by the existence of *argR* mutants whose repressibility is sensitive to high temperature (*argR*^{ts})[2,3] and to amber suppression[2].

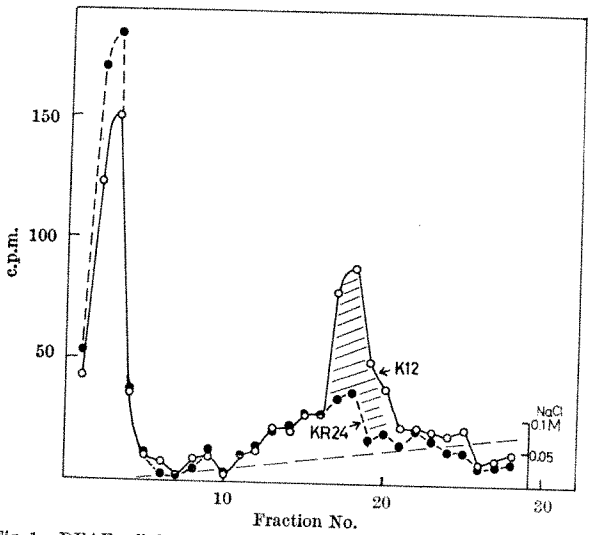


Fig. 1. DEAE-cellulose elution profiles of a mixture of fractions derived from the ribosomes of the wild type and an *argR*⁻ mutant, KR 24. The cells were grown in an M9 medium supplemented with 0·03 per cent yeast extract and nineteen amino-acids (not including lysine) (20 μg/ml. each). Fifty μCi of ^{3}H-lysine (○) was added to the wild type culture and 20 μCi of ^{14}C-lysine (●) to the KR 24 culture. The bacteria were labelled for three generations at 37° C and then mixed. Cell extracts were prepared by alumina grinding in 1·5 ml. of 0·01 M Tris (*p*H 7·4) and 0·1 mM magnesium acetate, and centrifuged on a sucrose density gradient and fractionated by the conventional method. The supernatant, 30S and 50S ribosome fractions were pooled separately. The 30S fraction was dialysed against 5 mM Tris (*p*H 7·4), and then incubated in 10 mM Tris (*p*H 8·5)–10 mM magnesium acetate–60 mM NH_4Cl at 25° C for 1 h. This fraction was passed through a DEAE-cellulose column (1 × 10 cm) which was equilibrated with the same buffer. The effluent was collected, dialysed against 5 mM Tris (*p*H 7·5), and applied to another DEAE-cellulose column (0·5 × 15 cm) equilibrated with 5 mM Tris. Fractions were eluted with a 40 ml. linear gradient of NaCl (0–0·08 M) containing 5 mM Tris (*p*H 7·5). The flow rate was 1·5 ml./fraction/30 min. An aliquot of each fraction was added directly to Bray's solution[10] and counted on a scintillation spectrometer. All fractionations were done at 4° C. The counts were normalized by multiplying the measured ^{14}C values by the ^{3}H/^{14}C ratio of the sample applied to the column.

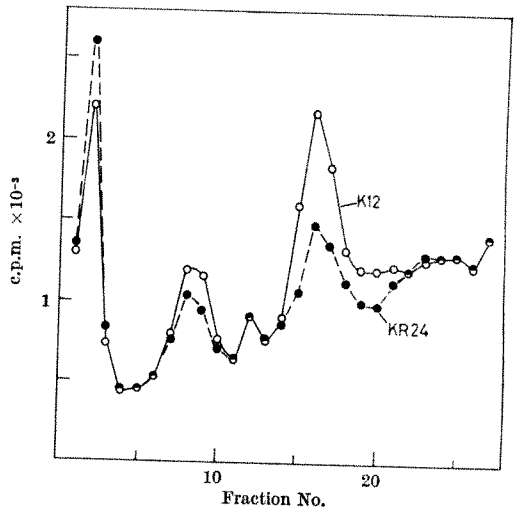


Fig. 2. Elution profiles of a mixture of crude extracts from the wild type and KR 24. The wild type was labelled with 4 μCi ^{3}H-lysine and KR 24 with 1 μCi ^{14}C-lysine. The labelling of cells and the preparation of crude extract were as described in Fig. 2, except that 5 mM Tris (*p*H 7·5) was used for extraction. The crude extract was dialysed against the same buffer, then applied to a DEAE-cellulose column (0·5 × 15 cm). Gradient elution and analysis of the effluent were as described for the second column chromatography of Fig. 1.

Isolation of the *argR* Product

Our previous work has suggested that the repressor protein might be involved in some way with the ribosome[7]. We have therefore examined the ribosomal proteins of *argR* mutants. Cells of wild type *E. coli* K-12 and an *argR* mutant were differentially labelled with ^{14}C- or ^{3}H-amino-acids, and ribosomal proteins analysed on carboxymethyl-cellulose columns by the method of Otaka *et al.*[8]. There was no significant difference between the principal ribosomal protein components, but comparison of the proteins of the 30S subunits showed a small but reproducible difference between the two bacterial strains.

One column fraction was rich in the radioactive label of the wild type. The detailed procedures used for its further purification are given in Fig. 1. We postulate that the

Table 1. AMOUNTS OF THE FRACTIONS R AND R′ IN MUTANTS DERIVED FROM KR 18 AND KR 24

Strain	Derived from	Temperature 24° C: Ornithine transcarbamylase	Temperature 24° C: Arginino-succinase	Temperature 24° C: Fraction R	Temperature 24° C: Fraction R′	Temperature 37° C: Ornithine transcarbamylase	Temperature 37° C: Arginino-succinase	Temperature 37° C: Fraction R	Temperature 37° C: Fraction R′	Phenotype
K-12 (wild type)		4·8	0·07	1	0	2·5	0·08	1	0	Repressible
KR 18	K-12	13	0·10	0	1	280	1·0	0	0–0·1	Temp.-sensitive repression
18-B-1	KR 18	5·9	0·05	1	0	2·7	0·05	1	0	Repressible
18-B-7	KR 18	1·1	0·10	1	0	1·0	0·06	1	0	Repressible
18-B-8	KR 18	1·8	0·04	1	0	1·5	0·05	1	0	Repressible
18-H-6	KR 18	655	2·5	0	0	394	2·2	0	0	Non-repressible
18-H-17	KR 18	900	3·0	0	0	548	2·4	0	0	Non-repressible
18-H-20	KR 18	618	2·2	0	0	525	2·0	0	0	Non-repressible
18-M-7	KR 18	7·2	0·05	0	1·5–2	248	0·67	0	0·7–1·2	Temp.-sensitive repression
KR 24	K-12	561	2·3	0	0	455	2·1	0	0	Non-repressible
24-M-49	KR 24					93	0·50	0·5	0	

Cells of KR 18 and KR 24 were mutagenized with nitrosoguanidine[11]. The isolated colonies were tested for their enzyme levels[12]. The mutants selected were purified and subjected to further analysis. Possible contaminants in the mutagenized cells were eliminated by examining the colony morphology on EMB agar and sensitivity to phage T_4. The cells were grown in M9 medium containing 0·5 per cent casamino-acids and 100 μg/ml. L-arginine and their enzyme levels were determined as previously described[9]. The amounts of the fractions R and R′ were estimated from the area of each peak in the DEAE-cellulose elution profile obtained as in Fig. 2. Specific activities of enzymes are expressed as μmoles of reaction product formed per h per mg of protein. The amounts of the fractions R and R′ are shown relative to that of the fraction R of the wild type.

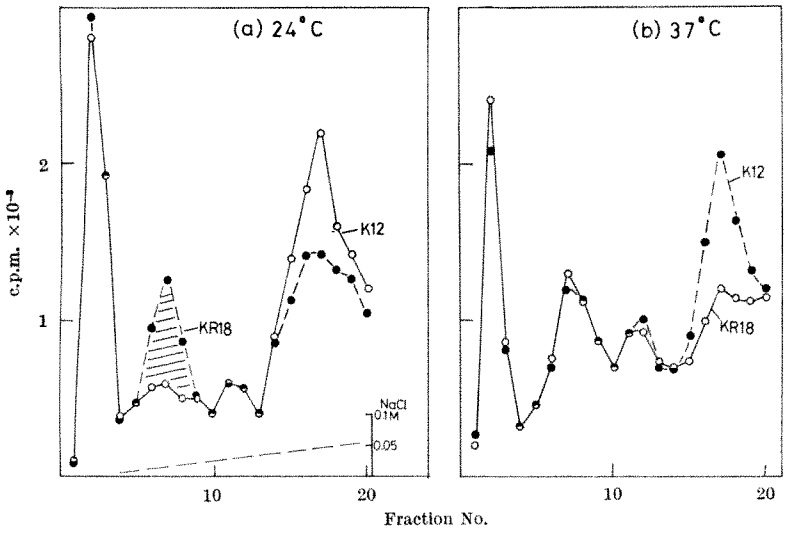


Fig. 3. Elution profiles of a mixture of crude extracts from the wild type and KR 18, grown at 24° C and 37° C. Cells were labelled either with ^{3}H-lysine or ^{14}C-lysine. The cells were labelled at 24° C and 37° C as indicated. All the analytical procedures were the same as in the legend of Fig. 2. (○), ^{3}H; (●), ^{14}C.

shaded area of this figure represents a protein fraction (termed fraction R) which is present only in the wild type cells and not in the *argR* mutant cells. The radioactivity of fraction R constituted only about 0·1 per cent of the total radioactivity of the 30S subunit. Cells of KR 18 (*argR*^{ts}) grown at 37° C yielded a profile similar to that of KR 24 (*argR*$^-$) shown in Fig. 1, thus also showing lack of fraction R. KR 18 cells labelled at 24° C, however, yielded a small extra peak in approximately 0·02 M NaCl eluate.

The amount of the repressor associated with the ribosome is only a small proportion of that found in the soluble fraction (see below) and the repressor fraction can easily be removed from ribosomes by buffers containing 0·06 M NH_4Cl. This suggests that its presence on the ribosome is fortuitous and results from a non-specific adsorption. Some *argR* mutants have ribosomes with altered sensitivities to streptomycin (unpublished observations); this suggests that such an adsorption may occur *in vivo* to cause some change in ribosome function, but its significance remains obscure.

An elution profile similar to that of Fig. 1 was obtained when a soluble fraction of the cell extract (the 100,000*g* supernatant) was applied to the DEAE-cellulose column by the procedure given in Fig. 1. The fraction R recovered from the supernatant or crude extract was much larger than that from the ribosome fraction. A chromatographic profile of the crude extract showing the presence of fraction R is indicated in Fig. 2. Double-label experiments conducted with reversibly labelled amino-acids (a "criss-cross" experiment) gave the same result, and experiments carried out with leucine and reconstituted protein hydrolysates gave similar profiles. Although radioactivity measurements were taken directly on the sample solution, counting on the 10 per cent trichloroacetic acid (TCA) precipitate heated to 95° C for 20 min gave essentially the same results. We estimate that fraction R is approximately 0·3 per cent of the soluble cellular protein.

As shown in Fig. 3, the crude extract of KR 18 exhibited a definite extra peak in eluates with lower concentration of NaCl when the bacteria had grown at 24° C. We again postulate that the shaded area of Fig. 3*a* represents a protein present only in the KR 18 cells which had grown at lower temperature (referred to as fraction R′). The elution profiles of *argR*$^-$ mutants lacking fraction R were similar to that of K-12 in eluates with lower concentration of NaCl when they had grown at 24° C, showing that fraction R′ is unique for KR 18.

The following evidence indicates that fraction R is the product of the *argR* gene and that fraction R′ is an altered form of the repressor. The fraction R present in the wild type K-12 strain has not been found in the *argR*^{ts} mutant, KR 18, or in some of the *argR*$^-$ mutants (four out of nine mutants tested). Other *argR*$^-$ mutants and another *argR*^{ts} mutant, KR 59, produce a protein fraction indistinguishable from fraction R. Only KR18 formed the fraction R′ at 24° C at which temperature enzyme synthesis was nearly repressed. We therefore assume that both the fractions R and R′ are able to repress the synthesis of arginine enzymes.

Fraction R was found in derepressed cells of wild type bacteria grown in medium lacking arginine which formed about 60 times more ornithine transcarbamylase than repressed cells. This suggests that there is no self-control over fraction R formation by the repression mechanism. To see if fraction R is deleted only in *argR* mutants, similar double-label experiments were done with mutants of other operons; *i*$^-$ and *trpR*$^-$ mutants, and their respective isogenic parental *E. coli* (supplied by Drs T. Horiuchi and T. Yura, respectively), were compared. No difference such as that shown in Fig. 2 was found between these mutants and their parents.

Table 1 demonstrates quantitative alterations of fractions R and R′ on further mutations of KR 18 and KR 24. The amounts of these fractions varied in accordance with the changes in the phenotypes of the mutants. Thus repressible revertants from KR 18 gave the same chromatographic profile as that of the wild type. Non-repressible derivatives of KR 18 lost the ability to form fraction R′ at 24° C as well as at 37° C. Strain 18-M-7, in which the enzymes are more repressed, produced fraction R′ even at 37° C while KR 18 formed little at this temperature. On the other hand, strain 24-M-49 seems to form some fraction R, for it is partially repressed.

The fraction R′, but not the fraction R, nearly disappeared when KR 18 cells were heated at 49° C for 20 min (Fig. 4). Heating for 15 min resulted in an intermediate

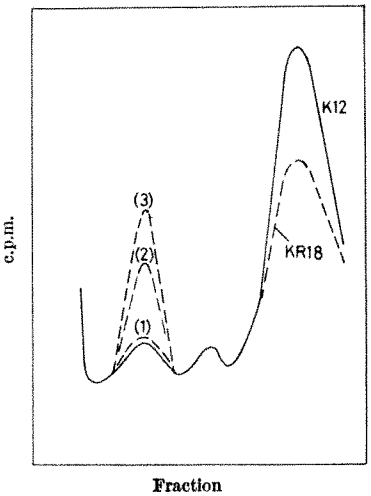


Fig. 4. Schematic elution profiles of the extracts from heated cells of KR 18. Labelling was performed as in Fig. 2. The labelled cultures were mixed, centrifuged, suspended in 1/20 volume of 0·01 M phosphate buffer (*p*H 7·1), and divided into several portions. One served as the unheated control. The other parts were heated at 49° C for 20 min with and without the addition of chemicals. Each sample was collected by centrifugation and ground with alumina. The subsequent analysis was as described in Fig. 2. Each curve represents schematically the summary of each analysis. The experiment was repeated two to four times. (1) Heated in absence or presence of 10^{-3} M D-arginine or of 10^{-3} M L-ornithine; (2) heated in presence of 10^{-3} M L-arginine; (3) unheated control.

disappearance of fraction R′. This figure also shows that L-arginine, but neither D-arginine nor L-ornithine, partially prevents the heat denaturation of fraction R′. The responses of fraction R′ to heat are consistent with the results of *in vivo* experiments with KR 18 (unpublished). When KR 18 cells grown at 24° C were heated in a buffer at 49° C for 10 min, a rapid enzyme synthesis ensued on return to 24° C. Addition of L-arginine to the buffer partially prevented the derepression by heat. We interpret these results to mean that the temperature-sensitive repressor is denatured by heat. The findings also provide evidence for the identity of fraction R′ as the altered repressor.

The approximate molecular weight of the repressor was estimated by gel filtration. The molecular weights of fractions R and R′ are about 45,000 and 25,000 respectively. Because the amounts of the two fractions are about equal, we might presume that fraction R is a dimer and that fraction R′ is a monomeric form of the wild type repressor. Disc electrophoresis of fraction R was carried out in a standard gel at *p*H 8·7 (ref. 9). Fraction R migrated toward the anode to about one-third of the distance between the origin and the dye front.

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Localization of Acyl Carrier Protein in *Escherichia coli*

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Acyl carrier protein has been localized in *Escherichia coli* by electron microscopy and autoradiography to a site just inside the cytoplasmic membrane.

Acyl carrier protein (ACP) functions as a carrier of acyl groups in the *de novo* biosynthesis of fatty acids. As such it plays an essential part in two kinds of fatty acid synthetases: multienzyme complexes and non-associated fatty acid synthetases.

In the fatty acid synthetase multienzyme complexes the individual enzymes participating in long chain acyl synthesis are present in a high molecular weight aggregate. This complex can be purified as a unit, but attempts to isolate the individual enzymes in an active form from this unit have been unsuccessful so far. Multienzyme complexes have been isolated from yeast[1], adipose tissue[2], liver[3] and *Mycobacterium phlei*[4]. Although protein-bound 4′-phosphopantetheine has been found in all of these, indicating the presence of an ACP-like component, only from the yeast synthetase has the ACP been isolated after disruption of the complex[5].

The non-associated fatty acid synthetases by definition fail to show any tendency to associate when studied *in vitro*. The individual soluble enzymes from these synthetases can be isolated by conventional protein separation techniques. By contrast with the multienzyme complexes, ACP in the non-associated fatty acid synthetases (as found in most bacterial[6,7] and plant extracts[8]) is present in the free state and can easily be separated from the enzymes involved in fatty acid biosynthesis. Yet in these fatty acid synthetases, as in the case of the multienzyme complexes, ACP must interact specifically and consecutively with acetyl-CoA-ACP transacylase, malonyl-CoA-ACP transacylase, β-ketoacyl-ACP synthase, β-ketoacyl-ACP reductase, β-hydroxyacyl-ACP dehydrase, and enoyl-ACP reductase[9]. In spite of this, the process of fatty acid biosynthesis in *E. coli* is apparently very efficient, for no free acyl-ACP intermediates are found in extracts made from cells grown in normal conditions. This observation led us to consider whether the synthetase from *E. coli* might exhibit some kind of structural organization *in vivo*. We therefore designed experiments which would localize ACP, a component of the fatty acid synthetase with a unique prosthetic group which facilitates detection, in the bacterial cell. We speculated that ACP might be lined up along the cytoplasmic membrane because recent studies from this and other laboratories have shown that acyl-ACP derivatives react not only with the soluble enzymes of the fatty acid synthetase but also with the membrane-bound enzymes involved in the biosynthesis of complex lipids[10–13].

To localize ACP in *E. coli* we used auxotrophs of this bacterium requiring either pantothenate or β-alanine. When these mutants are grown on limiting concentrations of radioactive pantothenate or β-alanine and allowed to remain in stationary phase for some time, the level of CoA drops dramatically and the pantothenate or β-alanine is present almost exclusively in ACP[14,15]. Thus by using β-^{3}H-alanine of high specific radioactivity we were able to label specifically one protein in the cell, ACP, and to localize it by electron microscopy and autoradiography.

Absence of ACP in Periplasmic Space

When *E. coli* Hfr 139, a pantothenate auxotroph, is grown on a limiting concentration of ^{14}C-pantothenate and allowed to remain in the stationary phase for several hours, the cells contain the incorporated ^{14}C-pantothenate almost exclusively in ACP. The cells used in the experiments of Table 1 contained 97 per cent of the radioactivity in ACP and 3 per cent in CoA. When these cells were subjected to the osmotic shock treatment described by Heppel[17], 92 per cent of the radioactivity (at least 90 per cent of the ACP) remained inside the shocked cells while 81 per cent of the 5′-nucleotidase activity was

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Table 1. OSMOTIC SHOCK TREATMENT OF ^{14}C-ACP CELLS

Fraction	Total protein (mg)	Total radioactivity (c.p.m.)	5′-Nucleotidase (μmoles P_i/20 min)
Cells	115*	382,000	938*
Stage 1 fluid	—	5,100	7
Stage 2 fluid	4	8,690	720
Shocked cells	109*	350,200	165*

* Determined on sonicates.

E. coli Hfr 139 were grown at 37° C in a New Brunswick shaker, model G-25, in medium E (ref. 16) containing 0·4 per cent glycerol as carbon source and supplemented with a limiting concentration of pantothenate (0·05 μM, specific activity 3·5 μCi/μmole) and 1·7 μM thiamine. Cells were harvested 4 h after they reached stationary phase at A_{600}=0·56. Parts of the cells were ruptured by French press and the extract was analysed for ^{14}C-ACP and ^{14}C-CoA (ref. 15). ^{14}C-ACP accounted for 97 per cent of the incorporated radioactivity. The remaining cells were subjected to osmotic shock treatment[17]. Stage 1 fluid—20 per cent sucrose supernatant; stage 2 fluid—supernatant after shock treatment in 5×10^{-4} M $MgCl_2$ (contains proteins released from periplasmic space). Sonic extracts of intact and osmotically shocked cells were made by treatment of cell suspensions for 90 s with a Branson sonifier, model LS 75. 5′-Nucleotidase was assayed with 5′-adenosine monophosphate according to Neu and Heppel[18].

released into the osmotic shock fluid. Thus ACP is localized either within the cytoplasmic membrane or in the cytoplasm and not—like some transport factors and enzymes such as 5′-nucleotidase and alkaline phosphatase[17]—in the periplasmic space between the cytoplasmic membrane and cell wall.

Preparation of Tritiated Cells

To localize tritiated ACP by electron microscopy and autoradiography, a number of control experiments were necessary to define certain parts of the cell. The preparation of cells labelled with various ^{3}H-labelled metabolites for autoradiography is described in Table 2. Cells were grown on [*methyl*-^{3}H]-thymidine to label the nuclear area, on ^{3}H-tryptophan or ^{3}H-glutamic acid to label proteins in general, and on different concentrations of β-^{3}H-alanine to label ACP and CoA. When the β-alanine auxotroph, M99-2, was grown on an excess of β-^{3}H-alanine (2·0 μM), it contained 8 disintegrations/day/cell with 89 per cent of the radioactivity in CoA and 11 per cent in ACP. When grown on limiting β-alanine concentrations the auxotroph contained 1·5 disintegrations/day/cell (32 per cent in CoA and 68 per cent in ACP) when harvested in log phase and 1·0 disintegration/day/cell (with 89 per cent as ^{3}H-ACP) when harvested in early stationary phase. The cells had to be harvested in early stationary phase because of increased reversion during the growth in β-alanine limiting conditions.

Electron Microscopy and Autoradiography

Electron microscopy with autoradiography of cells in which DNA was labelled with [*methyl*-^{3}H]-thymidine and proteins were labelled with L-^{3}H-tryptophan or DL-^{3}H-glutamic acid was done to establish that this method would adequately demonstrate the nuclear and cytoplasmic areas of the cell. The distribution of grains in the cells in Fig. 1 indicates a concentration of grains over nuclear areas in the thymidine labelled cells (*a*). A relative paucity of grains over nuclear material and increased numbers over the cytoplasm characterize preparations in which the label was in protein (*b*). The grain distribution in the β-alanine cells varied according to the β-alanine concentration and duration of growth. Those cells grown on excess β-alanine (*c*), in which 89 per cent of the label was in CoA and 11 per cent in ACP, had a grain distribution similar to cells in which proteins in general were labelled (*b*). In the cells grown on limiting β-alanine and harvested in early stationary phase (*d*), in which 89 per cent of the label was in ACP, many grains are centred over cell surfaces and a greater proportion are outside the cell than in the other preparations.

Histograms[20] were constructed to demonstrate the cellular distribution and median of grains in the different radioactive cells (Fig. 2) and these support the apparent surface localization of ACP. Thus the nuclear distribution of thymidine (*a*) is obvious. Both tryptophan (*b*) and glutamic acid (*c*) heavily labelled the cytoplasm and these patterns resemble the grain distribution seen with cells

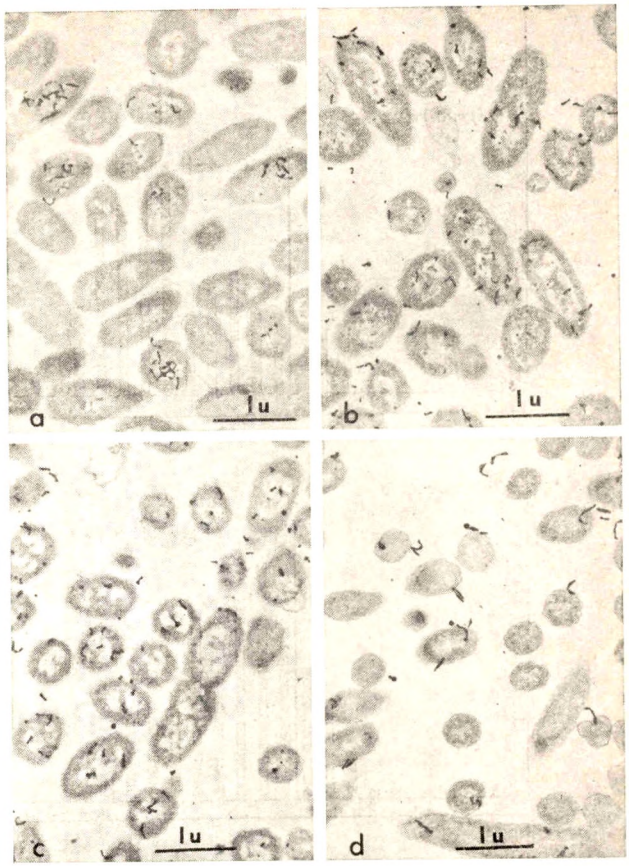


Fig. 1. Electron microscope autoradiography of tritiated cells. Washed cells prepared as described in Table 2 were resuspended in 1 ml. medium E, fixed in 1 per cent OsO_4 and processed for electron microscopy and autoradiography by a method to be published elsewhere (J. R. W. and H. van den B.). Loss of ^{3}H was monitored during fixation, staining and dehydration of the β-^{3}H-alanine cells. Cells grown in 2·0 μM β-^{3}H-alanine retained 53 per cent and cells grown in 0·05 μM β-^{3}H-alanine retained 80 per cent of ^{3}H after these procedures. Exposure time was 6 months. *a*, [*methyl*-^{3}H-thymidine; *b*, L-^{3}H-tryptophan; *c*, β-^{3}H-alanine (11 per cent of ^{3}H in ACP); *d*, β-^{3}H-alanine (89 per cent of ^{3}H in ACP).

grown on excess β-alanine (*d*). In the cells grown on limiting β-alanine the median of the grain distribution shifts towards the cell surface in conditions where the label is concentrated in ACP (*e* and *f*). The means and medians of these different preparations are listed in

Table 2. PREPARATION OF LABELLED CELLS

E. coli cultures	^{3}H-Metabolite	Conc. (μM)	Spec. act. (Ci/mmole)	A_{600} at harvest	Radioactivity (disintegrations/day/cell)
Hfr 139	[*methyl*-^{3}H]-thymidine	30·0	6·7	1·40	5·0
Hfr 139, Trp 3	L-^{3}H-tryptophan	40·0	2·5	0·2	41·0
Hfr 139	DL-^{3}H-glutamic acid	2·0	26·9	0·62	20·0
M99-2†	β-^{3}H-alanine	2·0	5·2	1·48	8·0 (89% as CoA)
		0·05	5·2	0·16	1·5 (32% as CoA)
		0·05	5·2	0·48	1·0 (11% as CoA)

All cells were grown in medium E with 0·4 per cent glycerol as carbon source. *E. coli* Hfr[139] was supplemented with 2·0 μM pantothenate and 1·7 μM thiamine. ^{3}H-Thymidine cells: Hfr 139 was grown in medium supplemented with [*methyl*-^{3}H]-thymidine, harvested and washed three times with medium E containing unlabelled thymidine. ^{3}H-Tryptophan cells: Hfr 139, Trp 3, a tryptophan auxotroph derived from Hfr 139 and donated by Dr D. F. Silbert, was grown in medium supplemented with L-^{3}H-tryptophan and washed as above with medium containing unlabelled tryptophan. ^{3}H-Glutamic cells: Hfr 139 was grown in medium supplemented with DL-^{3}H-glutamic acid. β-^{3}H-alanine cells: M99-2, a β-alanine auxotroph (donated by Dr W. Maas), was grown with either 2·0 or 0·05 μM β-^{3}H-alanine The cells grown on limiting β-alanine concentrations (0·05 μM) were harvested either in the logarithmic phase at A_{600}=0·16 or in early stationary phase at A_{600}=0·48 and washed as above. ^{3}H-CoA and ^{3}H-ACP were determined by DEAE-cellulose chromatography. β-^{3}H-alanine (specific activity 5·2 Ci/mmole) was obtained by reductive dehalogenation of α-bromo-β-aminopropionic acid with carrier-free tritium gas in dimethylformamide in the presence of 5 per cent palladium on alumina and an equimolar amount of triethylamine (carried out by New England Nuclear Corp., Boston). The α-bromo-β-aminopropionic acid was synthesized according to Schöberl and Braun[19].

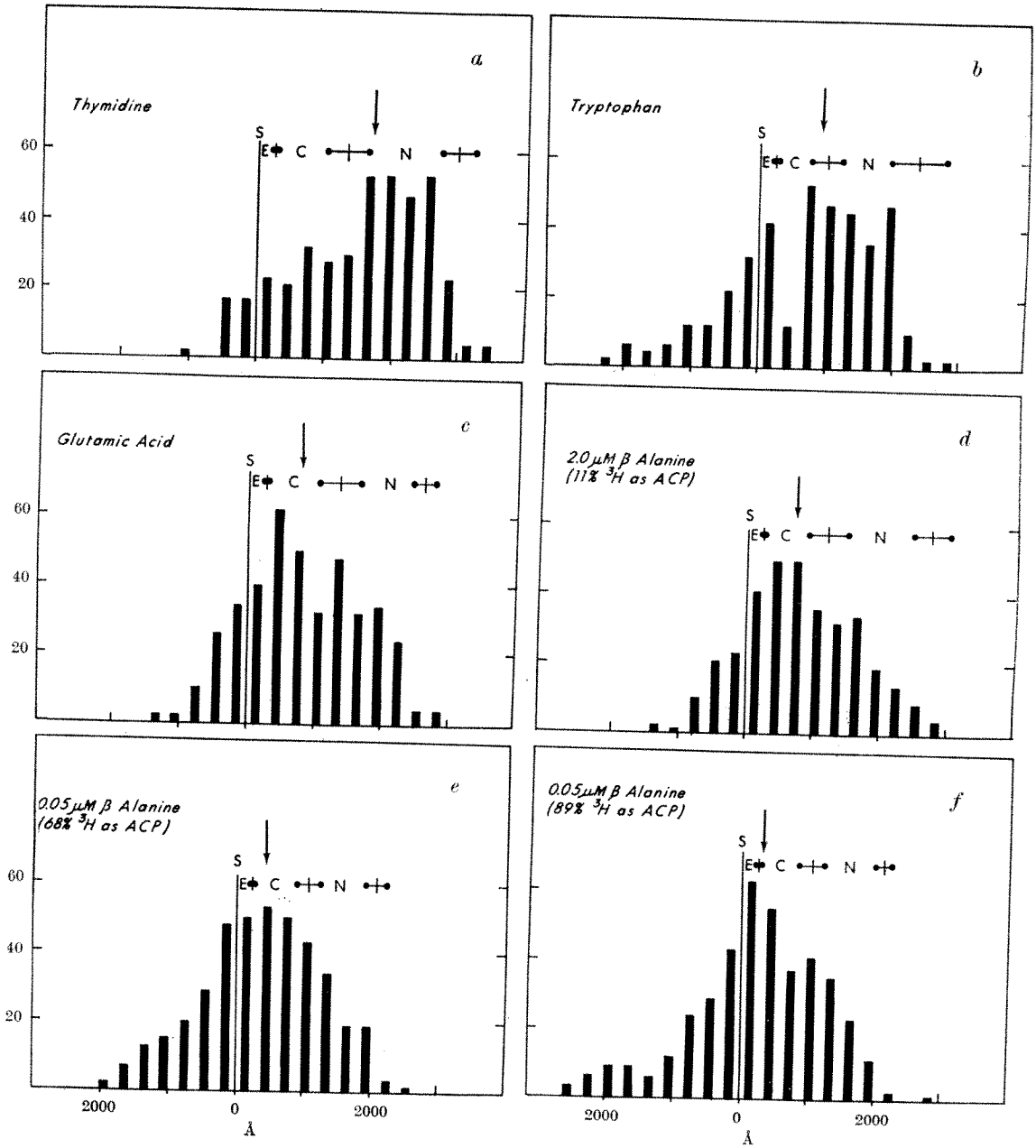


Fig. 2. Histograms of grain distributions in tritiated cells. The shortest distance from the midpoint of each grain to the cell surface (S) was measured with a dial caliper. Grains longer than 2500 Å, regardless of their location, were not included. From the tabulated measurements histograms[20] were constructed. The width and distance from the cell surface of the cell envelope (E), cytoplasm (C), and nuclear area (N) were obtained from measurements on fifty different cells in each group and are indicated ±s.d. The arrow indicates the median of the grain distribution in each group. *a*, [*methyl*-³H]thymidine; *b*, L-³H-tryptophan; *c*, DL-³H-glutamic acid; *d*, β-³H-alanine (11 per cent ³H as ACP); *e*, β-³H-alanine (68 per cent ³H as ACP); *f*, β-³H-alanine (89 per cent ³H as ACP).

Table 3. A χ^2 test (Table 4) comparing grain distributions indicates that each group differs significantly from the others except for the tryptophan, glutamic acid and excess β-alanine groups (2, 3 and 4 respectively), which do not differ from each other but do differ significantly from the remaining groups.

The results of these experiments indicate that ACP is located in the plasma membrane or very near the cytoplasmic side of the membrane. That ACP is not present in the cell wall is evident from the fact that bacterial L forms, which lack cell wall, are fully capable of synthesizing both fatty acids and complex lipids for membrane synthesis. The retention of at least 90 per cent of ^{14}C-labelled ACP inside osmotically shocked cells which had lost 81 per cent of the 5′-nucleotidase activity indicates that little if any ACP is present in the periplasmic space. The localization of ACP by electron microscopy autoradiography is based on visual inspection of the autoradiograms, the mean and median values for grain distribution in the various groups, and the χ^2 test of homogeneity of grain distributions. The χ^2 test results indicate the presence of four different patterns of grain distributions in the six experimental groups. This information, along with what we know about the cellular distributions of the radioactive constituents in three of the six groups, permits us to infer the source geometries responsible for the distribution of grains in the remaining three groups. The mean and median of the grain distribution for cells labelled with thymidine are, as expected, nearer the centre of the cells than the mean and median of the grain distributions for cells labelled with tryptophan or glutamic acid. These differences correspond to the distribution of nuclear material and cytoplasm. The mean and median grain distribution of β-alanine cells containing 89 per

Table 3. MEAN AND MEDIAN OF GRAIN DISTRIBUTIONS

Label	Mean ± s.e. (Å)	Median (Å)
[*methyl*-^{3}H]-thymidine	1,468 ± 69	1,715 (193)*
L-^{3}H-tryptophan	662 ± 70	900 (203)
DL-^{3}H-glutamic acid	824 ± 60	750 (200)
β-^{3}H-alanine		
11 per cent ^{3}H in ACP	713 ± 48	710 (349)
68 per cent ^{3}H in ACP	360 ± 43	420 (417)
89 per cent ^{3}H in ACP	232 ± 44	300 (409)

* Figures in parentheses indicate number of grains measured. Mean and median (in Å) of grain distributions, in relation to the cell surface, were determined in the experiments plotted in Fig. 2.

Table 4. χ^2 TEST OF HOMOGENEITY

Comparison	χ^2	Degrees of freedom	P
1*, 2	97·53	19	< 0·001
2, 3, 4	47·38	38	n.s.
2, 5	27·75	17	< 0·05
2, 6	49·27	16	< 0·001
4, 5	57·19	19	< 0·001
5, 6	42·49	17	< 0·001

1* = [*methyl*-^{3}H]-thymidine; 2 = L-^{3}H-tryptophan; 3 = DL-^{3}H-glutamic acid; 4 = β-^{3}H-alanine (11 per cent ^{3}H in ACP); 5 = β-^{3}H-alanine (68 per cent ^{3}H in ACP); 6 = β-^{3}H-alanine (89 per cent in ACP).

cent of the label in ACP are quite close to the cell surface, in fact just beneath the cell envelope in a position corresponding to the plasma membrane. Because the distribution of grains in each β-alanine group is different ($P < 0.001$) and the mean and median shift toward the cell surface as the ratio of ACP to CoA increases, and because we have independent evidence that ACP is not present in the cell wall or the periplasmic space, it seems reasonable to infer that ACP is associated with the plasma membrane. This association is such, however, that rupture of the membrane releases the ACP into solution.

The localization of ACP on or near the inside surface of the plasma membrane would be consistent with its role in the synthesis of fatty acids and phospholipids which are present almost exclusively in the cytoplasmic membrane of *E. coli*. The fact that ACP is not randomly distributed in the cell suggests a degree of organization for a central component of the fatty acid synthetase which has not been appreciated before. This finding suggests that the *E. coli* fatty acid synthetase may be organized *in vivo*, perhaps in a typical multienzyme complex which includes the ACP.

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Field and Laboratory Observations of the Crown-of-Thorns Starfish, *Acanthaster planci*

Although there have been many reports of the large scale destruction of Pacific coral reefs by the crown-of-thorns starfish, *Acanthaster planci*, little is known about the starfish's ecology and behaviour. We publish here reports of its population density in the Pacific Ocean, and studies of its locomotory and feeding behaviour in the laboratory.

Densities of *Acanthaster planci* in the Pacific Ocean

THERE have been widely publicized reports of damage to coral reefs by *Acanthaster planci* (Linnaeus), the crown-of-thorns starfish[1–4]. Between December 1968 and April 1970 I studied, by diving, the occurrence and abundance of *Acanthaster planci* on eighty-three widely distributed coral reefs in the Pacific (Table 1). Many dives were made on reefs of Australia's Great Barrier Reef complex, including some reefs previously surveyed[3].

Acanthaster was recorded as common if more than five specimens were seen in a 20 min dive, and as scarce if there were between one and five specimens. None was found on 81 per cent of the reefs visited, which included twenty-one reefs on the northern section of the Great Barrier Reef (between Thursday Island and Lizard Island). On 14 per cent of the reefs the starfish was recorded as "scarce". I found denser populations on John Brewer and Lodestone Reefs off Townsville and two patch reefs in Fiji, but none of these populations attained the level which Pearson described[5] as an "infestation" (that is to say, more than forty starfish counted during 20 min of searching).

Accounts of "plague" densities have suggested that they are a new phenomenon following human interference with

Table 1. NUMBER OF REEFS VISITED, GRADED ACCORDING TO THE NUMBERS OF *Acanthaster* SEEN

Region	Common	Scarce	None seen
Tahiti	—	—	4
Moorea	—	—	1
Tuamotu Atolls	—	—	5
S. Queensland	—	—	6
C. Queensland	2	8	8
N. Queensland (Thursday Is. to Lizard Is.)	—	—	21
New Guinea	—	—	4
New Britain	—	1	2
Buka (Sohano)	—	—	1
Solomon Islands	—	1	5
New Caledonia	—	—	3
Fiji	2	—	2
American Samoa	—	1	3
Hawaiian Islands	—	1	2
Total	4	12	67

the environment[3,4,6,7], but Mortensen[8], who studied *Acanthaster planci* in the Java Sea in 1929, wrote in 1931: "This species was found rather commonly on the coral reefs at the little island of Haarlem off Batavia, near Onrust . . .". It seems quite probable that Mortensen's record of "common" might have been applied to the modest densities which constitute plagues, according to the criteria established by Endean[2] and Pearson[5]. It has been widely held that Green Island experienced the first known plague on the Great Barrier Reef in 1960, but an experienced professional diver from Townsville, D. Tarca (personal communication and *Sunday Mail*, September 7, 1969), recalled that there were large numbers on the south-east side of Lodestone Reef in 1954. I have spoken to fishermen in the Solomon Islands who recall large concentrations of them many years ago. It was said of Maringe lagoon, Santa Ysabel, that night-time fishing on the reef was especially hazardous about forty years ago, because of the numbers of *Acanthaster* then. The recent outbreak of reports about *Acanthaster* may perhaps be explained by great public attention and increases in the numbers of divers rather than of starfish.

The suggestion that plague populations result from removal by human shell collectors of the natural predator, *Charonia tritonis* (Linnaeus)[3], seems unlikely to be supported by further research. Although this large gastropod occasionally preys on *Acanthaster*[3,4,6] it usually prefers *Linkia laevigata* (Linnaeus), a starfish which is normally much more abundant than *Acanthaster*. Given an unlimited supply, one *Charonia* attacked only one *Acanthaster* in six days, and several of the victims escaped with the loss of a few arms[4].

Another form of interference suggested is that mechanical damage, by blasting or dredging, leaves large areas of dead coral on which *Acanthaster* larvae can settle, without being engulfed by the plankton-feeding coral polyps, establishing "seed" populations that may develop by further breeding into plagues[4]. It seems well established that metamorphosis of echinoderm larvae can be delayed until they discover an area suitable for settlement[9] and the older photonegative larvae[10] may be expected to visit the bottom repeatedly, while being carried by water currents for several days. It is less clear, however, that the searching larvae are vulnerable to predation by coral. If they are photonegative, it is probable that most of their searching is carried out during daylight, when the coral polyps are not feeding. The shaded crevices of coral reefs offer many suitable surfaces for settlement, such as dead coral, shell fragments and algal-encrusted stones, which are free from filter feeders and would provide for the attachment of brachiolaria larvae and the protection of young metamorphosed starfish. Mechanical destruction of coral would seem to be unnecessary for the survival and metamorphosis of *Acanthaster*, and might be unfavourable through reducing the available food. It does not necessarily follow, moreover, that a localized "seed" population of adults would result in heavy settlement of larvae, for the larvae have a planktotrophic life of considerable duration, during which they must be widely dispersed.

Mortensen[8] reared the larvae of *A. planci* for sixteen days, by which time they had reached the brachiolaria stage, indicating that the pelagic life lasts at least two or three weeks. The great variations in abundance of *A. planci*, including occasional plagues, may therefore result from the vicissitudes of life in the plankton. Such variations are to be expected and have often been recorded in invertebrates having a long planktotrophic stage[10]. We are probably observing natural fluctuations in the population of an animal which appears to be one of the main predators of coral. There is no real evidence to support the view that human interference is affecting the phenomenon to any extent. The greatest toll on *Acanthaster* is likely to be from pelagic predators. Endean[2] mentions that reef fishes have been observed to consume the eggs. During the breeding season of *A. planci* in Queensland waters (December 20 to January 20 (ref. 2)) there are abundant ctenophores, medusae and other plankton predators in the area of the Great Barrier Reef. This is the season for mackerel fishing and for peak abundance of other plankton feeding fish.

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Locomotory Response of *Acanthaster planci* to Various Species of Coral

THE crown-of-thorns starfish, *Acanthaster planci*, which consumes coral tissues[1,2], is destroying coral communities in widespread localities in the Pacific[3–5].

During a recent survey in Micronesia we noted that some corals survive better than others in the face of starfish attack. Some aspects of the physiological basis of selective predation were subsequently investigated. We here describe the locomotory response of *Acanthaster planci* to certain species of coral.

Four starfish were used in experiments carried out in a large shallow aquarium. Trials were mostly conducted in the late evening and early morning when the animals were most active. A starfish was turned on to its aboral surface and a stiff wire screen suspended horizontally above it. Such a starfish invariably pulled itself on to the underside of the screen and walked across it. In this position, numerous tube feet projected through the screen, allowing pieces of coral to be held where the tube feet would encounter them. Pieces of coral were also placed on top of the screen in the path of a walking starfish. The responses of the tube feet to various species of coral are summarized in Table 1.

Although there were wide differences in response to different coral species, the reactions to any given coral were consistent, and usually of one type (I–IV), with all the tube feet responding in the same manner. Certain corals elicited more than one kind of response in different tube feet at the same time. This may have been caused by local variations in the surface of the coral. The starfish reacted more strongly to the calicinal side of *Pavona frondifera* than to the acalicinal (living) side. Except for *Porites* (*Synaraea*) *iwayamaensis*, the tube feet formed strong attachments to the washed skeletons of all corals tested. It is therefore probable that the withdrawal reaction is a response to living coral tissue.

To test the effect of tentacular nematocysts on the reaction of the tube feet, the octocorallian *Heliopora coerulea* and the scleractinian *P.* (*S.*) *iwayamaensis* were induced to expand by placing then in cold sea water and

then returning them to normal temperatures. When tube feet touched these corals, there was a much more rapid withdrawal than occurred with the same colonies in the contracted state. From this we conclude that nematocysts are an important factor in the withdrawal of tube feet.

Corals, such as *P.* (*S.*) *iwayamaensis*, which produced a weak reaction in an unfed *Acanthaster planci*, provoked an immediate response after the starfish had fed for several hours. Thus satiety in the starfish may potentiate the withdrawal reflex.

Similar withdrawal responses were seen when corals were placed in the path of a starfish moving over the bottom of the aquarium. The tips of the arms on the advancing half of the animal were curled up, their terminal tube feet extended and performing exploratory motions. When an obstacle was encountered, the arm or arms involved reared back, and the larger, more central podia brought to bear on it. In this way, the starfish climbed over the obstruction. If the obstacle were a living coral, this reaction was modified, the tube feet touching the coral being withdrawn and the ambulacral grooves often closed. The arms involved curled back as the animal advanced. If about to feed, the starfish will mount the coral with minimal use of the tube feet, gaining purchase with its heavy spines. Mounting was due chiefly to muscular action of the arms. Starfish not about to feed may simply climb over the coral, if it is small. If, however, the terminal tube feet continue to encounter living coral as the arms rear up, then the coral is skirted with the arms held back (Fig. 1).

The responses of the extruded stomach to mechanical and other stimuli were examined by pulling two starfish from the corals on which they were feeding. The gastric folds were then prodded with pieces of *Pocillopora* and *Acropora*. Localized contractions of the muscles in the gastric folds were seen. Similar contractions were produced by prodding with a blunt rod. But gentle stimulation with a sharp needle caused larger responses. Thus we conclude that the stomach is less sensitive to the nematocysts than are the tube feet.

Field observations suggest that some coral species are more likely to survive than others in the face of moderate to heavy *Acanthaster* predation. For example, the hydrozoan *Millepora* and the octocorallian *Heliopora*, and, among scleractinians, large heads of *Porites*, particularly *P. lutea* and *P.* (*S.*) *iwayamaensis*, predominate on reefs devastated by *Acanthaster*. There is also evidence that large heads of *P. eydouxi* (personal communication from T. F. Goreau) and some *Galaxea* are less likely to be eaten than other such corals.

Table 1. REACTIONS OF THE TUBE FEET OF *Acanthaster planci* TO CONTACT WITH VARIOUS SPECIES OF CORAL

Coral[7]	Touch		Walking	
	Living coral	Washed coral skeleton	Living coral	Washed coral skeleton
Pocillopora eydouxi	I (7)	V (1)	I (4)	V (1)
Pocillopora damicornis	I (1)	—	I (1)	—
Acropora formosa	I–II (7)	V (1)	I–II (4)	V (1)
Acropora hyacinthus	II (1)	V (1)	II (1)	V (1)
Porites (*Synaraea*) *iwayamaensis*	III–IV (6)	IV (3)	III–IV (4)	IV (3)
Porites lutea	III (5)	V (1)	III (3)	V (1)
Porites andrewsi	I (3)	V (1)	I (3)	V (1)
Pavona frondifera (oral surface)	I–II (4)	—	—	—
*Millepora platyphylla**	I–IV (1)	V (1)	I–IV (1)	V (1)
*Millepora tenera**	IV (2)	V (1)	IV (3)	V (1)
Heliopora coerulea†	I–IV (2)	V (1)	I–IV (2)	V (1)

The grades of reaction are: I, instant retraction when the tube foot contacted the coral surface; II, exploration of the coral surface followed by rapid retraction; III, exploration, placement of adhesive disk, followed by rapid retraction; IV, exploration, placement of disk with weak adhesion to the coral, such that the tube feet were not broken when the substrate was pulled away; V, exploration, placement of disk with strong adhesion to the coral such that the tube feet were stretched and often broken when the substrate was pulled away. Reactions are of tube feet encountering coral held above the wire screen (touch), and corals placed on the screen in the path of a moving starfish (walking). The numbers in parentheses represent the number of days on which a given species of coral was tested. On each occasion a freshly collected specimen of coral was used.

* Hydrozoa, Milleporina.
† Anthozoa, Octocorallia.

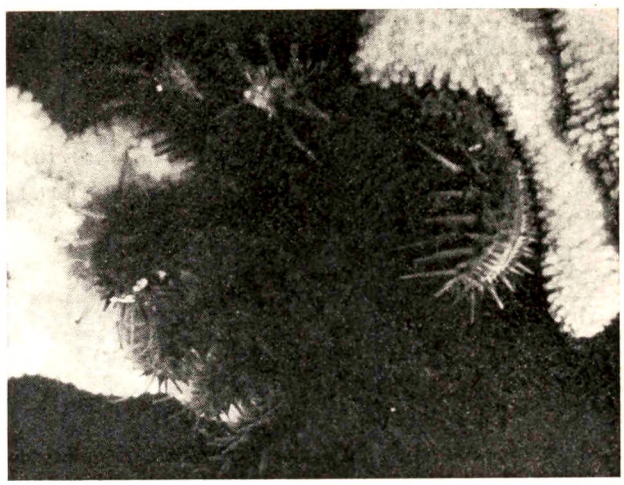


Fig. 1. Photograph showing *Acanthaster planci* walking between two pieces of *Pocillopora damicornis*. The living coral on the left has stimulated an avoidance reaction in one arm which is held back from the coral with the ambulacrum closed. The starfish is using its tube feet to climb over the washed coral skeleton on the right of the photograph.

Acanthaster planci feeds by everting its stomach through the mouth, and spreading the gastric folds over the coral surface by means of the tube feet[6]. The nematocysts seem ineffective against the stomach, but very effective against the tube feet. The peculiar humped position often observed in *Acanthaster* feeding on corals may arise from the necessity to keep tube feet away from the coral tissue while the stomach is being extended. Spreading by means of the tube feet suggests that it may be difficult for the starfish to insert its stomach into crevices. Corals growing in crevices, such as *Galaxea*, are to a certain extent protected. Furthermore, in the clefts often present in massive corals, areas of tissue often survive attack. This was particularly evident in *Platygyra lamellina*.

When climbing a coral, the starfish uses its arms and spines as much as possible. The branching staghorn coral, *A. formosa*, in spite of its strong defence mechanism, is more easily mounted than the smooth massive growths of *P. lutea*, which has weaker defences. The size of the polyps, and their arrangement in the skeleton, may be important, both in the localization of nematocysts and in the accessibility of tissue for digestion. For example, in the hydrozoan corals, most of the tissue is within the skeleton, and the nematocysts are concentrated in dactylozooids which present a formidable defence when they emerge. In addition, some corals, such as *P.* (*S.*) *iwayamaensis*, seem to be more palatable than others[6,7].

None of the resistant corals is immune from attack, but experiments described here and in the following letter[6] indicate that physiological factors, such as hunger and the time of day, affect the ability of the starfish to overcome the defences of the coral.

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Triggering of the Stomach Eversion Reflex of *Acanthaster planci* by Coral Extracts

RECENT observations of *Acanthaster planci* populations in Micronesia suggest that the animal may eat certain corals in preference to others. The starfish feeds by everting its stomach and applying it to the coral surface. Neither the stimuli evoking stomach eversion nor the mechanism by which the stomach is applied to the coral surface has been described (see Figs. 1 and 2 for explanations of the structure of *Acanthaster*).

The behaviour of *Acanthaster* in aquaria was studied: within a few hours this starfish, like a number of other asteroids[1,2], moved on to the glass side of the aquarium. It is thus possible to observe the activities associated with feeding, without disturbing the animal.

"Extracts" of coral tissue were prepared by scraping the coral surface, by crushing and grinding pieces of skeleton, or by washing off the tissue with a jet of seawater. Although each type of extract produced much the same reaction in the starfish, the latter method was used because it produced the least crude preparation. Standard "extracts" were prepared by repeatedly squirting the same 5 ml. of seawater at ten different spots on the surface of the coral. The extracts were rather viscid because of the production of mucus by the corals. Under the microscope, such extracts were seen to include zooxanthellae, cell debris, including discharged and undischarged nematocysts, and skeletal fragments. Extracts were presented to the starfish along an inter-radius, through a blunted 6 inch hypodermic needle, insinuated gently between the animal and the aquarium wall. The tip of the needle was slightly bent so that the extract could be directed into the oral region.

The injection of coral extracts into the mouth of the starfish resulted in highly reproducible responses that are distinct from the minor responses produced by similar injections of seawater (see Table 1 and Figs. 3 and 4).

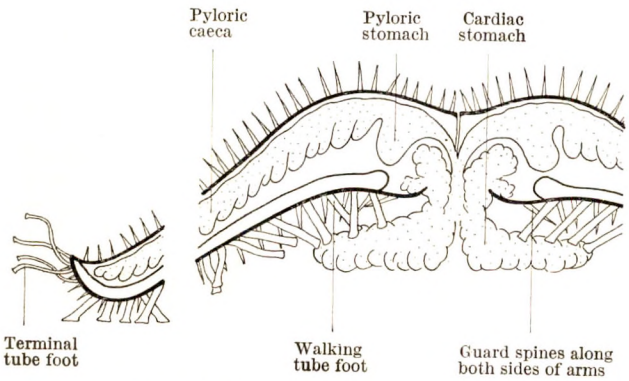


Fig. 1. A much simplified diagram of a section through the central disk and one arm of an *Acanthaster planci* everting its stomach. The mouth is distended and its guard spines held back. The aboral side of the disk is depressed and the whole disk is raised above the substrate as the cardiac stomach is everted through the mouth and then spread out by the tube feet.

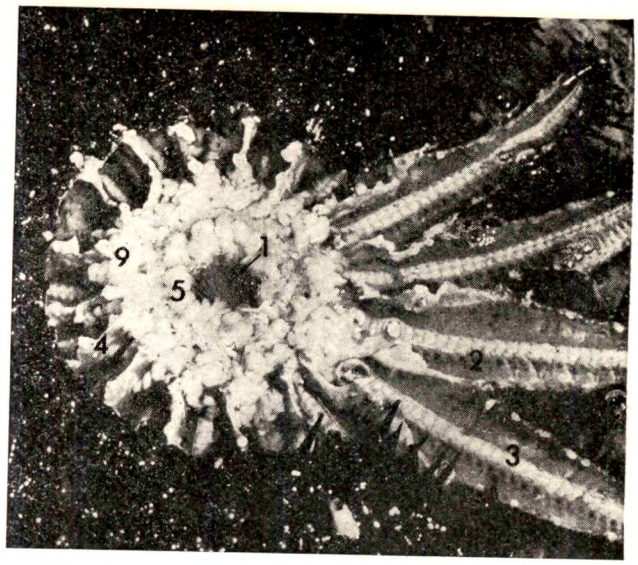


Fig. 2. Aboral dissection of *Acanthaster planci*. The roof of the central disk, and of four arms, has been removed to expose the folds of the cardiac stomach (5) and the pyloric stomach (4) surrounding it. The pyloric stomach gives off a pouch or caeca into each arm. These caecae have been removed from the dissected arms to show their skeletal elements, the ambulacral ossicles (3) and the balloon-like ampullae (2) that provide hydrostatic pressure for the tube feet. Some of the spines that guard the mouth can be seen through the mouth, at the centre of the disk (1). When the cardiac stomach is everted through the mouth, these spines are moved out of the way.

Extracts from *Acropora formosa* cause intense activity of the circum-oral tube feet within 1 min of introduction. During the next 2 to 4 min this activity spreads outwards along the ambulacral grooves. A widening of the oral opening occurs 5 to 7 min after injection and, at the same time, the horizontal spines bordering its margin are turned outwards, enlarging the opening further. The gastric folds can now be seen. Activity of the tube feet surrounding the mouth continues, and some of the more central ones are extended across the mouth region. Extrusion of the gastric folds is accompanied by flattening, then depression, of the aboral side of the central disk. This "lowers" the stomach to the central tube feet, which

Table 1. RESPONSE OF *Acanthaster planci* TO VARIOUS CORAL EXTRACTS

Donor species	Type of extract	Number showing response: Withdrawal —	Withdrawal +	Withdrawal ++	Stomach eversion —	Stomach eversion +	Stomach eversion ++	Stomach eversion +++
Acropora formosa	1 and 2	3	1	0	0	0	4*	0
	3	11	1	0	1	2	9*	0
Pocillopora eydouxi	3	1	3	0	0	0	2	2
	4	5	0	0	0	1	3	1
	5	0	1	2†	0	1	0	1
Porites spp.	3	0	3	5‡	6	1	1	0
Sea water	—	2	2	0	4	0	0	0

Responses were elicited by placing 3 ml. of the various extracts into the oral opening of *Acanthaster* specimens of diameter 10 to 12 inch tip to tip.

Types of extracts used: (1) scrapings of coral surface resuspended in seawater and allowed to settle for 5 min; (2) whole branch of coral (2 inches long) ground up with 10 ml. seawater and allowed to settle; (3) 5 ml. of seawater sluiced repeatedly over the coral surface as described in the text; (4) supernatant from centrifuged type 3 extract; (5) precipitate from preparation of type 4 extract, resuspended in 5 ml. of seawater. Responses were graded, to 3+ on an empirical scale.

* One animal in this series proceeded through the usual sequence of tube foot activation, to expansion of oral opening, but then moved away, still in a feeding configuration, and was found shortly afterwards feeding on a piece of coral left in the aquarium.

† One animal after a violent withdrawal reaction moved away, as though escaping from an irritating stimulus.

‡ Two experiments with type 4 *Pocillopora* extract failed to elicit withdrawal responses, but produced only ++ and +++ stomach eversions. One experiment with heat-treated type 5 extract failed to elicit either withdrawal or eversion response.

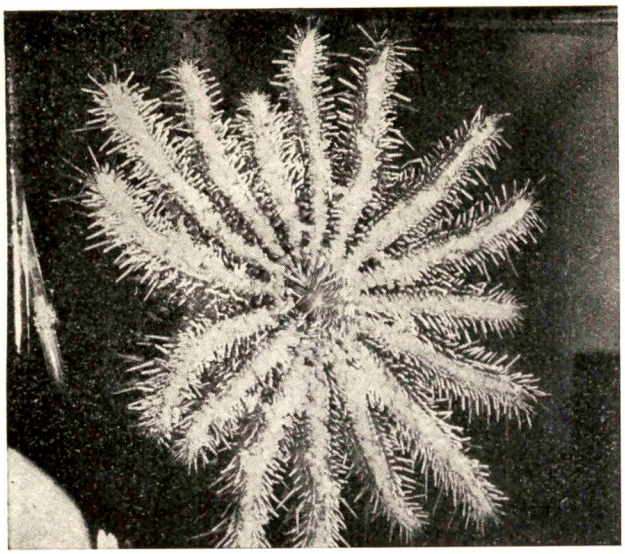


Fig. 3. A 10 inch specimen of *Acanthaster planci* in resting position on wall of aquarium. Note disposition of tube feet and position of circumoral spines covering closed oral opening.

then retract. The adhesive disks of the podia form attachments which pull on the gastric folds, spreading them out. Subsequent activity of the podia pulls and pushes the gastric folds into position until, after about 10 to 15 min, the everted part of the stomach covers an area slightly larger than the central disk. The folds seem to be extruded by hydrostatic pressure in the coelom, but observations of *Acanthaster* feeding on corals indicate that the tube feet are chiefly responsible for moulding the stomach to the contour of the substrate. The intensity of the response decreases after about 20 min, so that most animals return to the resting condition within 30 min after injection of the extract.

Extracts of *Pocillopora eydouxi* cause a similar response, but with two differences. First, there is an initial retraction of the tube feet that come into contact with the injection mass. This quickly gives way to the activity described above. Second, extracts of *Pocillopora* produce responses persisting considerably longer than those seen with *Acropora*.

Extracts from two species of *Porites* (*P.* (*Synaraea*) *iwayamaensis* and *P. lutea*) evoke a pattern of response in which tube foot withdrawal predominates. Three to five minutes after injection of such extracts, all portions of the ambulacral grooves near the mouth are closed up, and the animals remain suspended only by their "finger-tips". It usually takes 10 to 15 min for the animal to return to its original state. In two out of eight instances this initial aversive response was followed by gradual opening of the mouth and extrusion of the stomach, which in one case reached maximal extension after about 30 min.

When extracts of *Pocillopora eydouxi* are centrifuged for 20 min at 2,000*g*, the resultant supernatant is water-clear, and retains its ability to cause stomach extrusion. The response, however, appears less intense. The initial withdrawal reaction is abolished. Boiling the supernatant for 20 min does not further decrease the extrusion responses. When the sediment from the centrifugation is resuspended in seawater and given to the starfish, an initial withdrawal response is obtained, usually followed by stomach extrusion. Heat treatment of the centrifugate abolished the withdrawal reaction, but did not prevent stomach eversion.

Extracts produced by grinding up portions of the gastric folds of *Acanthaster planci* have no effect when presented to intact starfish in the aquaria, but similar extracts prepared from the pyloric caeca of the same specimen caused a strong and enduring gastric eversion response. This suggested either the storage of active components from past meals in the caecal pouches, or, possibly, the presence of chemical control substances elaborated by the animal.

There are indications that the responsiveness of *Acanthaster* to chemical evocation of stomach eversion is not constant, but varies with the physiological state of the animal. As with other asteroids, the time of day[3,4], and nutritional status[5], are two factors which give rise to such changes. Thus, during daylight hours, animals usually seem less reactive than at night, and the peak of activity seems to be between midnight and dawn. Animals not deliberately stimulated by administration of coral extract invariably are found on the aquarium walls in the resting state during the day. On the other hand, between midnight and 0200 h on four successive nights, 64 per cent of all observations of the same animals showed specimens with partly opened mouth and visible gastric folds. In a few instances, the stomach was partially everted at this time. Nutritional status likewise seems to affect responsiveness. Thus it was observed that, one day after being fed with live coral, all specimens tested responded feebly to preparations that had previously elicited marked stomach eversion. Similarly, the intensity of tube foot withdrawal increases in these circumstances[6].

Although evocation of feeding behaviour by chemical means in coelenterates has previously been described, the only comparable observations in echinoderms relate to stimulation of locomotion by extracts from prey organisms[7,8] and to the evocation of opening of the mouth by acetylcholine[9].

Two of us (R. W. B. and M. R. J.) have been able to stimulate stomach eversion in *Asterias forbesi* and in *Astropecten vulgaris* with extracts of the bivalve molluscs *Venus mercenaria* and *Crassostrea virginica*. In these cases, as well as in the case of *Acanthaster*, the active elements producing stomach eversion seem to be water soluble, heat stable chemicals of low molecular weight. The initial withdrawal response was seen only in *Acanthaster*-treated extracts of certain coral species. The active elements seem to be insoluble and heat labile. It seems likely that nematocysts are responsible, particularly as there is other evidence to indicate that nematocysts cause tube food withdrawal[6].

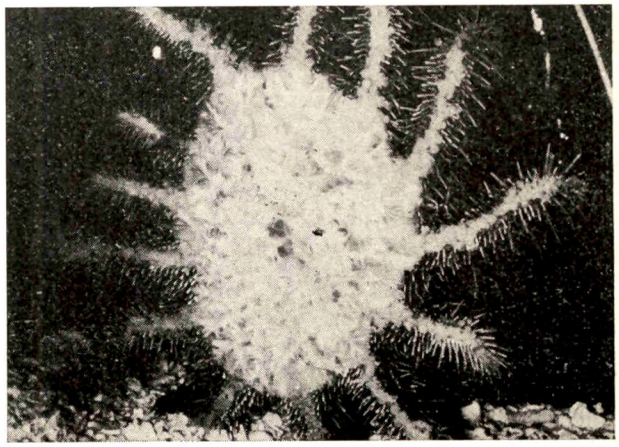


Fig. 4. Maximal stomach eversion in the specimen shown in Fig. 3 15 min after administration of 3 ml. of type 3 *Pocillopora eydouxi* extract. Note position of tube feet visible beyond edge of expanded stomach. The yellow suction disks of many other tube feet applied to the folds can be made out through the membranes. Note also the highly developed network of haemal canals within the gastric folds, corresponding perhaps to similar but smaller structures in lining of the cardiac stomach in other asteroids[10].

It is possible that there is a further factor operating so that *Acanthaster* finds some extracts more acceptable than others. *Porites* (*S.*) *iwayamaensis* has weak nematocyst defences, unlike most other corals which the starfish tends to leave alone. Thus it is significant that extracts of *P.* (*synarea*) *iwayamaensis* always evoke a withdrawal reflex[6], but seldom stomach eversion.

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Spaced Receiver Observations of Radio Pulses

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Preliminary observations have been carried out to look for radio signals associated with gravitational waves. Although inconclusive, the observations from five receivers in the British Isles suggest the value of pressing on with further experiments.

Towards the end of last year, the evidence for the detection of pulses of gravitational waves[1] had reached a significance sufficient to arouse interest in many areas of astronomy and physics. It occurred to one of us (J. V. J.) that if Weber's flux were $\sim 10^4$ erg cm^{-2} s^{-1} (ref. 1) at the Earth, an exceedingly small fraction ($\sim 10^{-19}$) of this energy, which might be radiated into ~ 1 MHz bandwidth of the radio spectrum, would be detectable with the very simplest of radio antennae (a single dipole for example) and a receiver of even modest performance. This suggestion, made casually at a meeting in Cambridge, was immediately taken up by the Mullard Radio Astronomy Observatory. It was realized that the detection of isolated radio pulses, of say a few per day, in the presence of man-made interference, lightning or other geophysical phenomena, required simultaneous observations at widely spaced sites. It was further realized that observations at two or more frequencies could in principle provide valuable evidence as to whether any events observed were galactic in origin, for the interstellar plasma would cause dispersion. Any such events need not, of course, necessarily be associated with gravitational pulses but could originate, for example, in supernovae explosions[2] or in the rather energetic events which Scargle and Harlan[3] suggest are responsible both for the wisp activity and the pulsar spin-up in the Crab nebula. It may be of relevance that coincident radio pulses have already been observed, on a baseline ~ 150 km and during hours of darkness, in the course of early work[4] on scintillation phenomena; as far as we know, these events have not been explained and further events of this type might easily have been overlooked in the course of subsequent radio astronomical investigations.

We are here reporting some preliminary observations which have been carried out simultaneously with receivers sited at five locations distributed over England, Scotland and Ireland, covering a range of baselines between 110 and 500 km. These sites are shown in Fig. 1. At 151 MHz all five sites were equipped with almost identical systems. Each station had two half-wave dipole antennae mounted on the ground, 5λ apart, approximately $(\lambda/4)$ above a metal ground plane on an EW-baseline; they operated as phase-switched interferometers[5]. Bandwidths varied

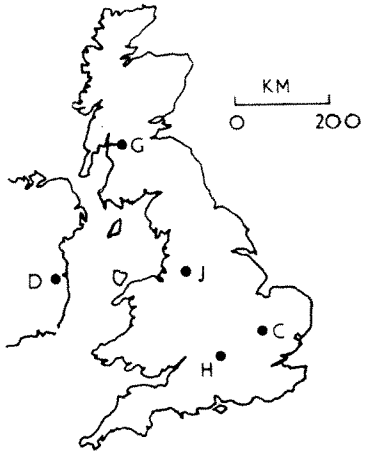


Fig. 1. Location of the observing stations. C, Cambridge (151, 81 MHz); D, Dublin (151, 70 MHz); G, Glasgow (151, 81, 47 MHz); H, Harwell (151 MHz); J, Jodrell Bank (151, 45 MHz).

among the stations, between 200 kHz and 1·5 MHz, and integrating time constants of between 0·3 and 1·0 s were used. The central lobes of these five interferometers were aligned to account for the differences in geographical longitude. Recordings were made on chart paper at speeds ranging between 6 inch and 24 inch per hour, and time marks enabled an accuracy ranging from ±24 s to ±6 s to be attained for the times of the recorded events. Similar interferometers at lower frequencies also operated at certain stations as noted in Fig. 1. Most of the stations were operating by January 1, 1970, and the observations have been analysed until the end of May.

Many two-fold and multiple coincidences were obtained from the outset, but it soon became apparent that most of these were occurring during daylight hours and were attributable largely to Type III solar bursts. We therefore use in the analysis only events which occurred between sunset and sunrise.

At all stations, occasional pulses of radio emission were observed at a rate of a few per night and with durations ranging up to several minutes. To avoid making any prior assumptions about the likely intrinsic duration of any interesting radio "event" or its dispersion through the receiver bandwidth, all such bursts of radio emission were classified as "events". The only acceptance criterion applied was that each burst should exceed three times the normal "peak-to-peak" noise level in the records. This corresponded to a minimum detectable flux density $\sim 10^{-18}$ ergs cm^{-2} s^{-1} Hz^{-1} per cent.

The times of the events recorded at the five individual stations at 151 MHz have been compared for evidence of the simultaneous observations of the same event at two or more stations. Coincidence resolving times of 1 min and 20 s have been used, these values taking account of the accuracy of timing and also the possibility of slight differences of arrival times at the five stations. These could arise from interstellar dispersion and the fact that the central frequencies of the receivers were not all precisely the same, on account of local interference problems. A dispersion measure $DM = \int n_e \, dl$ of 400 cm^{-3} pc, for example, produces a frequency sweep rate of 1 MHz s^{-1} at 151 MHz; this dispersion measure is about the highest

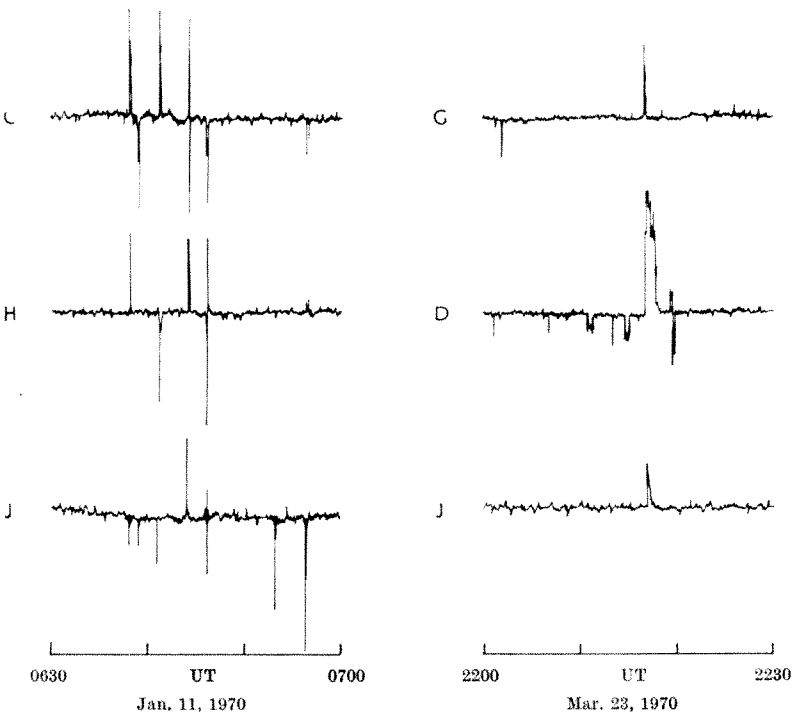


Fig. 2. Tracings of interferometer records for the three stations involved in two of the "triple-coincidence" events.

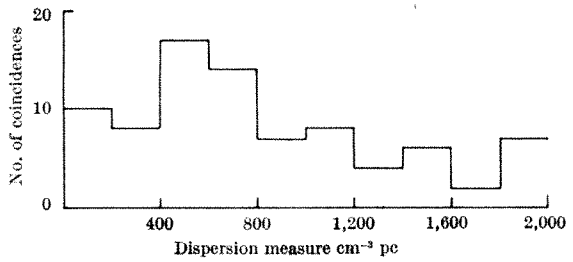


Fig. 3. Histogram of delayed coincidences as a function of dispersion measure.

so far observed for any pulsar[6]. The numbers of two-fold coincidences expected to occur by chance were estimated by a similar intercomparison of the times of events at pairs of stations, when the times of events at one station were delayed by 3 min. In this way, allowance could be made for the very variable single-station counting rates and occasional operating failures at individual stations.

During five months of operation, some seventy-two "prompt" two-fold coincidences were observed within a 1 min resolving time, compared with forty-seven "delayed" coincidences. When the coincidence resolving time was reduced to 20 s, these numbers fell to thirty-five "prompt" and twenty-two "delayed" coincidences. The numbers of "prompt" two-fold coincidences are thus barely distinguishable from those expected by chance.

Five "prompt" coincidences occurred between three stations, however; these twice involved sequences of more than two pulses. Examples are shown in Fig. 2. Inspection of these three-fold events suggests that they are not attributable to a distant source, for (i) the precise pulse shapes differ at the individual stations and (ii) the polarity changes in the pulse sequence are inconsistent with the motion of a celestial source through the interferometer lobes. It is also significant that no four or five-fold coincidences have ever been observed. The 151 MHz data alone have therefore not so far yielded any evidence for radio pulses of extraterrestrial origin.

Recordings at the other frequencies have been scrutinized for evidence of delayed pulses. The technique used has been to assume a particular value of DM, compute the radio pulse delays expected at different frequencies and then, starting with event times recorded at the highest observing frequencies, look for corresponding events at the appropriate delay at lower frequencies. A computer program performs this analysis for successive increments of 25 in values of DM up to 2,000 electrons cm^{-3} pc. In this way, a plot of "delayed coincidences" versus DM is obtained and any contribution of genuine radio pulses from a source at a particular value of DM appears as a peak on this distribution. The results of this analysis are shown in Fig. 3. There is seen to be an excess in the region $DM \sim 600$ cm^{-3} pc. Although this has a statistical significance of about 1 per cent if it is assumed that the data of Fig. 3 follow a Poisson distribution, we feel that further observations are required before the excess can be regarded as convincing. Such a value for the dispersion measure would not be wildly unreasonable, however, for a source at the galactic centre[7] on the basis of an assumed mean interstellar electron density of 0·06 cm^{-3} (ref. 8) and a distance of 10 kpc. Higher values of DM, on the other hand, might be difficult to observe with the present equipment

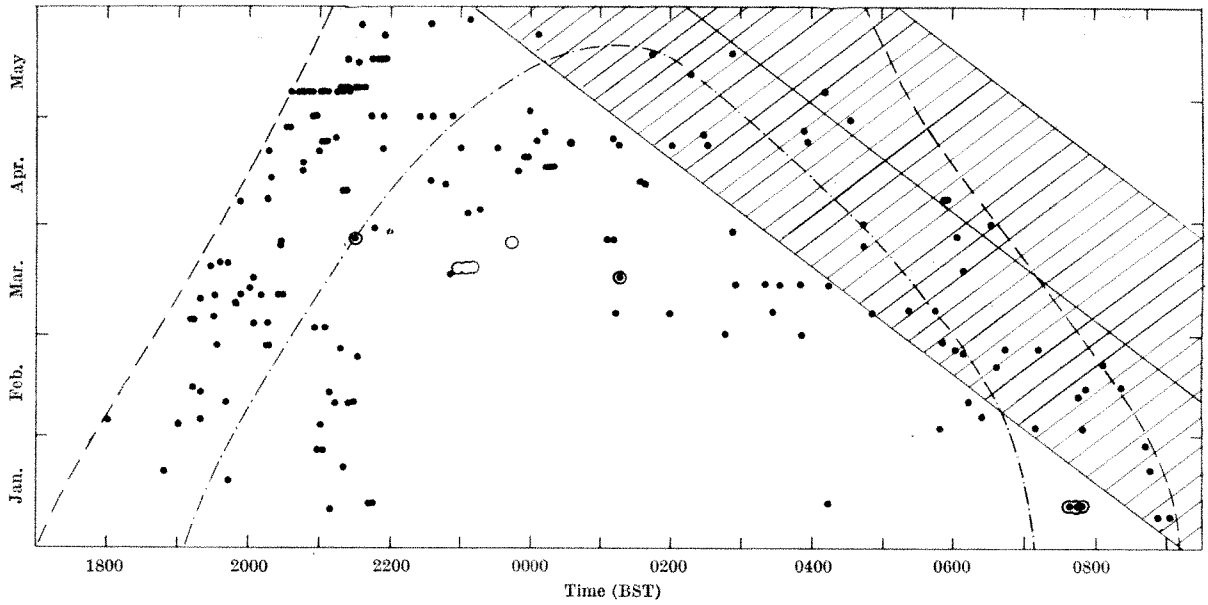


Fig. 4. The distribution of 151 MHz event arrival times during the hours of darkness for one station. ●, Events; ○, triple coincidences; - - -, time of sunrise and sunset; · · · · ·, start or end of astronomical twilight; ———, transit time for galactic centre. The shaded area indicates when the galactic centre was above the horizon.

because of increase of the pulse length at the lower frequencies.

Weber's latest investigations[7] indicate that his gravitational events probably originate in the direction of the galactic centre which, because of its low maximum elevation ($< 10°$) at our latitudes, was not favourably placed for our simple dipole antennae. For this reason, four of the existing stations, and a fifth on Malta, have now been equipped with suitably orientated directional antennae specifically designed for galactic centre observations. This new program commenced in May but no results are yet available. We understand that Drs Partridge and Ruffini of Princeton are carrying out similar galactic centre observations at microwave frequencies.

Although unlikely to be astronomical, the origin of the coincident events and of most of the individual events is of interest. In general, the rate of events was found to be greatest for a few hours after sunset or a few hours before sunrise (Fig. 4). While some events undoubtedly originate in local interference, particularly in the evening, this is unlikely to be the explanation of the high pre-dawn rate. The cause of these increased rates is not at present understood, although ionospheric scattering of solar bursts, or reflexion of terrestrial signals by meteor trails, are possibilities. The occurrence of coincident events does not correlate in any obvious way with electrical storms, unusual geomagnetic activity, meteor showers or transits by man-made satellites. Further observations designed to clarify the nature of this phenomenon are in progress.

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Contribution to the Co-rotating Magnetic Field Model of the Pulsar

by

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A new general solution of the electromagnetic field equations convenient for rotating field problems is used to analyse the case of low plasma number density. Two localized pulse emitting regions rotating with the star at approximately twice the velocity of light radius are predicted.

RECENT observations[1,2] of the polarization of the radio pulses from pulsars lend qualified support to the idea of a co-rotating magnetic field as proposed by Gold[3] and discussed by several authors[4–8]. Gold[4] has shown that radio frequency radiation from the Crab pulsar is much too small to explain its observed rate of slowing down and has suggested that loss of relativistic gas from the star's magnetosphere constitutes the principal cause of drag torque. Pacini[5] pointed out earlier that radiation of the rotating dipole into a vacuum produces the right order of magnitude for drag torque. He dismissed then the possibility that even approximate vacuum conditions could prevail, although he and Rees[8] have now qualified this assertion. Ostriker and Gunn[6] have shown that the measured rate of slowing down of the Crab pulsar, its present age and the estimated energy supply to the surrounding nebula are all in very good agreement with the attribution of the pulsar energy loss to dipole radiation

into an approximate vacuum. We analyse the case where the plasma number density is low, by which we mean $\lesssim 10^{19}$ m^{-3} in the case of the Crab pulsar. This leads to straightforward electrodynamics. It provides simple explanations of the transmission of energy from the pulsar to the surrounding nebula, and of many features of the structure of both optical and radio pulses. In particular, it predicts that there should be two localized emitting regions, a feature that has not so far been explained. They are located approximately two velocities of light radii away from the star and on opposite sides of it. We start by giving our own solution of the field problem which presents the results in a form more amenable to the subsequent analysis.

In the stationary coordinate system orientated on the rotation axis of the star, Maxwell's equations apply with no complications of a general relativistic nature. Provided that approximate vacuum conditions apply, and we justify this retrospectively, spatial homogeneity may be assumed and the field problem then reduces to solving the complex vector Helmholtz equation

$$\nabla^2 \boldsymbol{A} + k^2 \boldsymbol{A} = 0 \tag{1}$$

where $e^{i\omega t}$ time variation has been assumed, $\boldsymbol{A}$ is a solenoidal vector which may be either the electric or the magnetic field and k is the complex wave number for the medium. We solve the equation in spherical polar coordinates as follows. (i) Write it out in cylindrical polar coordinates (ρ,φ,z). (ii) Remove the simultaneity by introducing

$$A_1 = (A_\varrho + iA_\varphi); \quad A_2 = (A_\varrho - iA_\varphi) \tag{2}$$

(iii) Transform to spherical polar coordinates (r,θ,φ) retaining the field quantities A_1, A_2, A_z. (iv) Separate variables. This yields the general solution

$$\begin{matrix} A_1 \\ A_2 \\ A_z \end{matrix} = \sum_{m,n} \begin{matrix} 1 \\ 2 \\ z \end{matrix} C^{z}_{mn} j_n(kr)\, P_n^{\begin{matrix} (m+1) \\ (m-1) \\ m \end{matrix}} (\cos\theta) e^{i(m\varphi + \omega t)} \tag{3}$$

where the Cs are constants, the js spherical Bessel functions, and the Ps associated Legendre functions. This solution is particularly suitable for simple rotating fields $(m = 1)$. The solenoidal condition has to be applied, but as the curl of any solution is also a solution, this poses no great difficulty.

For the case of the magnetic dipole orientated at right-angles to and rotating about the polar axis, we reject the spherical Hankel function corresponding to the advanced solution which is non-physical and retain the retarded solution, so that the full field becomes

$$\begin{aligned} E_\theta &= \frac{M\omega}{4\pi r^2}\,(kr - i)e^{i(\varphi + \omega t - kr)} \\ E_r &= 0,\ E_\varphi = i\cos\theta\, E_\theta \\ H_\varphi &= \frac{M}{4\pi(\mu_0 r^3}\,((kr)^2 - ikr - 1)\, e^{i(\varphi + \omega t - kr)} \\ H_r &= \frac{2\sin\theta}{\omega\mu_0 r} E_\theta,\ H_\theta = -i\cos\theta\, H_\varphi \end{aligned} \tag{4}$$

where M is the dipole moment and the real part of the solution is understood. The drag torque on the dipole may be obtained by computing the Maxwell stresses over a sphere centred on the origin, E_r is zero, so only the tension associated with the H-field contributes and the torque T is given by

$$T = \underset{r\to 0}{\text{Limit}} \int_0^{2\pi}\int_0^{\pi} \mu_0 H_r H_\varphi r^3\, d\varphi \sin^2\theta\, d\theta \tag{5}$$

Assuming k is real, most of the terms vanish identically or over the φ integration and the result is

$$T = \frac{M^2}{6\pi\mu_0} k^3 \tag{6}$$

The same result can be obtained by computing the radiated power and dividing by the frequency, but this method emphasizes that there is a real drag torque on the dipole. The radiation component of (4) agrees with Deutsch's result[9]. The intermediate field produces little complication. If there is a time independent axi-symmetric component of the field, it has no radiation associated with it and no electric field in the rest coordinate system. Its magnetic field can be linearly superposed on equation (4). It can be seen by inspection that the field lines are static in the rotating coordinate system. The rotating magnetic field lines in the plane $\theta = \pi/2$ are shown in Fig. 1. In the case of the Crab pulsar, M is estimated as 10^{21} Wb.m., ω $(= kc)$ is 60 π s^{-1}, and using equation (6), $T \sim 10^{29}$ Nm and the radiated power $\sim 2 \times 10^{31}$ W. These figures show good agreement with the experimental evidence[6]. If it could be assumed that the moment of inertia of the star and its dipole moment remained constant in time, then equation (6) would predict that the rate of slowing down was proportional to the cube of the frequency. The star is expected to change shape, however, and variations from this law are therefore expected. It can also be concluded from considerations of Maxwell stress that if the magnetic dipole is not perpendicular to the rotation axis, the torque vector remains at right-angles to the dipole and co-planar with the dipole and the rotation axis. This may tend to align the dipole with the rotation axis, depending on the mechanical properties of the star, and such alignment would decrease the slowing down rate[10,11].

It is now necessary to explain why the plasma should be very nearly transparent. For any solenoidal field in which the φ dependence is $e^{i\varphi}$ it may easily be shown that

$$\nabla \times (r \sin\theta\, \hat{\varphi} \times \boldsymbol{A}) = -i\boldsymbol{A} \tag{7}$$

The plasma will be in equilibrium, neglecting gravitational and inertial effects, provided its motion is such that

$$\boldsymbol{E} + \boldsymbol{v} \times \boldsymbol{B} = 0 \tag{8}$$

Inside the velocity of light cylinder, synchronous rotation of the plasma is one possibility, and using equation (7)

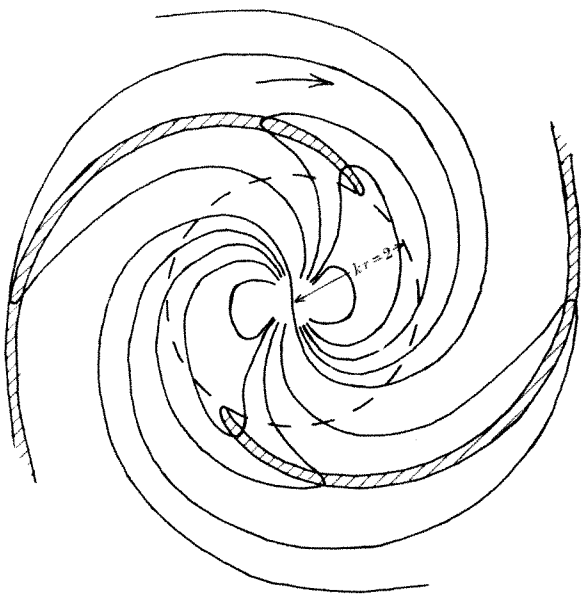


Fig. 1. Rotating magnetic field lines in the plane $\theta = \pi/2$. The plasma disk rotates at half synchronous speed and becomes relativistic near the radius $kr = 2$. It is only when the shaded zones pass through the plasma outside this radius, however, that it is forced to become highly relativistic. It is proposed that the leading edges of the zones are the pulse emitting regions.

it may easily be shown that this does ensure

$$\nabla \times (\boldsymbol{E}+\boldsymbol{v}\times\boldsymbol{B})=0 \qquad (9)$$

To satisfy equation (8) completely, however, a space charge density σ is required such that

$$\sigma = -\varepsilon_0 \nabla \cdot (\boldsymbol{v}\times\boldsymbol{B}) = 2\omega\,\varepsilon_0 B_z \qquad (10)$$

where terms of order $(v/c)^2$ have been neglected. Equilibrium is thus possible provided there are enough charged particles to satisfy equation (10). The number density required near the surface of the star is $\sim 10^{19}$ m^{-3}. A charge redistribution similar to equation (10) is also required by an axi-symmetric component of magnetic field[12]. There is little doubt that such a co-rotating equilibrium region will exist. Assuming that the number density is $\lesssim 10^{19}$ m^{-3}, however, its boundary will be close to the nominal surface of the star, simply because of a shortage of charged particles. We demonstrate below that for this case, the number density in the remainder of the magnetosphere will then be very low, except near an equilibrium surface, because all charged particles, except those near the equilibrium surface, will be attracted to the co-rotating region. This being the case, the free space field solution (4) will be approximately valid outside the co-rotating region, with the minor qualification that the charges required by equation (10) mean that the pure magnetic dipole source is supplemented by an electric quadrupole source with a negligible effect on the field beyond the immediate vicinity of the co-rotating region.

In order that equation (8) should be satisfied, a necessary condition is

$$\boldsymbol{E}\cdot\boldsymbol{B}=0 \qquad (11)$$

This quantity is Lorentz invariant, and it is sufficient, therefore, to calculate it in the rest coordinate system. Outside the co-rotating region, the solution (4) applies and

$$\boldsymbol{E}\cdot\boldsymbol{B} = -\frac{M^2\omega\cos\theta}{(4\pi)^2 r^5} \qquad (12)$$

which is an elegant result independent of φ and t. In the plane $\theta=\pi/2$, equation (11) is satisfied. For $\theta<\pi/2$, $\boldsymbol{E}_{\parallel}$ is always opposite in direction to $\boldsymbol{B}$. For $\theta>\pi/2$, $\boldsymbol{E}_{\parallel}$ is always in the same direction as $\boldsymbol{B}$. But all magnetic field lines are closed loops passing through the co-rotating region and none cross the plane $\theta=\pi/2$. Thus charged particles not in the plane $\theta=\pi/2$ cannot be in equilibrium. Most particles will be accelerated rapidly along their field lines until they reach the co-rotating region, although some may be deposited in the plane $\theta=\pi/2$ or escape from the magnetosphere altogether. If an axi-symmetric magnetic field is present the surface satisfying equation (11) becomes fixed in the rotating coordinate system, inclined at an angle to the $\theta=\pi/2$ plane, and no longer a plane for large kr. Nevertheless it still exists and the argument remains substantially unchanged.

Continuing the analysis for the case of no axi-symmetric magnetic field, application of equation (8) to particles in the plane $\theta=\pi/2$ yields

$$E_\theta + \mu_0 (v_\varphi H_r - v_r H_\varphi) = 0 \qquad (13)$$

and this is satisfied by $v_r=0$, $v_\varphi=-r\omega/2$, that is, by rotation of the particles at half the synchronous speed. Clearly, not only is equilibrium possible in this plane, but also relativistic speeds are not required until kr reaches ~ 2, when equation (13) is no longer valid.

Outside the circle defined by $kr=2$, it is appropriate to examine the formula for $\boldsymbol{v}$

$$\boldsymbol{v} = \frac{\boldsymbol{E}\times\boldsymbol{B}}{B^2} + \frac{(\boldsymbol{v}\cdot\boldsymbol{B})\,\boldsymbol{B}}{B^2} \qquad (14)$$

obtained by taking the cross product of equation (8) with $\boldsymbol{B}$. The second term is simply the component of velocity along the instantaneous direction of $\boldsymbol{B}$ which is left unspecified by both equations (8) and (14). This formula breaks down when other forces, gravitational, inertial or reaction due to relativistic particle radiation become important. For a relativistic particle following approximately the circle $kr=2$, the synchrotron radiation reaction becomes important first, at a value of γ given by

$$\frac{e^2 r^2}{6\pi\varepsilon_0 c^4}\left(\frac{\omega}{2}\right)^4 \gamma^4 \sim er\left(\frac{\omega}{2}\right) B \qquad (15)$$

where e is the electron charge and $\gamma=(1-v^2/c^2)^{-\frac{1}{2}}$. Substituting approximate values yields $\gamma\sim 10^7$. A value of this order is required before equations (8) and (14) become invalid. (The centrifugal forces are negligible until $\gamma\sim 10^9$.) The component of velocity along $\boldsymbol{B}$ can be reduced without violating equations (8) and (14). Clearly this will happen first and will involve comparatively modest values of γ. Assuming this component of velocity is zero, equation (14) yields

$$v = \frac{r\omega}{2}\left[1+\left(\frac{H_\varphi}{H_r}\right)^2\right]^{-\frac{1}{2}} \qquad (16)$$

When equation (16) yields $v>c$, $(E>cB)$, the equation is clearly no longer valid, and the particles must become highly relativistic. Note that $E\sim 10^9$ V m^{-1} at $kr=2$. The boundary curves $E=cB$ are fixed in the rotating coordinate system and obey the equation

$$\tan(\varphi+\omega t-kr) = [kr-\{(kr)^2-4\}^{\frac{1}{2}}]^{-1} - kr \qquad (17)$$

The zones are shown in Fig. 1. Because they rotate at synchronous speed, while particles leaving the circle $kr=2$ are rotating at about half synchronous speed, many of the particles will be caught by the leading edges of the zones and concentrations of highly relativistic particles must be expected there.

Identification of the leading edges of the zones as the pulse emission regions would explain the optical observations of the Crab pulsar[13,14] on the basis of the proposed simple geometric model[14]. It also fulfils the basic requirements to explain the radio pulses. A build-up of number density sufficient to make correlated radio emission probable might be expected. The field lines in the emitting region are normal to the direction of propagation. Thus the synchrotron emission will propagate as the extraordinary mode across the field lines and produce predominantly linearly polarized radiation. The circularly polarized Whistler mode, which propagates along field lines, will find conditions unsuitable, which means that the strong pulses occurring approximately every 10^4 ordinary pulses[2] must have a different explanation.

For $kr\gg 2$, all particles encountered by the field will be accelerated to highly relativistic speeds and take energy from the field through mechanisms such as those discussed by Ostriker and Gunn[6]. The effect on the field will be to give k a small imaginary part resulting in a spatial exponential decay in the solution (4). Thus the electromagnetic momentum will not be radiated away to infinity but will be absorbed by the surrounding nebula.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Pulsar Distributions without Magnetic Decay

SEVERAL authors have recently argued for the necessity of decay of the surface magnetic field of neutron stars with a characteristic time of a few million years in order to explain the details of the observed pulsar sample. Gunn and Ostriker[1] have summarized these arguments, pointing to the three empirical pieces of evidence which imply such decay: (1) the scarcity of long period pulsars, (2) the apparent decrease in surface magnetic field with age, and (3) the decrease in pulsed radio luminosity with age.

Setti and Woltjer[2] have indicated that magnetic decay is not necessary for the explanation of the relative scarcity of long period pulsars, but have not tried to explain the other two observations. In this report we point out that the assumption of a period-dependent pulsed radio luminosity can, in fact, explain the age dependence of both the surface field and pulsed radio luminosity, although an additional assumption must be made to reconcile the model with the two observed young pulsars, NP 0531 and PSR 0833.

Gunn and Ostriker[1] have found good agreement with the observed pulsar sample by assuming that the radio luminosity is period-independent; in other words, that it is proportional to the square of the surface field only, and that this field decays with a characteristic time of a few million years. If, instead, it is assumed that the radio luminosity is a fixed fraction of the total electromagnetic energy loss rate of the rotating neutron star, such radio luminosity becomes explicitly period-dependent. In all electromagnetic models[3–5], the electromagnetic luminosity is proportional to the square of the surface field and inversely proportional to the fourth power of the rotation period. Setti and Woltjer[2] have shown that the scarcity of long period pulsars is an immediate consequence of this assumption (in fact, a rather wide range of inverse period indices will yield consistency with the observed period distribution at large periods without the additional assumption of a decaying field).

The present model can account for the other two characteristics of the pulsar sample as well. For pulsars the periods of which are much larger than their initial values, the radio luminosity is proportional to the inverse square of the product of the surface field and the age. That the radio luminosity should decrease as a function of age is an obvious consequence of this relation. A further consequence of this assumption is a selection effect for lower field pulsars as a function of age, owing to the inverse square dependence of the radio luminosity on the field. Thus, if the detection threshold is age-independent, the high-field pulsars will become unobservable at a younger age than otherwise identical low-field objects.

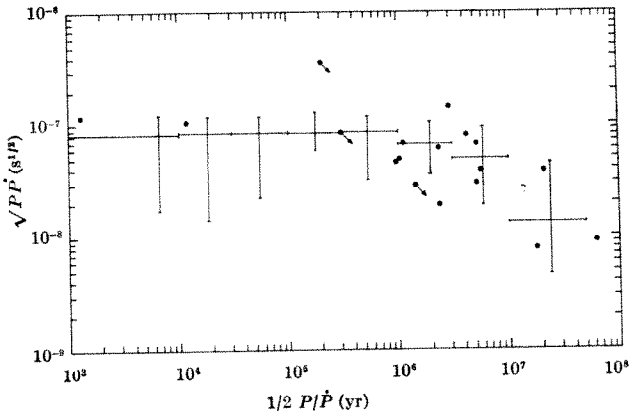


Fig. 1. Surface magnetic fields of pulsars as a function of age. The dots represent pulsars with measured P and $\dot{P}$ (ref. 6). The error bars represent the median and 1σ levels for the surface field in each age group. These quantities were computed for pulsars with random position, age and surface field in 100 trial galaxies of radius 12 kpc and thickness 200 pc. The probability distributions in position, age and surface field were uniform, with an arbitrary pulsar birth rate, and a maximum field value corresponding to $P\dot{P}=2\times10^{-14}$ s.

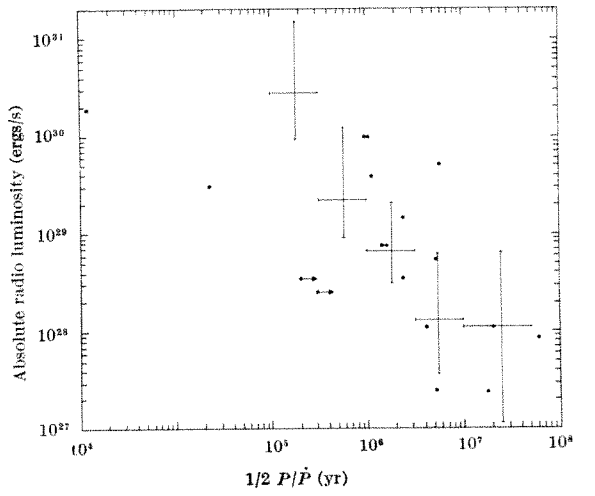


Fig. 2. Absolute pulsed radio luminosities as a function of age. The dots represent pulsars with measured P and $\dot{P}$ and the error bars represent the median and 1σ levels for the radio luminosities in each age group, based on calculations as described in the text and in the caption to Fig. 1.

In Figs. 1 and 2 we have compared quantitatively the observed pulsar sample with the consequences of this assumption. The error bars in each age group represent the calculated standard 1σ deviations from the median in 100 trial galaxies of pulsars generated at random in space, time and surface field. The pulsar birth rate and the probability of the pulsar "beam" intersecting the Earth are assumed arbitrarily to be one pulsar birth per 100

years in the galaxy, and a probability of 1/4. The observation threshold is then adjusted such that the period distribution above the maximum at $\sim 0{\cdot}6$ s is matched. The results are found to be insensitive to the birth rate and observing probability in the sense that they may both be varied substantially and the threshold adjusted such that the period distribution above $\sim 0{\cdot}6$ s is reproduced.

The calculated and observed magnetic fields agree at all ages, as they must if this model is tenable. At ages less than $\sim 10^6$ yr, however, the model predicts many more pulsars (and with higher luminosity) than the two, NP 0531 and PSR 0833, which are actually observed; this is the same problem which faced Setti and Woltjer[2] for periods below $\sim 0{\cdot}6$ s. Among the variety of possible additional assumptions which could account for both the small number of observed young pulsars and their low radio luminosity, one is particularly interesting. If, for young pulsars, the pulsar beam is extremely narrow (so that the probability for observation of the beam is much smaller than the $\sim 1/4$ assumed earlier), then we would expect to see no pulsars younger than $\sim 10^6$ yr on the beam. If also there is a roughly isotropic pulsed component four or five orders of magnitude less intense than the component on the beam, this would explain why NP 0531 and PSR 0833 (both young close pulsars) are observed at a lower intensity than expected for the pulsar beam, and why more distant pulsars which are younger (for example, the pulsars which might be associated with Cas A and SN 1572) are below the threshold of observability.

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Volcano on Jan Mayen Alive Again

On September 19–20 the Beerenberg volcano on Jan Mayen Island in the Norwegian Sea (71° N, 8°–9° W) became reactivated after being dormant for several hundred years, perhaps thousands of years. Reports by whalers at sea of activity on the southern slope of the volcano in 1732 and 1818 have been subject to doubt. But steam and carbon dioxide occur in fractures, and earthquakes are common.

The present eruption was preceded for 1 or 2 days by earthquakes with epicentres which were, however, some distance to the north-east. On the morning of September 20, smoke and steam rose to 30,000 feet on the north-east flank of the volcano, apparently coming from a 5–6 km long fracture (Fig. 1) with five main parasitic crater centres. Geologists from the Norsk Polarinstitutt were rushed to the island and are observing the activity from land, sea and air. After 2 days, explosive activity decreased, but a great amount of basaltic lava continues to emerge, and cascade down the slope to build up a new coastal platform at least 500 m wide and 3·5 km long. Because most of the volcano is covered by glaciers, a great deal of meltwater has caused floods (Jökulhlaup) that have formed deltas in other places. The lava has formed a dense basaltic rock with conspicuous olivine phenocrysts up to cm size. Phenocrysts of plagioclase (An 60) occur, but these are smaller.

The geology of Jan Mayen has been studied extensively in recent years, in particular by British geologists[1]. The formation of the island was started by extensive submarine basaltic flows. Rocks of phyric basaltic, trachy-basaltic and even trachytic composition then built up the island as lava and pyroclastics emerged from vents and fractures, generally following two principal directions, an older N 50° E and a younger N 40° E. The present fracture seems to coincide with the latter, and falls in line with the earlier development of the Beerenberg volcano as described by Fitch, Roberts and Hawkins[2,3].

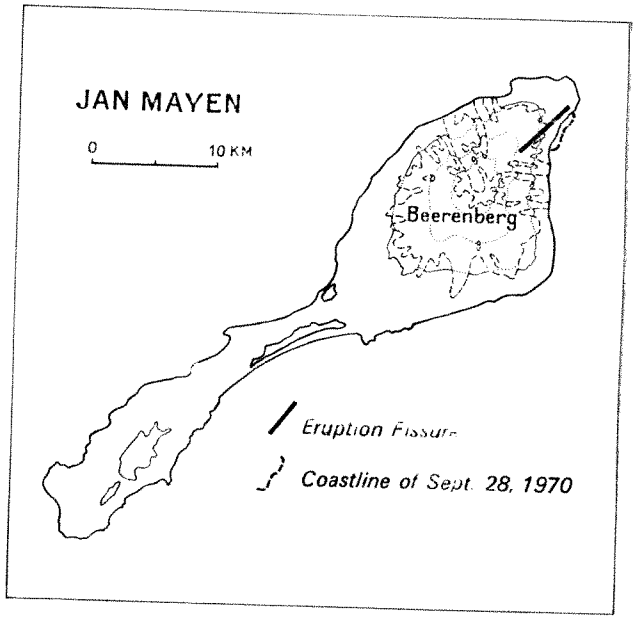


Fig. 1. The volcano on Jan Mayen Island.

The structural setting of Jan Mayen is intricate. According to Johnson[4], it is located on the intersection of an important NW–SE striking fracture zone and an N–S trending easterly marginal ridge of the Icelandic Plateau that occupies the major part of the Mid-Atlantic ridge in the region. Johnson interprets the morphological and earthquake data in favour of a displacement of the crest of the Mid-Atlantic ridge 200 km laterally eastwards along the Jan Mayen fracture zone. It should be noted, however, that the island of Jan Mayen itself is trending SW–NE, parallel to the direction of the crest both to the south of Iceland and to the north of the fracture zone (the Mohn ridge), and occupying a central position within the ridge.

The renewal of the volcanic activity of Jan Mayen calls for a close study of the island itself, as well as of the structural relationship of the Mid-Atlantic ridge in the Norwegian Sea.

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Temporal Patterning of Post-Pleistocene Glaciation

MOST post-Pleistocene glaciation has apparently been confined to four phases at intervals of around 2,600 years[1]. The exact timing of these phases will be examined in relation to recent carbon-14 age corrections and additional glacial chronologies.

The earliest glacial phase, the Cochrane–Cockburn, occurred in North America and has been dated by ^{14}C methods at around 8,000–9,000 years BP, probably nearer 8,000 years BP (ref. 2). The correlation of the Cochrane–Cockburn glacial phase with Swiss[3], Austrian[4] and Swedish[5] glaciation is still uncertain. The second phase, the Atlantic/sub-Boreal transition, is now well documented from North America[6–9] and from Europe[5,6,10] as occurring around 4,500–5,000 ^{14}C years BP and culminating between 4,600–4,800 years BP. The third phase, the sub-Atlantic minimum, culminated around 2,600–2,800 ^{14}C years BP in North America[1,7] and in Europe[1,4,7,11,12] and probably in Australia[13]. The last phase, the "Little Ice Age", had a world-wide culmination around AD 1,550–1,900 (ref. 1) which was synchronous for North America, Arctic–Eurasia and the Southern Hemisphere.

The mid-points of the culmination of these four glacial phases were 8,000 (tentatively), 4,700 and 2,700 ^{14}C years BP and 225 years BP. Studies of conversion of ^{14}C to true time[14,15] indicate that from 0–2,500 BP, ^{14}C and true time were the same; 2,500–5,500 ^{14}C years BP there was an underestimate of true time; and around 8,000 BP, ^{14}C and true time were, again, about the same. Correction factors of 1·03 for 2,700 BP, of 1·14 for 4,700 BP and of 1·03 for 8,000 BP were estimated (from ref. 15) to give corrected dates of 8,250, 5,350 and 2,780 BP. These dates, together with the recent date of 225 BP, give intervals of 2,900, 2,570 and 2,555, of which the first is not very reliable. Neglecting the first interval, the last three glacial phases are separated by around 2,600 years. An arithmetic progression starting with 22 years (the complete sunspot cycle) and a first term of four results in a sequence of 88, 440 and 2,640 years. The estimate of an 11 year half cycle is based on the mean length of 11·04 years for 214 BC–AD 1382 and 1830–1957 (ref. 16) and neglecting the interval 1382–1830 as a sunspot dearth period.

The apparent cyclic recurrence of the four glacial phases and, with the possible exception of the first phase, their synchronism in Europe, North America and elsewhere, suggest they have a similar cause. The most likely explanation of the recent glacial phase was lower temperature and a shorter ablation season[17,18]. If all four phases were caused by a periodic decline in temperature, then the decline would have been modified by the chief trend in temperature during the Holocene, because there is a decreased severity of glaciation from the first to second phase and an increased severity to the third and fourth phase. This pattern may reflect a periodic temperature decline which was of decreasing expression as a result of the predominant rising temperature of the early to mid-Holocene, and of increasing expression as a result of the subsequent temperature decline following the Thermal Maximum. The periodic appearance of glacial phases throughout the Holocene and their occurrence near the Thermal Maximum suggest that the mechanism underlying these phases is independent of that determining the predominant Holocene temperature trend.

There seem to be two major Holocene temperature patterns: (1) the predominant trend of increase in temperature followed by a slight decrease, exemplified by retreat of Pleistocene ice and the subsequent renewal of alpine glaciation and by the rapid rise and later fall in ocean level; and (2) a secondary trend of recurrent cold phases with greatly increased glaciation at higher altitudes and latitudes. As noted earlier, these two patterns are probably causally independent, but are additive in their expression. The first pattern has often been interpreted as a result of changes in the Earth's orbit relative to the Sun[19]. The second pattern may be related to cyclic intervals of decreased solar activity[1]. The most recent glacial phase has been correlated with an extended period of decreased solar activity[1]. Because the preceding two, and probably three, phases had a similar regular occurrence[1] and a similar short duration of from four to six centuries[1,2,6,7,10,20], they may also be related to recurrent periods of reduced solar activity. A glacial phase before the Cochrane–Cockburn, the Windermere, which occurred at 10,150–10,810 ^{14}C years BP, also had a relatively short duration of around four to six centuries[17].

If the two temperature patterns noted here continue, the following changes may be expected: (1) a period of slow cooling to around AD 2450, followed by an interval of more cooling lasting some 3,000 years, according to the first pattern[19]; and (2) a period of cooling and glaciation around AD 4300 according to the second pattern. A conservative estimate of cooling for the first pattern was made at 3·1° and 2·7° C for latitudes 25° and 65° N (ref. 21). For England, cooling as a result of the second pattern showed a decline of nearly 2·0° C from AD 1000–1300 to the "Little Ice Age"[22,23]. If cooling of around 3·0° C as a result of the first pattern is insufficient to trigger a glacial era, then it is conceivable that the temperature decline of nearly 2·0° C estimated for mid-latitudes for the second pattern could contribute the cooling necessary for continental glaciation. A possible new glacial era may begin, therefore, somewhere around AD 4300, the next predicted 2,600 year temperature decline, or sooner, if the rapid cooling predicted from the first pattern to occur after AD 2450 is sufficient.

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Deformation of Particles during Briquetting

THE briquetting of small particles usually consists of the application of pressure in controlled conditions so that the individual particles hold together to form a

coherent compact. Many mechanisms have been suggested to explain why the particles cohere to form a compact, but none have been based on satisfactory observation of the behaviour of the individual particles under stress. Our present studies have involved such detailed observation and we describe here the essential difference in behaviour of particles of a number of different materials.

Some materials which are generally regarded as brittle are considered to behave plastically when the particles are small and subjected to high degrees of stress (microsquashing). Considerable study has been made of the behaviour of coal particles in this connexion[1–4]. The critical size for microsquashing rather than for brittle fracture to occur is uncertain but may well vary over a considerable range depending on the material of the particle and the nature of the stress system applied to it. Newman[5] considered that there was evidence of plastic flow in coal particles of the order of 1 mm linear dimension. Microsquashing has been considered[6] to be important in the achievement of sufficient area of contact between coal particles in a compact for the whole to remain coherent after release of the briquetting pressure.

One of the difficulties inherent in an experimental study of the compaction process is the observation in sufficient detail of the behaviour of individual particles. Most observations have been made using optical instruments characterized by limited resolution and depth of field. During the present study a scanning electron microscope has been used to observe both particles and sections of briquettes. This microscope has a resolution of approximately 500 Å and a depth of field very many times greater than the size of the particles being observed.

Three materials were chosen for study, namely sodium chloride, a medium volatile coal (NCB classification 502, 85 per cent carbon) and sucrose. It was expected that the coal would behave in a manner intermediate between that of the sodium chloride, which was expected to be plastic[7,8], and that of the sucrose, which was expected to be brittle[9]. A sample of each material in the size range 251–295 μm was prepared; such particles are approximately in the middle of the range over which it has been suggested in the past that microsquashing might occur. The briquettes were formed in a cylindrical floating die under a pressure of $1{\cdot}55 \times 10^3$ kg cm^{-2} applied at a rate of 13·8 kg cm^{-2} s^{-1} at room temperature.

Briquettes formed from each of the three materials were broken and a portion cleaved from the centre was fixed to the mounting stub of the scanning electron microscope. Because the powders were badly conducting materials each specimen was covered with a very thin layer of silver to prevent the charging up of the surface. This has no effect on the observation of detail, for the layer thickness is much less than the resolution of the instrument. At the same time samples of the unbriquetted powder were examined in similar fashion.

The photographs in Fig. 1 were taken at low magnification in order to give a better general impression of the change taking place in individual particles during compaction. The specimens were, however, observed at a magnification of the order of 40,000, at which fine detail down to the limit of resolution is clearly visible. Even under high magnification the uncompacted particles showed no evidence of cracking; the briquette sections showed no more detail than is given at the lower magnification. The difference in behaviour of the three materials is shown in Fig. 1.

Figs. 1*a* and *b* are of sodium chloride particles before and after compaction respectively; *c* and *d* are a comparable pair for the coal used; *e* and *f* are for the sucrose. In the case of the sodium chloride there is no evidence for any fragmentation of the individual particles, the compact suggesting a mass of deformed cubes. This supports the view that the densification of sodium chloride is by plastic deformation[10,11]. In the case of the sucrose there is extensive fragmentation of the particles, each seemingly losing its original identity. The behaviour of the coal is intermediate between that of the sodium chloride and the sucrose. Some fragmentation does occur, but it is nowhere as extensive as with the sucrose and large portions of the original particles can be seen. It seems unlikely that plastic flow on the scale suggested by Newman[5] is taking place in this particular case. It may well be that the underlying mechanism is coal fragmentation combined with plastic deformation of the small fragments or of regions of the large particles.

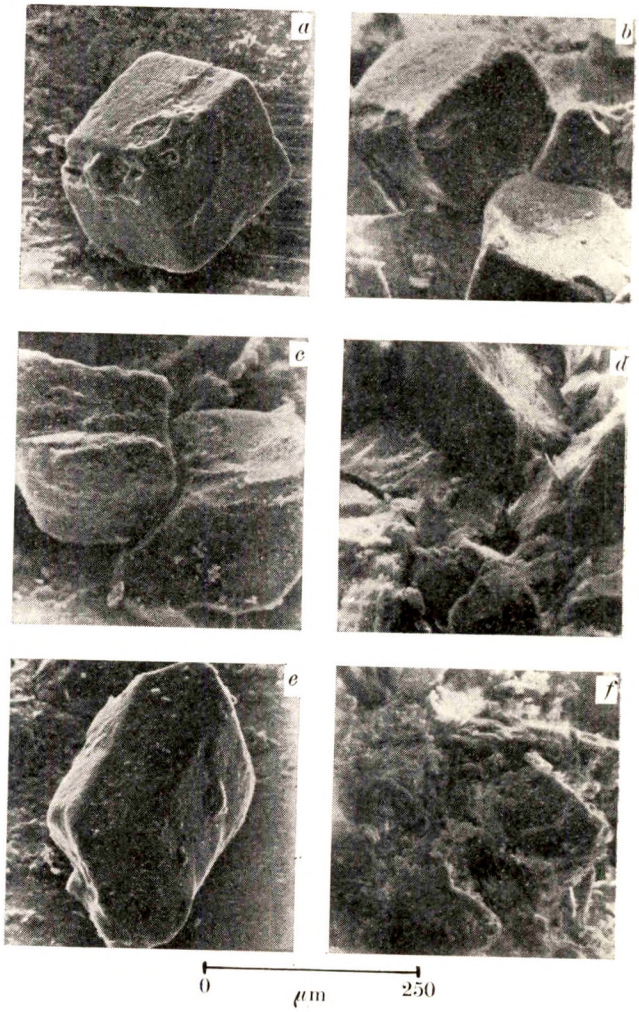


Fig. 1. *a* and *b* are sodium chloride particles before and after compaction; *c* and *d* are a comparable pair for the coal used; *e* and *f* are for sucrose.

The above observations are supported by measurements of specific surface of the particles before and after compaction. There is very little change in the case of sodium chloride and a ten-fold increase with sucrose.

The observations described in this note indicate the nature of the changes likely to be taking place in particles of the materials used. Previous workers[10,11] have deduced that plastic flow together with some fragmentation must be taking place in sodium chloride compacts, but there has never been any direct evidence. The present work provides strong support for a mechanism involving plastic flow. The behaviour of this material contrasts strongly with that of sucrose, and coal particles behave

in a fashion intermediate between these two extreme cases.

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Crystal Structure of 18αH-Oleanane

DISTILLATES boiling in the range 340°–550° C contain molecular types belonging to the triterpane family, C_nH_{2n-8} (ref. 1). So far the structures of three compounds, corresponding to chromatography peaks labelled E, F and H (ref. 1, Fig. 5), have been solved by X-ray crystallography using trial and error methods. Triterpane E has been shown to be a spiro-triterpane[2,3], while triterpane H has been shown to be a member of the hopane family.

Triterpane F crystallized, surprisingly, in both triclinic and orthorhombic forms and the triclinic form was readily shown to be the parent hydrocarbon 18αH-oleanane. The orthorhombic form of F, however, contained two molecules in the asymmetric unit and posed the formidable task of finding sixty independent carbon atoms. Even so the structure has been successfully determined by the use of direct methods with no preliminary assumptions made about the molecular architecture.

Orthorhombic F has a unit cell $a = 20{\cdot}44$ Å, $b = 20{\cdot}90$ Å, $c = 12{\cdot}20$ Å; space group $P2_12_12_1$; $Z = 8$. 2,610 significant structure amplitudes were collected on a PAILRED diffractometer by courtesy of Dr G. J. Bullen (University of Essex), brought to a common scale and used to calculate a set of normalized structure factors. As an indication of the severity of the problem, the magnitude of the triple product $E_HE_KE_{(H-K)}$ had to exceed 28·7 for centrosymmetric reflexions for a 97 per cent probability of phase correctness and to be greater than 22·3 for non-centrosymmetric reflexions for a variance of 0·5 square radians[4].

With four reflexions to select the origin and enantiomorph and four symbolically phased reflexions (two real and two complex), a total of only eighteen reflexions could be obtained by the Σ_2 formula which satisfied the above criteria. Nevertheless, two definite phase relationships were obtained involving the two real and one complex symbolic phases and a third, less certain relating the two complex phases. In view of the very limited amount of data, this third relationship was ignored and sixty-four starting phase combinations developed for the eighteen initial reflexions. The tangent formula[5] was used to calculate phases for about 190 reflexions with $E \geq 1{\cdot}6$ for each starting set followed by the calculation of an E map. It became clear that the residuals calculated in the tangent formula were consistently lower for one combination of the symbolically phased real reflexions and that the third relationship was essentially true. The starting set which gave the lowest residual of all yielded the correct structure and the resulting E map contained no less than fifty-two clearly resolved atoms, that is twenty-six atoms in each molecule; indeed, in retrospect the remaining eight atoms were also present in the map but with much weaker peaks. An Fo map phased directly on these fifty-two atoms showed clearly the remaining atoms and the first structure factor calculation for all sixty atoms resulted in an R index of 28 per cent; two further cycles of least-squares refinement have reduced it to 18·2 per cent, and further refinement is in hand.

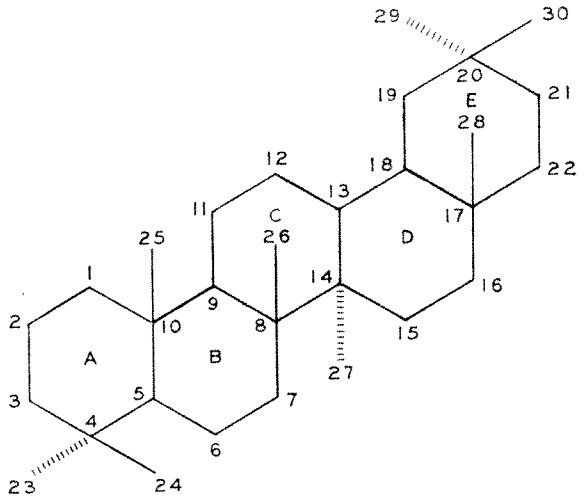


Fig. 1. 18αH-Oleanane.

The power of the symbolic addition procedure and tangent formula has been amply demonstrated by the relative ease with which the correct solution was revealed. Although sixty-four starting phase combinations were to be investigated sequentially, the correct solution was obtained before all cases were calculated and it is noteworthy that, had the third phase relation been used, the number of alternative starting sets would have been drastically reduced.

It is therefore remarkable that not only was the structure obtained by starting with a small number of phases and relatively weak phase relationships but also for the clarity with which the E map revealed nearly 90 per cent of the structure with only about 190 reflexions having $E \geq 1{\cdot}6$. This is in contrast with the suggestion[6] that about nine to ten reflexions per atom are required for good resolution. In addition no recycling procedure as advocated by Karle[7] proved necessary. The diffraction data for this structure may, of course, possess special features but this was not in any way apparent from the intensity statistics. We therefore attribute the success of solving this structure to a cautious approach by accepting only those phases which have a high probability of correctness and to very strict rejection criteria in the tangent formula. While this led to a small number of reflexions available for the calculation of the E map, the map for the correct solution was exceptionally clear and had relatively few noise peaks. Most of these peaks were weak and the few strong ones in obviously non-bonded

positions. An analysis of the phase angles of 135 complex reflexions gave an average error of 28°. It should be mentioned that two likely solutions were obtained in the process. Both contained about forty atoms corresponding to complete rings B, C, D and incomplete A and E rings of both molecules. One structure could be discarded on packing consideration and the other would not refine. It did, however, bear close resemblance to the true structure and corresponded to a starting set wherein one of the symbolically phased reflexions had changed by π.

This is, we believe, the first time that the parent hydrocarbon 18αH-oleanane (Fig. 1) has been positively identified, although derivatives are known and triterpane E has been reported[2,3] as a spiro modification of it. In the structure both molecules lie approximately parallel to the $1\bar{1}0$ plane on the same level as each other with the mean plane of their rings at about 45° to the *ab* plane. There is a displacement between them of approximately $\frac{1}{4}(a-b)$. A full description of this structure will be published elsewhere.

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Ionization in Carbon Monoxide Flames

It is now generally believed that ionization in hydrocarbon flames results chiefly from a chemi-ionization process involving the reaction

$$CH + O \rightarrow CHO^+ + e^- \quad (1)$$

There is evidence that the same process occurs in hydrocarbon/oxygen flames[1,2], and in hydrogen[3] and carbon monoxide[4] flames to which small amounts of hydrocarbons have been added.

The presence of ions in flames in which the fuel and additive includes carbon but not hydrogen, for example, carbon monoxide plus carbon tetrachloride, would require an ion formation process other than (1) above. In fact it has been reported that carbon tetrachloride gives a slightly greater response than methane in the carbon monoxide flame[5]. Hydrogen and water are also said to give a sensitive response in this flame[6]. More recently, however, it has been shown that the ion current in a carbon monoxide flame containing small quantities of added ethylene is proportional to the square of the ethylene concentration and this result has been interpreted as consistent with reaction (1) on the assumption that both reactants for the ionization step are provided by the hydrocarbon[7].

In view of these apparently conflicting results we have carried out a series of experiments on the carbon monoxide flame, initially using 99·5 per cent pure carbon monoxide (J. T. Baker Chemical Co., Phillipsburg, New Jersey, USA) burning at a 0·10 inch inner diameter platinum jet at a flow rate of 35 ml./min. Additives could be continuously introduced into the flame at a controlled rate either by a direct pressure control system (for hydrogen) or by passing a portion of the total carbon monoxide flow through a saturator (for water, carbon tetrachloride and liquid hydrocarbons). Ion currents were measured with a Keithley model 300 operational amplifier (Keithley Instruments Inc., Cleveland, Ohio) with the platinum jet 300 V positive with respect to ground. A full description of the flame assembly, amplifier and saturator used in these studies will be published elsewhere[8]. With this arrangement we could obtain no significant signal for either hydrogen, water or carbon tetrachloride (May and Baker, research grade) when added to the carbon monoxide flame, and at a carbon tetrachloride concentration of approximately 1·4 volumes per cent the flame was extinguished.

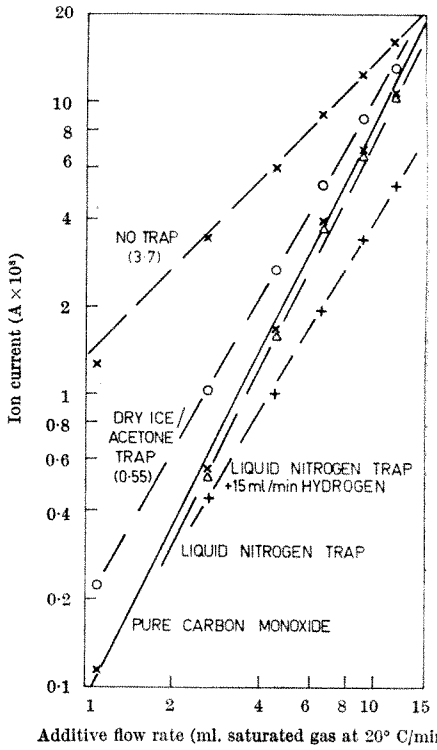


Fig. 1. Response of the carbon monoxide diffusion flame to ethanol for pure carbon monoxide (solid curve) and impure carbon monoxide (broken curves), plotted on log–log scales. Figures in brackets indicate corrections made for the residual background current due to the methyl formate impurity.

The results for carbon tetrachloride and hydrogen have been repeated using a second sample of 99·5 per cent pure carbon monoxide (Matheson Company Inc., East Rutherford, New Jersey, USA) at flow rates from 35 to 90 ml./min. Again no response could be obtained for carbon tetrachloride (irrespective of the addition or not of up to 15 ml./min of internal oxygen), or for hydrogen at low rates of addition. At a hydrogen flow rate of 20 ml./min and carbon monoxide of 50 ml./min a small signal ($1{\cdot}7 \times 10^{-9}$ A) was obtained, and at still higher hydrogen flow rates the signal increased rapidly with hydrogen addition. The appearance of the flame indicated that solid carbon formation was occurring[9] and this may well account for the marked increase in ionization observed in these conditions.

In the case of ethanol (Fig. 1) our results with pure carbon monoxide are similar to those previously reported for ethylene[7]. Benzene, toluene and *n*-heptane, however, all gave a substantially first order response at ion currents greater than 1×10^{-8} A (Fig. 2), although the toluene

results in particular suggest a square law dependence at lower concentrations. This change in kinetic dependence may arise from cracking reactions which give relatively high yields of some hydrogen-containing species which are precursors to reaction (1) above, or are directly involved in this reaction. This is possible because the ion formation process is a minor one, only about 1 ion being produced per 10^6 molecules of additive. The addition of up to 30 volumes per cent of hydrogen, however, to a carbon monoxide/ethanol flame resulted in only a marginal change towards first order dependence and a net reduction in the ion current (Fig. 1).

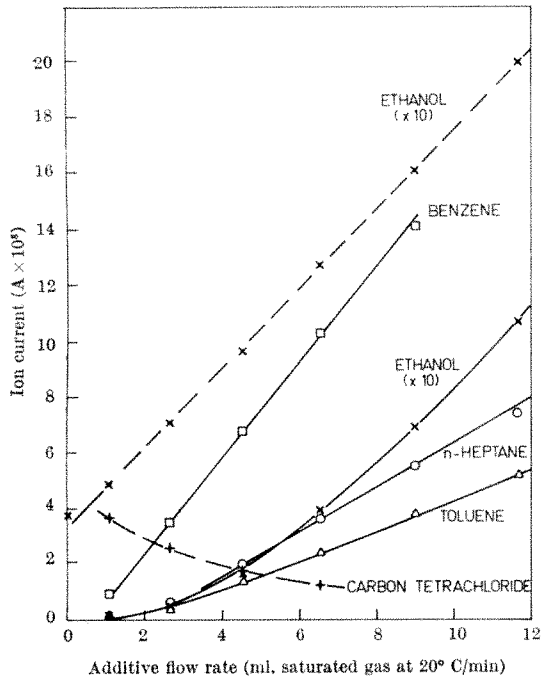


Fig. 2. Response of the carbon monoxide diffusion flame to various additives for pure carbon monoxide (solid curves) and impure carbon monoxide (broken curves).

In later experiments using a third sample of carbon monoxide (Matheson Company Inc., East Rutherford, New Jersey, USA) it was observed that the response was almost linear with ethanol concentration, but a large background signal was obtained in the absence of any organic additive. The introduction of carbon tetrachloride in this case reduced the level of the background signal as shown in Fig. 2 (broken curves). Trapping out the impurity giving rise to this signal, by using either a liquid air or dry ice/acetone bath, reduced the signal to zero irrespective of the addition of either carbon tetrachloride, hydrogen or water to the flame. Removal of the impurity in this way also resulted in the ethanol response reverting to a square law dependence as previously found with pure carbon monoxide (Fig. 1). Mass spectrometric analysis suggested that the major impurity in the carbon monoxide was methyl formate. This has been confirmed from the infrared spectrum of a sample of the trapped impurity, and its concentration has been determined by gas chromatographic analysis as 1·6 volumes per cent.

The above results show that neither hydrogen, water nor carbon tetrachloride produce significant ionization in the carbon monoxide flame, and this is consistent with ions being produced by reaction (1) above. The response due to hydrogen-containing organic compounds, however, appears to be complicated by kinetic factors arising from the effect of the cracking processes occurring in the flame.

I thank Miss C. Drummond who carried out most of the experimental work reported here, Dr I. R. Wilson for valuable discussions on the kinetic aspects of these results, and the Shell Group of Companies in Australia for the award of a research fellowship.

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Aquoluminescence from Gamma-irradiated Alkali Halides

We report here the observation of a faint bluish green glow with a peak around 5000 Å when gamma-irradiated NaCl, NaBr, KCl or KBr is dissolved in distilled water. To observe the glow the room must be dark and the eye must be acclimatized to the darkness for a few minutes. A high speed (400 ASA) photographic film is blackened by direct exposure to the glow in about 10 min (Fig. 1).

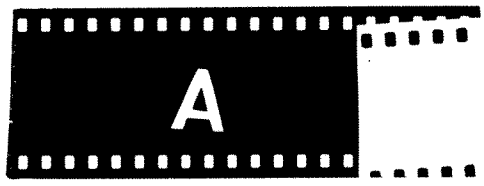


Fig. 1. Blackening of photographic paper by aquoluminescence.

We propose to call this emission aquoluminescence. The glow can be received on a photomultiplier and the output displayed on an oscilloscope. Fig. 2 for NaCl shows a typical trace. The maximum intensity develops in about 0·1 s while the decay time extends to about 2–3 s.

The following mechanism is proposed.

$$e^-_{\text{trapped in lattice}} + \text{water} \rightarrow e^-_{aq} \qquad (1)$$

$$e^-_{aq} + Cl\cdot \rightarrow Cl^-_q + h\nu \qquad (2)$$

The emission of a photon in a reaction of the above type does not appear to have been reported hitherto.

From a study of the reduction in intensity of aquoluminescence due to pre-thermal annealing of the irradiated salt, we have obtained data for (*a*) the energy barrier for the trapped electron (1·1 eV for NaCl), (*b*) the first order rate constant for thermal annealing ($3{\cdot}5 \times 10^{-2}$ s^{-1} at 423 K) and (*c*) the maximum number of defects produ-

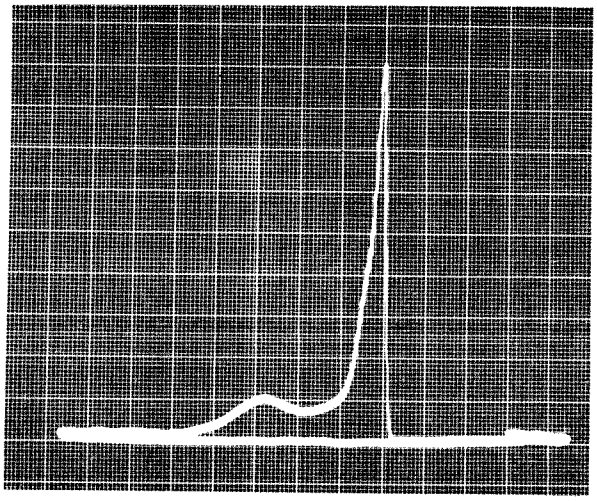


Fig. 2. Oscilloscope pattern of aquoluminescence from γ-irradiated NaCl.

cible by gamma irradiation (2×10^{19}/mole in NaCl). These results agree with values reported earlier[1-3]. The phenomenon thus seems to provide a relatively simple method for studying irradiation effects in crystals.

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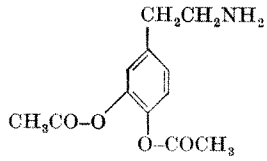
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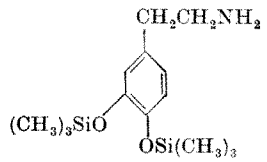
Possible Dopamine Derivatives capable of Crossing the Blood–Brain Barrier in relation to Parkinsonism

The exact neurophysiological role of dopamine is still unclear, but it has been implicated in the functioning of the extrapyramidal motor system (EPS)[1,2]. Experiments on animals and humans, however, indicate that the smooth functioning of the EPS depends on a fine balance between several biogenic amines[3-5]. Nevertheless, deficiencies of brain dopamine resulting from degeneration of the substantia nigra seem to be associated with the extrapyramidal symptoms of Parkinson's disease[1-6]. In accordance with these observations, attempts have been made to raise brain dopamine levels in patients suffering from Parkinsonism. Dopamine itself cannot be used, for it is incapable of crossing the blood–brain barrier, and therefore dopamine precursors, in particular L-3,4-dihydroxyphenylalanine (L-dopa), have been used[7-10]. Although such treatment apparently relieves the symptoms of hypokinesia, rigidity and posture, tremor is little affected and the necessity for large daily dosage and the side-effects of nausea and hypotension remain as serious drawbacks to the therapeutic use of L-dopa. Stock[11] has recently suggested that 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine may be a potential anti-parkinsonism agent since this tyrosine hydroxylase co-factor may be deficient in patients suffering from Parkinsonism[12].

Implicit both in Stock's suggestion and in the use of L-dopa is the assumption that dopamine precursors capable of crossing the blood–brain barrier will be enzymatically transformed *in vivo* into dopamine, thereby increasing brain dopamine levels. I propose that such levels could more easily be increased by administration of labile, lipophilic derivatives of dopamine. The modification of pharmacological activity by the synthesis of labile derivatives which undergo either enzymatic or non-enzymatic hydrolysis *in vivo* to regenerate the parent drug is well known, and the principle has been successfully applied to noradrenaline[13]. The 3,4,β-triacetate and 3,4,β-*tris*-(trimethylsilyl) derivatives of noradrenaline cause the sustained release of the parent catecholamine in mouse brains[14]. It therefore seems likely that similar derivatives of dopamine, for example I[15] and II[16], may penetrate into



I



II

the central nervous system and be converted to dopamine in the vicinity of the basal ganglia, for both *in vitro* and *in vivo* experiments[1,2] indicate that dopamine is preferentially taken up by these parts of the brain.

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Sunlight-induced Pyrimidine Dimers in Human Cells *in Vitro*

Pyrimidine dimers have been implicated in much ultraviolet light induced damage in bacteria[1], and it has been shown that they can be induced in mammalian cells with short wavelength ultraviolet light[2-7]. In placental mammals, pyrimidine dimers are not repaired by direct monomerization by photoreactivating light[2,8,9], although a dark repair mechanism, somewhat similar to that found in bacterial cells, has been demonstrated[10].

It has recently been reported[11–13] that cells of people with the hereditary disease, xeroderma pigmentosum, have a low capacity for DNA repair replication[14,15] and do not excise the ultraviolet-induced pyrimidine dimers[16,17]. These patients are very sensitive to ultraviolet light and exhibit a high incidence of skin cancer[18–20]. This report demonstrates that normal exposures to natural sunlight, which is known to induce skin cancers but contains no detectable wavelengths shorter than 290 nm at sea level, can induce significant formation of pyrimidine dimers.

Human amnion (AV_3) cells were plated (8×10^5 cells/plate) into 60 mm Petri dishes containing ^{3}H-thymidine (1 μCi/ml.; 2·0 Ci/mmole specific activity) in Eagle's minimal essential medium (MEM) (10 per cent calf serum) and incubated for 24 h. Twenve hours before exposure to sunlight, the labelled medium was discarded and enough non-labelled cells in non-labelled medium were added to the labelled monolayer of cells to form a second layer of cells on a series of plates. This was done to mimic the dead, horny layer of cells found on the epidermis of human skin. The cells were then carefully washed and 2 ml. of Hanks buffered saline was placed on each plate, which was exposed for various times to the sunlight. Plates were placed in a special constant temperature bath at 4° C. for the duration of the exposure. Subsequent pyrimidine dimer analysis was done according to methods previously described[2,21].

Table 1. EFFECT OF SUNLIGHT ON THE INDUCTION OF THE THYMINE-CONTAINING DIMERS IN HUMAN CELLS GROWN *in vitro*

Treatment	$\frac{\wedge}{XT/T}$ (c.p.m.)	Per cent of thymine as dimer
Experiment 1, July 26, 1968*		
Two cell layers		
Sunlight 2 h	89/121,700	0·07
Sunlight 3 h	123/98,200	0·12
Sunlight 5 h	318/137,000	0·23
Sunlight through 1 cm plate glass	0/216,500	0·00
Dark control	0/167,800	0·00
Experiment 2, August 11, 1968†		
One cell layer		
Sunlight 2 h	822/582,700	0·11
Sunlight 3 h	1,053/607,000	0·17
Sunlight 5 h	1,040/283,500	0·36
Two cell layers		
Sunlight 2 h	441/583,700	0·07
Sunlight 3 h	1,029/682,100	0·15
Sunlight 5 h	1,225/463,500	0·26

* Average dosimetry readings of sunlight, taken at five different times from 1030–1530, were $6{\cdot}5 \times 10^5$ erg/cm^2/s under 1 cm of plate glass and $7{\cdot}8 \times 10^5$ erg/cm^2/s without glass.

† Average dosimetry readings of sunlight, taken at five different times from 1030, were $6{\cdot}8 \times 10^5$ erg/cm^2 s under 1 cm of plate glass and $8{\cdot}4 \times 10^5$ erg/cm^2/s without glass.

Table 2. ABSENCE OF EFFECT OF SUNLIGHT ON THE MONOMERIZATION OF ULTRAVIOLET LIGHT-INDUCED PYRIMIDIME DIMERS

Treatment*	$\frac{\wedge}{XT/T}$ (c.p.m.)	Per cent of thymine as dimer
Ultraviolet alone†	1,376/920,000	0·14
Ultraviolet + 2 h sunlight‡ (cells in MEM)	737/536,400	0·13
Ultraviolet + 4 h sunlight (cells in MEM)	898/629,300	0·14
Ultraviolet + 4 h dark incubation (cells in MEM)	978/858,837	0·11
Ultraviolet + 2 h sunlight (cells in Hank's)	1,180/800,300	0·14
Ultraviolet + 4 h sunlight (cells in Hank's)	588/370,300	0·15

* Average dose rate of sunlight on August 4, 1969, taken from five readings between 1030 and 1530 was $5{\cdot}7 \times 10^5$ erg/cm^2/s (determined through 1 cm of plate glass with a YSI model 65 radiometer).

† Cells were irradiated with 300 erg/mm^2 of ultraviolet light at 2537 Å. Dose rate was 20 erg/mm^2 s.

‡ Cells were exposed to sunlight through 1 cm of plate glass.

To test for the possible monomerization of pyrimidine dimers by sunlight, ^{3}H-thymidine labelled cells (1 μCi/ml.; 2·0 Ci/mmole specific activity; 24 h incubation) were irradiated with 2537 Å ultraviolet light in the laboratory (300 ergs/mm^2 at 20 ergs/mm^2/s). After irradiation, 2 ml. of Eagle's MEM was added to each plate and the plates, in a water bath at 21° C. were then exposed to various doses of sunlight under plate glass (1 cm thick) and later analysed for pyrimidine dimers.

Assuming that the sunlight-induced photoproducts, which had the same chromatographic R_f values as thymine containing pyrimidine dimers, are in fact thymine-containing dimers, it follows that these can be induced by sunlight (Table 1). The quantity of pyrimidine dimers formed during 5 h of exposure at those dates and places is equivalent to approximately 600 ergs/mm^2 of 2650 Å ultraviolet light[2]. The human amnion (AV_3) cells do not photoreactivate ultraviolet induced damage by monomerization of pyrimidine dimers by sunlight (Table 2).

These empirical findings support the findings and hypothesis of Harm[22] that sunlight-induced lesions in DNA are the same as those made by 254 nm irradiation. The results of this study are of some empirical interest, for it has been suggested that ultraviolet-induced cancer might be explained by the somatic mutation theory[23,24]. If, in fact, mutations can cause cancer in humans, and if mutations are the result of defective repair of ultraviolet-induced pyrimidine dimers, as in bacteria[25], then these findings demonstrate that there is a molecular basis for such an hypothesis.

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Decreased Host Cell Reactivation of Irradiated SV40 Virus in Xeroderma Pigmentosum

Bacteria possess enzyme systems for repairing in the dark the damage caused by ultraviolet irradiation[1] in which damaged regions of DNA are excised and replaced[3]. Damage to an infecting phage can also be repaired. This capacity, known as host cell reactivation (HCR), is manifested by the lowered survival of ultraviolet irradiated phages in radiation-sensitive mutant host bacteria[3,4]. Mammalian cells also possess repair systems. In xeroderma pigmentosum, a genetic disease associated with unusually high sensitivity to sunlight and a greatly increased susceptibility to carcinomas and melanomas of the skin, the capacity of fibroblast cells for repair is

much lower than that of normal fibroblasts[5]. Recent evidence suggests that xeroderma pigmentosum cells are defective in the first reaction of the DNA repair mechanism[2,6]. Previous investigations have raised the possibility of a host cell reactivation system in mammalian cells[7–13]. We have shown directly that normal human cells have HCR capacity by comparing the ultraviolet sensitivities of two viral functions of the DNA tumour virus SV40—transformation and T-antigen induction—in normal and xeroderma pigmentosum cells.

Human cell cultures were derived from punch biopsies of normal (non-tumorous) skin from a 5 year old male (XP-1) and a 7 year old female (XP-2) with xeroderma pigmentosum and from a clinically normal 12 year old male (M. G.). Cells were grown in Dulbecco's modification of Eagle's medium supplemented with 10 per cent unheated calf serum, and they were used within the first twenty generations in culture.

The SV40 virus induces permanent alterations in the morphology and growth properties of several mammalian species in tissue culture including human cells[14]. Transformants can be recognized by their ability to form dense, piled-up colonies against the background of normal contact-inhibited cells. This lack of normal growth restraint induced by the virus provides the basis for the quantitative assay of transformation[15] (see legend to Fig. 1). Two to three weeks after infection with SV40, densely growing colonies of epithelioid cells could be recognized as early transformed colonies. After about 24 days, the cultures were fixed for 30 min with 10 per cent formalin and stained for 60 min with Harris haematoxylin. Transformed colonies were scored under a low power dissecting microscope. The transformation frequency was expressed as the total number of transformed colonies obtained per total number of cells inoculated (corrected for plating efficiency). The transformation frequency with the control SV40 was approximately 0·25 per cent for each cell strain. Thus, if the ultraviolet repair enzyme or enzymes deficient in xeroderma pigmentosum cells were necessary for the process of transformation, their presence at markedly lower levels still permitted a normal transformation frequency in the xeroderma pigmentosum cells.

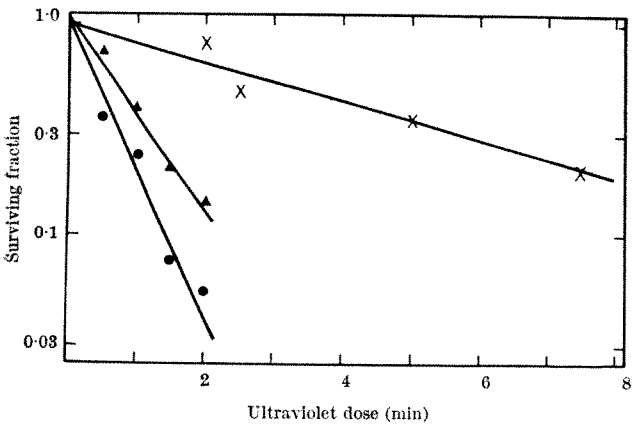


Fig. 1. Ultraviolet survival curves of SV40 transformation in normal and xeroderma pigmentosum cells. The virus used was a plaque purified pool of the small plaque (SV-S) variant of SV40 (ref. 6) which titred 5×10^8 plaque forming units (p.f.u.) per ml. in African green monkey kidney cells. The results are the average of two experiments. Transformation frequency with unirradiated virus for M. G. (×), 0·025 per cent; XP-1 (●), 0·03 per cent; XP-2 (▲), 0·023 per cent. Thin layered samples of SV40 were exposed to ultraviolet light for different times at room temperature in conditions of constant agitation of the fluid. The energy at the surface of the sample was 40 erg/mm²/s at a wavelength of 2537 Å measured with an ultraviolet dosimeter (Ultraviolet Products). Each aliquot was then reconstituted to its original volume with sterile distilled water. In the transformation assay, Petri dishes containing around 5×10^5 normal or xeroderma pigmentosum cells were exposed to normal SV40 (200 p.f.u./cell) or to equal amounts of ultraviolet-irradiated virus. The cells were then subcultured to several new Petri dishes, each at 10^4 or 5×10^4 cells per dish. The plating efficiencies for the cell strains were 60–85 per cent and were not decreased by exposure to SV40. Because SV40 can grow to a limited extent in human cells, all SV40 infected cultures were continually maintained in the presence of 0·5 per cent calf SV40 antiserum (Baltimore Biologic Laboratories). This concentration was capable of neutralizing between 10^5 and 10^6 p.f.u./h at 37° C.

The ultraviolet survival curves for transformation are shown in Fig. 1. These have been normalized to the transformation frequency of unirradiated virus. The data are adequately described by single hit, exponentially decreasing functions. The slope of the ultraviolet survival curve for the normal cell strain was very similar to that obtained for other normal human cells tested in identical conditions[16]. The ultraviolet survival curve of transformation in both types of xeroderma pigmentosum cells was considerably steeper than that of the normal cell strain, M. G. The slope for the most ultraviolet sensitive cell strain (XP-1) was about eight times greater than the slope for the normal fibroblasts.

SV40 T antigen is known to appear in acutely infected cells and to persist in transformed cells. It can be localized in the cell nucleus by an indirect fluorescent antibody test[17]. With infection by normal SV40 the number of human cells synthesizing T antigen during the acute infection is maximal at 72 h (ref. 18). The fraction of normal and xeroderma pigmentosum cells containing the antigen at this time was scored. The ultraviolet survival curves are compared in Fig. 2. As with transformation, the ultraviolet sensitivity of T antigen induction was greater in the two radiation-sensitive human cells. The slopes for XP-1 and XP-2 were calculated to be 9·0 and 5·0 times greater than normal, respectively.

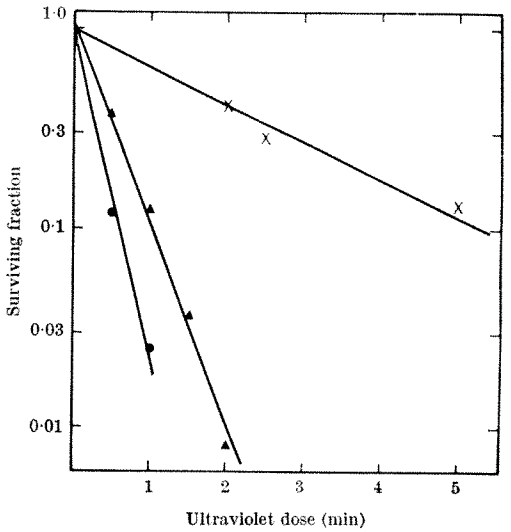


Fig. 2. Ultraviolet survival curves of SV40 T antigen in normal and xeroderma pigmentosum cells. T antigen was measured 72 h after infection. The results are the average of two experiments. The T antigen level with unirradiated virus in M. G. (×), 5·1 per cent; XP-1 (●), 6·2 per cent; XP-2 (▲), 6·9 per cent. At appropriate times, cells grown on coverslips in the presence of SV40 antiserum were fixed in acetone at −20° C for 10 min, and then incubated for 30 min with hamster SV40 T antiserum, washed carefully, and then incubated for the same time with fluorescein-conjugated goat anti-hamster globulin. Each coverslip was scored by two observers. At least 200 positive cells or 20,000 total cells were examined for each point.

Our findings are consistent with the hypothesis that xeroderma pigmentosum cells have little or no ability to reactivate ultraviolet-damaged SV40. The kinetics of T antigen induction in normal human and mouse cells are delayed by ultraviolet-irradiated virus[16], and we therefore studied the kinetics of T antigen formation with ultraviolet-damaged SV40 in the xeroderma pigmentosum cells. Fig. 3 shows the percentages of T antigen positive cells at different times after infection of normal and xeroderma pigmentosum cell strains by the control SV40 and by the ultraviolet-irradiated SV40. The T antigen levels for control virus

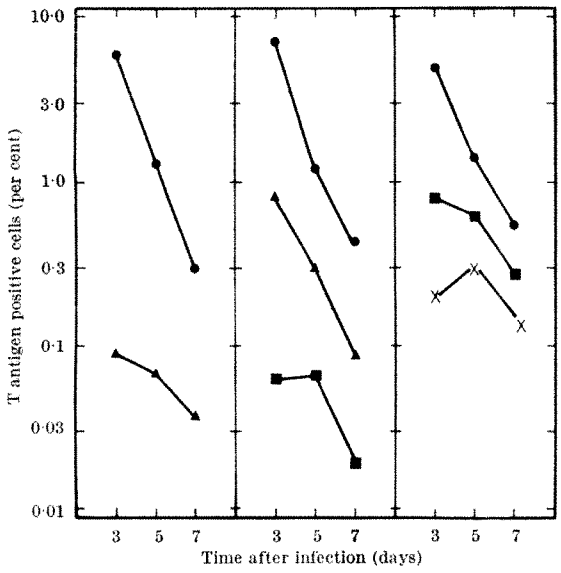


Fig. 3. T antigen levels measured at different times after infection by control and ultraviolet-irradiated SV40. Ultraviolet doses: ●, 0 min; ▲, 1 min; ■, 2 min; ×, 5 min. Left, XP-1; middle, XP-2; right, M.G.

were 5–7 per cent in each cell strain after 3 days and thereafter declined at similar rates. There was a definite delay in the induction of T antigen in the normal cells by the ultraviolet-irradiated virus. With the xeroderma pigmentosum cells, also the timing of the induction of T antigen by ultraviolet-irradiated SV40 was altered. The time course of T antigen appearance was similar in xeroderma pigmentosum and normal cells, although the absolute T antigen levels were much higher in the latter. This suggests that although the probability of host cell reactivation of damaged virus in the xeroderma pigmentosum cells was much lower than in normal cells, if reactivation did occur it did so with a time course similar to that seen in the normal cells.

Skin fibroblast cells from patients with certain genetic diseases associated with a high risk of tumour formation have a markedly increased susceptibility to transformation *in vitro* by SV40[15,18]. Our tests on xeroderma pigmentosum cells have not revealed an abnormally high transformation frequency with unirradiated virus, although both xeroderma pigmentosum patients had skin tumours, and one (XP-1) died of leukaemia (personal communication from R. M. McAllister). Thus the SV40 screening test may not detect the increased tumour susceptibility of xeroderma pigmentosum patients.

We have compared the survival of two functions of the tumour virus SV40 in normal and xeroderma pigmentosum cells at identical virus multiplicities. The results clearly showed a markedly lower survival of both transformation and T antigen functions in the xeroderma pigmentosum cells. By analogy with studies in bacteria (see review by Rupert and Harm[19]), this host cell effect on ultraviolet-damaged virus is most consistent with the hypothesis that ultraviolet-damaged SV40 is repaired by enzymes in the normal human cell that are missing or at much lower levels in xeroderma pigmentosum cells. The results also indicate that enzymes present in normal human cells can reactivate an ultraviolet-irradiated DNA tumour virus. The data obtained with strains XP-1 and M. G. indicate that the normal cell can have 8–9 times more HCR capacity than the repair-deficient cell. This is similar in magnitude to the difference between the abilities of normal and radiation-sensitive bacteria to reactivate ultraviolet-irradiated T1 bacteriophage[3].

Strain XP-1 produced steeper survival curves than XP-2 for both T antigen and transformation functions. That consistent differences were found in the ability of the two xeroderma pigmentosum strains to "reactivate" damaged SV40 implies that these cells have relative and not absolute defects in their DNA repair capacity. Similarly, relative differences in repair replication have been found for several xeroderma pigmentosum cell strains[5]. The repair replication ability of strain XP-1 was tested by Dr J. Cleaver and found to be about 5 per cent of the normal using his assay methods (personal communication). Our results showing about 12 per cent of the normal HCR in XP-1 indicate that there may be a close correlation between the capacities for repair replication and HCR in human cells.

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New Form of Molecular Defect in Xeroderma Pigmentosum

XERODERMA pigmentosum is a rare hereditary disease of the human skin. After slight exposure to sunlight the skin develops widespread poikiloderma with spotted dyschromia, telangiectasia and atrophy of the epidermis including multiple hyperplasia and the following malignancies of epidermal and dermal origin: basal cell and squamous cell carcinoma, melanoma, fibroma, sarcoma, histiocytoma, neurofibroma and angioma[1,2]. The molecular basis of light sensitivity and accelerated light induced carcinogenesis of xeroderma pigmentosum may involve a decreased tolerance of individual cells to light[3]. The initial direct damage is not increased, but the ability of cells to tolerate light induced DNA damage is decreased: investigation of cultured fibroblasts of xeroderma pigmentosum patients showed that these cells are unable to repair ultraviolet-induced single-strand DNA lesions[3]. The molecular defect lies in the initial stage of the repair process, probably at the endonuclease level[4,5].

Skin grafts, 0·2–0·3 mm thick, were cut from unaffected skin with a dermatome and divided into small pieces (2 × 2 mm). These pieces were irradiated with an unfiltered

xenon lamp[6] at various energies (total output: $2{\cdot}1 \times 10^6$ erg cm^{-2} s^{-1}, measured by a compensated thermopile 'CAI'; Kipp & Zonen). Immediately after irradiation, the specimens were incubated in tissue culture medium 199 (Difco) for 4 h at 37° C with 1 μCi/ml. [*methyl*-^{3}H]-thymidine. They were then fixed in formalin, sectioned, stained with acid haemalaun and autoradiographed using Kodak 'AR 10' stripping film and an exposure time of 7 days.

Three sporadic cases of xeroderma pigmentosum, of the autosomal recessive type, showed the same degree of heavy labelling in the basal cells as in the control group consisting of six normal persons, 20–30 years old. There seems to be no difference between patients with xeroderma pigmentosum and normal persons as far as the DNA replication in the S-phase of cell cycle is concerned. The repair activity of the lightly labelled xeroderma pigmentosum epidermal cells (basal cells, keratinocytes and granular cells) and fibrocytes of the upper dermis is reduced to about 10–20 per cent of the values of the control group (Table 1). These findings agree with those of Cleaver[4] and Setlow[5].

Table 1. REPAIR AND MITOTIC ACTIVITIES IN EPIDERMAL CELLS

	Irradiation time (s)			
	0	40	90	120
Repair activity				
Mean values of control group	0	57·1	61·4	60·0
Standard deviation	—	3·50	5·20	5·71
Xeroderma pigmentosum				
S. A.	0	4·5	5·5	3·5
A. W.	0	3·0	7·0	10·5
K. B.	0	3·0	5·5	4·5
Pigmented xerodermoid				
H. K.	0	44·0	53·5	54·5
M. H.	0	26·0	54·5	66·5
Mitotic activity				
Mean values of control group	36·8	28·4	19·2	12·6
Standard deviation	4·44	4·34	3·40	5·32
Xeroderma pigmentosum				
S. A.	27	19	12	9
A. W.	35	24	20	12
K. B.	44	29	20	9
Pigmented xerodermoid				
H. K.	32	9	5	2
M. H.	25	6	6	3

Repair activity as expressed by the number of lightly labelled epidermal cells out of 200 cells investigated. Mitotic activity is expressed by the number of heavily labelled (S-phase) cells out of 1,000 basal cells investigated.

We have found two female cases with normal brown pigmentation in which light produces an alteration of the skin resembling xeroderma pigmentosum. These actinic skin changes occurred between 35 and 40 years of age, but now, some years after the onset, extensive senile elastosis with poikiloderma, multiple keratosis and malignancies including melanotic freckles have developed. From the clinical point of view this condition has to be classified somewhere between extensive solar keratoses and a mild form of xeroderma pigmentosum. We therefore call it pigmented xerodermoid. Nevertheless, epidermal cells exhibit normal repair activity (Table 1). In spite of good pigmentary protection and the extensive lesions in skin exposed to the Sun, with multiple keratomata and malignancies, the early manifestation leads us to separate this phenomenon from what is usually known as actinic or solar damage. Further investigations at the cellular level show a normal percentage of heavily labelled basal cells in the unirradiated specimens of pigmented xerodermoid. Although in normal skin the reduction in premitotic synthesis of DNA is reduced in proportion to the irradiation energy, in our two cases of pigmented xerodermoid we find a strong and almost total depression of DNA synthesis after application of a relatively low dosage of ultraviolet light. Repair replication as detected by the incorporation of labelled thymidine is therefore not disturbed.

We conclude that the first steps in the repair process involving the enzymes endonuclease, exonuclease and DNA polymerase are functionally normal. But we suggest, in consequence, that the increased sensitivity to light involving semiconservative DNA replication in epidermal basal cells may explain the extensive damage in areas of skin exposed to light. The specific defect in these two cases must reside in the final steps of the repair process such as the action of the ligase or could involve a DNA polymerase system of semiconservative replication that is not necessary for the repair process. A third explanation could be that another, separate process correcting ultraviolet induced DNA damage is involved. This might be the recombinational form of repair which interferes with post-irradiation semiconservative replication rather than repair and may be depressed by caffeine[4,7].

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Toxicity of Poly I·Poly C for Rabbit Eyes

A SYNTHETIC polyriboinosinic acid·polyribocytidylic acid complex (poly I·poly C) is known to be a potent inducer of interferon[1] and to mediate resistance to various viral and nonviral infections *in vivo* and *in vitro*. Recently poly I·poly C was reported to be effective in herpetic keratitis in rabbits without producing any evidence of local or systemic toxicity[2]. While studying the effects of intravenous poly I·poly C on herpetic kerato-uveitis in rabbits, however, we noticed ocular changes in many animals and here describe the ocular toxicity in rabbits which received only intravenous poly I·poly C.

Thirty-eight New Zealand white rabbits (2–4 kg) were divided into four groups—A, B, C and D. Poly I·poly C was obtained from three commercial firms (Biopolymers, Inc., Dover, Lot M–6; Miles Laboratories, Elkart, Control No. 11-4-321; P-L Biochemicals, Inc., Milwaukee, Lot 2026) and was reconstituted from its lyophilized or solution form as directed. On days 0, 2 and 4, 1 ml. of solution containing 1 mg of poly I·poly C was injected into the ear veins of the rabbits in groups A, B and C, each group receiving the same batch of poly I·poly C from a single source. The control group D received 1 ml. of phosphate-buffered saline (*p*H 7·2) (PBS) by the same route. The eyes of each rabbit were examined with the slit-lamp and ophthalmoscope. The examiners did not know which animals had received poly I·poly C or saline. The level of circulating interferon induced by intravenous injection of 1·0 mg poly I·poly C was approximately eight times greater than that produced by 0·1 mg (ref. 3). A similar dose (1·0 mg) was used by Park *et al.* in the successful treatment of herpetic ocular infections in rabbits with systemic poly I·poly C (ref. 4).

Among animals receiving poly I·poly C, one of ten animals in group A and two of ten animals in group B died within the first two weeks. In group C two of twelve animals died within 12 h after the first injection, three others died within the first week, one died the second week, and one became ill the first week and remained ill throughout the seven weeks of the experiment. All of the other animals in the three groups remained well, and

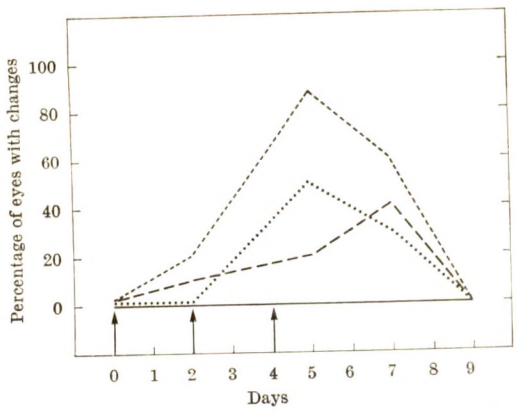


Fig. 1. Frequency of aqueous flare in the anterior chambers of rabbits inoculated intravenously (at arrows). Groups A (. . .). B (- - -) and C (— — —) received poly I·poly C from various sources; group D (———) received PBS.

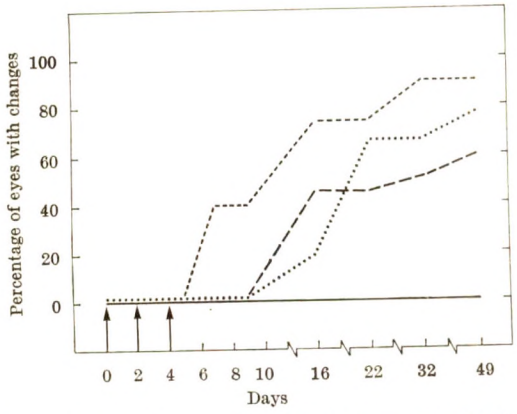


Fig. 2. Occurrence of lens opacities following intravenous injection (at arrows) with poly I·poly C. Cataracts were not observed in the control group (D). For explanations, see Fig. 1.

none of the six animals in control group D died or appeared ill at any time.

In surviving animals treated with poly I·poly C hyperaemia of the iris, aqueous flare (a manifestation of protein in the aqueous humour), and aqueous cells (thought to be white blood cells by virtue of their size and colour) were all noted in each of the three poly I·poly C-injected groups. The frequency with which aqueous flare developed (Fig. 1) paralleled the development of other inflammatory signs. These changes appeared soon after the first injection of poly I·poly C and reached their maximum between the fifth and seventh days. Inflammatory signs were not observed after the ninth day. No signs of inflammation were noted in control group D. In a previous investigation[5], the vascularity of the optic disk was increased by poly I·poly C, but in this study no such effect was noted, perhaps because the drug was given on alternate days instead of daily.

Opacification of the lens began as an increase in sheen posteriorly. This progressed to a posterior subcapsular plaque with posterior cortical changes (Fig. 2). In our initial experiment[5], clear lens fibres were seen behind the opaque lens fibres when the animals were followed for at least 20 weeks (Figs. 3 and 4), suggesting that the toxicity was for developing lens cells only.

Opacities in the anterior vitreous were noted as early as five days after injection of poly I·poly C (Fig. 5). In most instances they appeared to be crystalline in form and may well have been aggregates of poly I·poly C or its derivatives; cellular elements could not be ruled out, however. The opacities persisted for over a month. No changes in the vitreous were noted in control group D.

We considered the possibility that the observed ocular changes induced by poly I·poly C in these experiments were due to endotoxin contamination, but the ocular effects of typhoid endotoxin (100 μg), though similar in a similar model, were much less marked than those induced by the poly I·poly C. Although there were some differences in the degree of ocular toxicities of the three lots of poly I·poly C tested (Figs. 1 and 2), the numbers of animals tested with each lot were not great enough to make any valid comparisons. The 50 per cent mortality in group C, however, was clearly much greater than in groups A and B.

Various side effects of poly I·poly C have recently been reported. An increased mortality rate has been noted in mice; pyrexia and embryotoxic effects have been encountered in rabbits; and toxic effects on thymus and spleen have been noted in mice and rats[6–8]. Clearly, it is important thoroughly to evaluate the toxicity of poly I·poly C before any clinical trials in man.

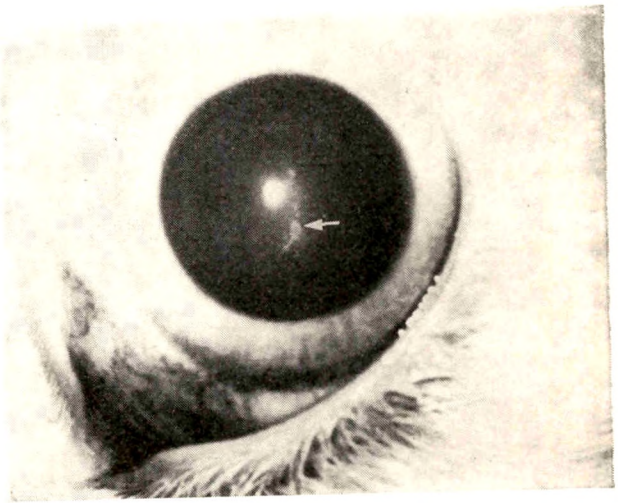


Fig. 3. Lens opacity (arrow) in a rabbit 208 days after intravenous administration of poly I·poly C. On slit lamp examination this was in the posterior cortex and there was a relatively clear zone between the lesion and the posterior lens capsule. The white circular spot is a reflexion of the photographic flash.

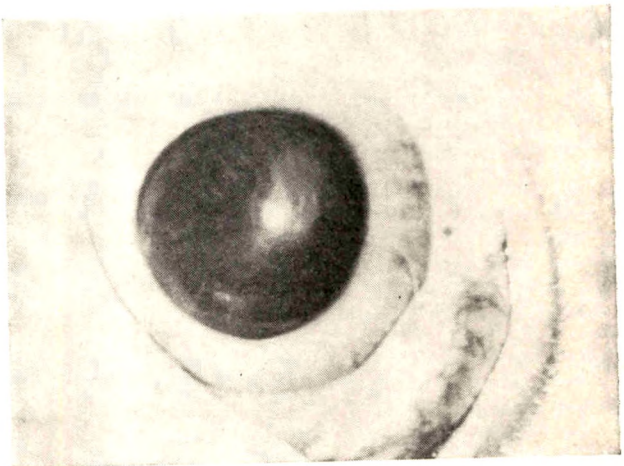


Fig. 4. Photograph of a lens opacity in a rabbit 208 days after the first injection of poly I·poly C. This opacity involved both the anterior and posterior cortex, sparing the inner portion of the lens. Both the central and peripheral lens cortex were involved.

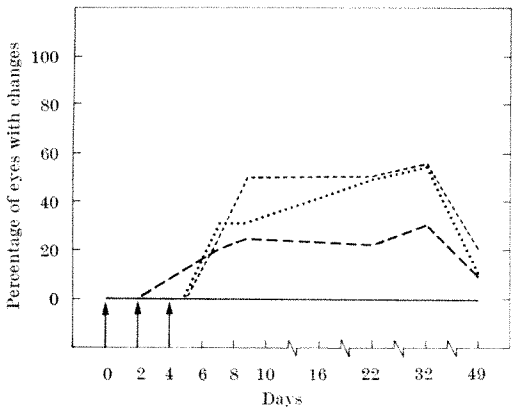


Fig. 5. Frequency of opacities in the vitreous following intravenous poly I·poly C injection. These opacities were discrete needle-like white crystals which were semitransparent, and were unlike the diffuse or focal vitreous opacities seen in infections of the posterior eye. For explanations, see Fig. 1.

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Cell Surface Charge Increase by both Tumour Inhibitory and Tumour Growth Promoting Polyanions

Many tumour growth inhibiting substances have been studied in various tumour systems: aliphatic diamines, hydroxy-amines, aromatic bases with two terminal groups[1], the synthetic polypeptides of lysine such as positively charged poly-DL-lysine[2], polyornithine[3], histones[4], lysine-rich histone fractions[5], DEAE-dextran[6,7], polyethylenimine[8,9], polyvinylamine bisulphate and polypropylenimine[9]. The synthetic double-stranded ribonucleic acid—a complex of polyinosinic acid and polycytidylic acid—(poly I·poly C), which is highly negatively charged, has also been demonstrated to inhibit the growth of several transplantable malignant murine tumours of known or unknown viral aetiology[10–17]. Some reports describe the protective action of poly I·poly C on non-viral diseases as well[10,18]. Certain polysaccharides isolated from lichens significantly inhibited the growth of implanted sarcoma-180 in mice and caused complete tumour regression in many cases[19], whereas acid mucopolysaccharides promoted tumour growth[20–29].

An increase in concentration of acid mucopolysaccharide (AMP) has been observed in human patients with proliferating malignant tumours[20,21]; and subjects with disseminated malignant diseases, particularly those with leukaemia, have increased urinary excretion rates of AMP, the major component of which is similar in composition to chondroitin sulphate (CS)[22]. Ozzello *et al.*[23] demonstrated the promoting activity of AMP *in vitro* on the growth of human mammary carcinoma cells, and Takeuchi[24–29] reported that the acid mucopolysaccharide chondroitin sulphate had some promoting effect on the tumour growth. Acid mucopolysaccharides contain hexosamine and hexuronic acid as the structural components[30] but the growth promoting activity of 2 per cent D-glucosamine hydrochloride, 0·2 per cent D-glucuronic acid, and 0·2 per cent N-acetyl-D-glucosamine was not so marked as that of chondroitin sulphate[28]. Takeuchi also observed[29] that the cationic proteins, egg albumen, calf serum and muramidase (lysozyme, EC 3.2.1.17) tended to counteract the promoting activity of chondroitin sulphate on tumour growth. Following current trends (see ref. 44) he suggested that the marked inhibitory effects of these basic proteins on the chondroitin sulphate activity resulted from a decrease in its negative charge.

The α-carboxyl groups (pK approximately 2·8) of neuraminidase-susceptible N-acetylneuraminic acid (NANA) on the tumour cell surface account for the bulk of the charge[31–34] (about 43 per cent of the electron charges[35]). Positively charged amino groups[35], sulphydryl groups[36] and ribonuclease susceptible phosphate groups[37] are also present in the cell periphery and the cells at physiological pH exhibit a net negative charge equivalent to approximately 10^7 electron charges per cell (see ref. 35 for review). The invasiveness and decreased adhesiveness of tumour cells (and other properties), at least in some systems, were attributed to the high electron charge density (anodic electrophoretic mobility, zeta potential) of tumour cells[38–44].

It was recently reported that positively charged polyornithine of high molecular weight inhibited the transplantability of mouse leukaemia, the inhibitory effect being reversed by the highly negatively charged mucopolysaccharide heparin[3]. My electrokinetic studies demonstrated that only the lysine polypeptides of high molecular weight and high charge density, which were reported to inhibit tumour transplantation/development, changed the tumour cell surface negative charge to zero or high positive values, presumably because of adsorption of the polycations on the cell surface; heparin inhibited or reversed this effect[44].

The recent spate of evidence on the highly negatively charged polymer poly I·poly C possessing tumour-inhibiting properties[10–18] and my observations that poly I·poly C increased the net negative charge of the Ehrlich ascites tumour cells[45,46], and the human platelet[45,46] seemed significant, but contradictory to the surface charge hypothesis—"associating high surface charge density with the invasive properties and the unsocial behaviour of tumour cells". These test systems offered an ideal situation to investigate the effect on the surface charge of cells treated with the negatively charged acid mucopolysaccharide (approaching raised levels in human patients) which promoted tumour growth and compare with effects produced by tumour-inhibiting polyanions.

Solutions of polyinosinic acid (1 mg/ml.) and polycytidylic acid (1 mg/ml.) were prepared in phosphate buffered saline (0·01 M phosphate buffer in 0·15 M NaCl containing 5×10^{-3} M Mg^{2+} as $MgCl_2$). Equimolar amounts of poly I and poly C were mixed at 37° C to prepare poly I·poly C. A decrease of about 40 per cent in the absorbance at 245 nm was observed as would be expected from the average absorbances of the unannealed

materials. The molecular weight of the poly I·poly C was believed to be several millions: too large to enter into a 2 per cent polyacrylamide gel in electrophoresis experiments (personal communication from Dr H. Levy of the US National Institutes of Health).

The sample from Bethesda was stored in a freezer and used to prepare test solutions in standard saline (2 mg/ml.). Chondroitin sulphate was supplied by Sigma (London). Electrokinetic studies were carried out as described[35,44].

Earlier, it had seemed plausible that the negative charge on the tumour cell membrane played a part in its invasive properties by way of electrostatic interactions between the positively charged tumour-inhibiting substances and the negative charges on the tumour cell surface. Blocking these sites and imparting the tumour cells, a high positive charge by treating with the polycations could, conceivably, suppress their invasiveness and the other allied properties[44]. The data in Table 1 demonstrate that the negative charge of the tumour cell membrane increased on treatment with both the polyanions poly I·poly C (tumour inhibitory) and chondroitin sulphate (tumour growth promoting).

Table 1. POLYANIONS POLY I·POLY C (TUMOUR INHIBITING) AND CHONDROITIN SULPHATE (TUMOUR GROWTH PROMOTING) INCREASE THE NET NEGATIVE SURFACE CHARGE OF CELLS

Cell type	Treatment	Electrophoretic mobility (μm/s/V/cm (with s.d.)	Change (per cent)
Ehrlich ascites tumour (of mice)*	Control	−1·05 ± 0·06 (105)	
	Poly I·poly C	−4·48 ± 0·09 (200)	×4 the original charge
	Chondroitin sulphate	−1·31 ± 0·06 (150)	+24 per cent
Human blood platelets†	Control	−0·88 ± 0·05	
	Poly I·poly C	−1·18 ± 0·08 (ref. 46)	+34 per cent

* Cambridge Radiotherapeutics strain of tumour[35,36,44].
† Normal healthy individuals.

Numbers in parentheses represent the numbers of cells examined. Evaluation of the electrokinetic parameters: zeta potentials, surface charge densities and the numbers of electron charges per cell, standard deviations, and so on, by the digital computer have been discussed recently[44]. The P values for all these experiments evaluated for the "unpaired" t tests: $P < 0.001$. Only values of electrophoretic mobility are included in this table. The medium was 0·145 M NaCl (pH 7·2 ± 0·2); mobilities were determined at 25° C.

It therefore seems an oversimplification to attribute both invasive properties and tumour growth promoting activities to the high electron charge density of the tumour cell membrane.

Great caution in the interpretation of the electrokinetic data on cells (chemically treated or otherwise) must be emphasized: the concept of charge alone will not explain all the phenomena observed.

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Susceptibility of *E. coli* K-12 to Actinomycin D after Infection with Phage M13

Escherichia coli cells are normally impermeable to the antibiotic actinomycin D[1] which inhibits DNA-dependent RNA synthesis[2] and the consequent inducible enzyme synthesis. Leive has shown that after treatment with EDTA, *E. coli* cells are more susceptible to actinomycin D[3] and that a lipopolysaccharide is released from the cell wall[4]. We have confirmed the observation[5] that *E. coli* cells also release a lipopolysaccharide into the medium after infection with the small filamentous phage M13 (unpublished results). Here we show that M13 infected cells are permeable to actinomycin D. It should be noted that M13 infected cells continue to grow and multiply while releasing progeny phage particles[6,7].

E. coli HfrC was grown in modified M9 medium[6] to log phase and then infected with M13 phage at a multiplicity of 10–20. The infected as well as control healthy cells were treated with 20 μg/ml. actinomycin D in the growth medium. Fig. 1 shows that the cells continued

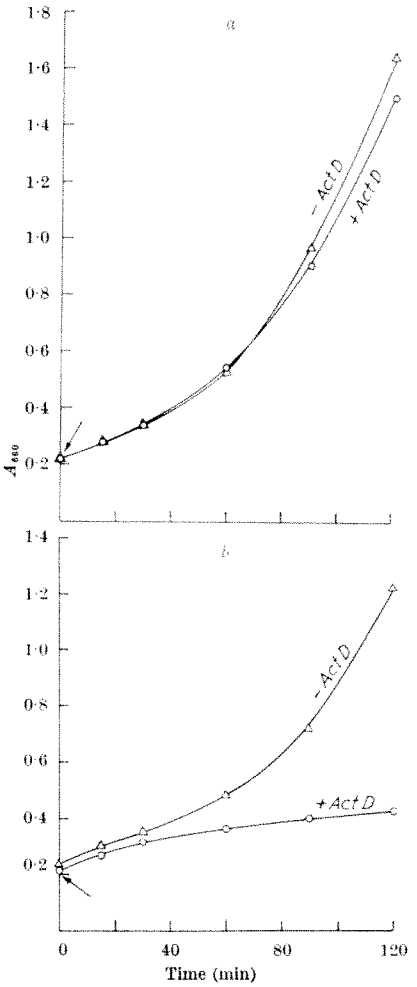


Fig. 1. Kinetics of growth of healthy and M13-infected *E. coli* HfrC in the presence of actinomycin D (20 μg/ml.), which was added simultaneously with the phage in the case of infected culture. The absorbance at 660 nm was measured at different times. (*a*) Normal cells, (*b*) infected cells with and without actinomycin D.

to grow for about 30–40 min when the antibiotic was added simultaneously with the phage. Then the growth of the infected cells almost levelled off while the growth rate of the healthy cells was not affected by the presence of the antibiotic. The addition of the antibiotic at different times after infection indicated that the infected cells become permeable to the antibiotic at about 30–40 min after infection. It has also been found that infected cells rapidly lose the ability to form colonies after treatment with the antibiotic even if it is removed before plating on nutrient agar. Control experiments indicated that the viability of healthy cells is not affected by the presence of the drug.

Fig. 2 is a photomicrograph of the M13-infected cells with and without treatment with actinomycin D. The healthy and infected cells were almost of comparable size, about 2·4 μm long, whereas the antibiotic treated infected cells became three to four times as long, suggesting lack of cell-septum formation. Actinomycin D has a similar effect on *B. subtilis* cells, which are normally permeable to the antibiotic[1]. With *E. coli*, there is no evidence of cellular lysis and the viability studies indicate that the elongated cells cannot form colonies. If we assume that actinomycin D specifically inhibits RNA synthesis without affecting DNA synthesis, then presumably the elongated cells are multinucleate and cell division is prevented. Various antibacterial agents of completely different modes of action are known to produce filamentation in *E. coli*[8–10]. The mechanism is not completely understood.

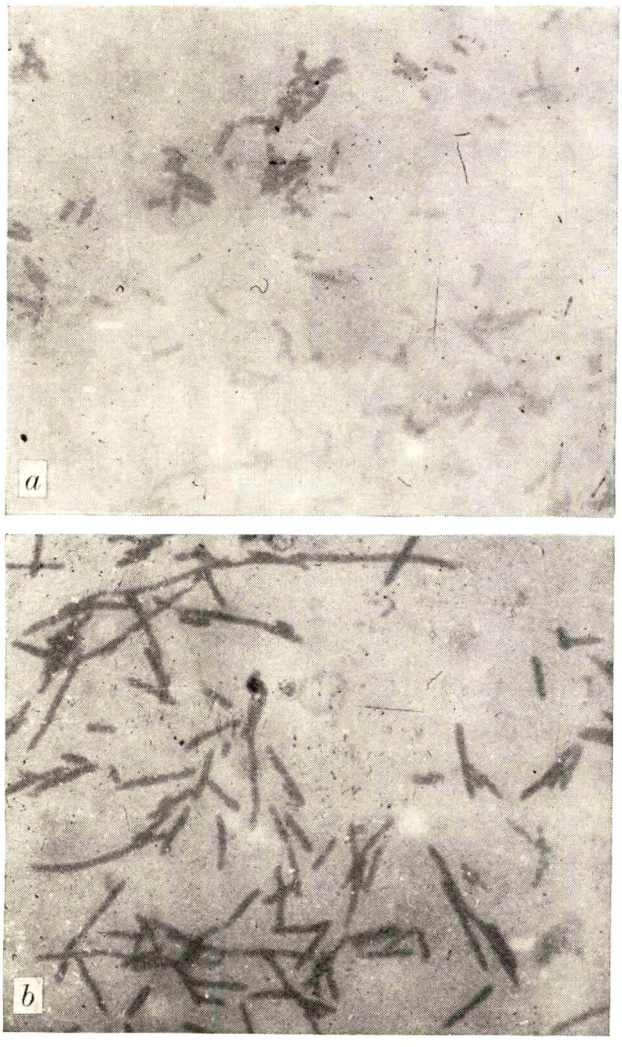


Fig. 2. Photomicrograph of *E. coli* cells stained with crystal violet. *a*, Infected cells; *b*, infected cells treated with actinomycin D for 2 h. The drug was added along with the phage (×*c.* 1,400).

The relationship between lipopolysaccharide release and actinomycin D susceptibility may be fortuitous. The site of the permeability barrier to actinomycin D in *E. coli* is presumably the cell wall, for the *E. coli* spheroplasts are permeable to the former[11]. We have recently found, however, that infection with M13 does not produce all the effects of EDTA treatment in *E. coli*. For example, periplasmic enzymes and acid-soluble ultraviolet absorbing materials are not released (unpublished results).

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Collagenolytic Activity of Sarcoma Tissues in Culture

The possibility that the invasiveness of malignant tissues may result from collagenolytic action of tumour cells was early considered[1–3]. This idea, however, was not supported by subsequent efforts to isolate a specific enzyme from neoplastic tissues or by attempts to obtain lysis of collagen enclosed in perfusion chambers embedded in tumour bearing animals[4].

With the demonstration that certain non-tumour epithelial tissues could produce an enzyme specific for the hydrolysis of reconstituted collagen in physiological conditions[5], interest in the collagenolytic capacity of tumour cells was renewed. First observations indicated that only epithelial cells were able to produce the lytic enzyme[6,7]. More recently, reports of degradation of collagen by non-epithelial tissues have appeared[8–10], but because most of these tissues were either abnormal or infiltrated with inflammatory cells, a question regarding the collagenolytic capacity of normal connective tissue cells still exists. There has been no convincing evidence of collagen lysis by normal tissues of mesodermal origin such as fascia, dermis, muscle or nerve. The role of infiltrating lymphoid or granular cells has not yet been thoroughly investigated.

Riley and Peacock[11] reported collagen lysis in cultures of a series of neoplastic and normal human tissues. They concluded that collagen breakdown was primarily associated with epithelial and not with deep connective tissue elements. The tumours of connective tissue origin which they tested did not produce lysis of collagen.

We report here the results of culturing fragments of human tumours on a collagen substratum in an effort to determine whether neoplastic tissues of mesenchymal

Table 1. TUMOUR BIOPSIES TESTED

	Total	Active	Inactive	Positive/total cultures
(*a*) Tumours of epithelial origin				
Metastatic carcinoma	4	1	3	19/19
Squamous carcinoma	7	2	5	15/24
Senile keratosis	6	0	6	32/44
Adenocarcinoma	3	0	3	17/23
Basal cell carcinoma	1	0	1	1/5
Wilms tumour	1	1	0	
Hepatic cell carcinoma	1	1	0	
Ovary, serous carcinoma	2	0	2	7/13
Thyroid carcinoma	1	1	0	
Transitional cell carcinoma	1	1	0	
Melanoma	3	2	1	7/7
Totals	30	9	21	
	Total	**Active**	**Inactive**	**Positive/total cultures**
(*b*) Tumours of non-epithelial origin				
Angiosarcoma	1	1	0	
Fibrosarcoma	2	1	1	2/10
Osteosarcoma	1	0	1	1/6
Rhabdomyosarcoma	2	0	2	7/8
Totals	6	2	4	

The figures represent numbers of collagenolytically active and inactive specimens and the ratio of the positive to the total number of fragments cultured from the active specimens.

origin differ in collagenolytic activity from those of epithelial origin.

Collagen was dissolved by weak acid from rat tail tendons and reconstituted as a film on glass coverslips according to the method of Bornstein[12]. Small samples of tumour proper having the gross appearance of viable tissue were dissected from human tumour biopsies. These were cut into 1 mm cubes for culture. Each small fragment was then placed in the centre of a collagen film and covered with a sheet of sterile 'Cellophane' dialysis membrane moistened in Hanks solution. This was assembled into a multipurpose culture chamber[13] which was then filled with Eagle's basal medium containing 10 per cent bovine embryo serum and incubated for periods of from 1 to 8 days. To see how much digestion had taken place, we fixed the collagen film in Bouin's fluid and coloured it with van Gieson's stain. Areas around active fragments where collagen had been hydrolysed remained unstained (see Fig. 1).

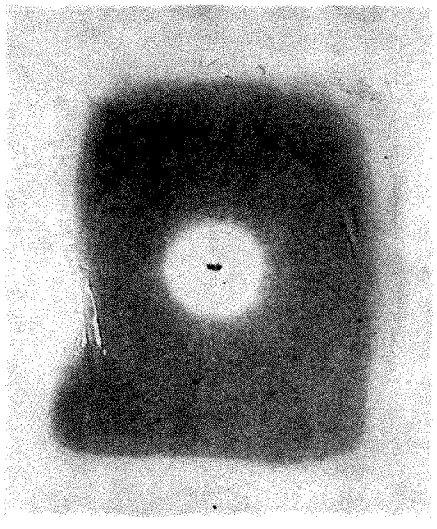


Fig. 1. Appearance of a collagen film stained after culturing for 3 days with a rhabdomyosarcoma fragment. The tumour fragment is in the centre of a clear area where the collagen has been removed by enzyme digestion.

Table 1 summarizes more than 300 cultures from 36 specimens removed from patients at the University of Texas M. D. Anderson Hospital and Tumor Institute at Houston. Most of the samples of carcinoma (21 out of 30) exhibited lysis of the collagen film. These findings support those of Riley and Peacock[11]. On the other hand, four of the six sarcomas tested also produced collagen breakdown (Fig. 1). The collagenolytic capacity of these neoplastic forms of striated muscle and fibrous connective tissue is in distinct contrast to normal tissues of the same types which have, in repeated tests, consistently failed to digest collagen.

Although there were tissues in both categories of tumour which did not digest collagen, this fact cannot be considered evidence that the tumour cells were incapable of elaborating collagenolytic enzyme. Numerous experiments have shown that the liberation of collagenase can occur only from synthesizing cells[5,6]. The lack of collagen lysis in some of the negative cultures may be because the tissues isolated from the tumour for culture did not survive. In such cultures, there was histological evidence of necrosis of the tumour cells. In cultures which did digest collagen, living cells were seen either in phase contrast examination of the cultures themselves or in histological sections of the cultured fragments. In the circumstances of these tests, therefore, the number of negative cases in the two categories of tumour is not of prime importance. What is of particular significance is the fact that some of the tumours of non-epithelial origin were capable of producing an enzyme that hydrolysed collagen, just as do some normal and malignant epithelial tissues.

If normal mesodermal tissues are unable to lyse collagen, it seems that the neoplastic transformation of mesenchymal tissues induces synthesis of a collagenolytic enzyme.

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Lymphocyte-mediated Transport of Aggregated Human γ-Globulin into Germinal Centre Areas of Normal Mouse Spleen

GERMINAL centres develop early in the immune response, and although knowledge of these structures is far from complete, they are known to be intimately associated with the capacity to produce specific antibodies after antigenic stimulation[1]. The localization of antigen in these centres coincides with the appearance of specific serum antibody[2], which is found in a close relationship to reticular cells in these areas[3]. It produces a characteristic dendritic intercellular pattern of immunofluorescent staining or autoradiography when isotopically labelled. This localization of antigen in germinal centres did not occur in rabbits

rendered tolerant to haemocyanin or human serum albumin[4], but injected immune complexes produced an identical pattern of localization of antigen in germinal centres in normal rat lymph nodes[5].

More recent studies[6] have established that alteration of heterologous IgG either by heat aggregation or by complexing as antibody with specific antigen is necessary for its localization in the germinal centres of auricular lymph nodes of normal guinea-pigs after injection into the ear. Such localization was not seen in animals given native, monomeric heterologous IgG. Nevertheless it does not depend on the antigenicity of the injected aggregate, for it was also seen in animals rendered specifically tolerant.

Thus germinal centre localization of antigen is mediated by specific antibody molecules through the configurational changes that they undergo on complexing with antigen, but the localization mechanism itself is obscure. With regard to localization, aggregated human IgG behaves identically to the antibody component of immune complexes[6]. It therefore provides a convenient substitute for complexes in further studies of the localization mechanism.

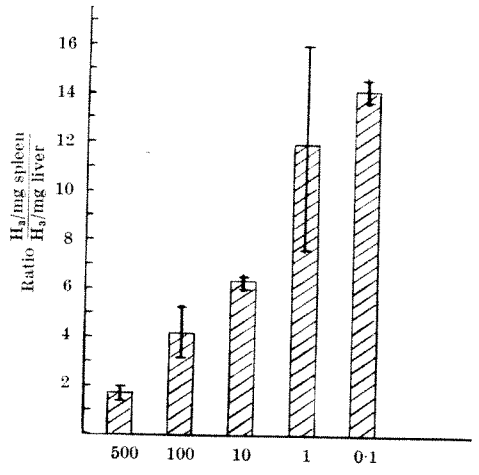


Fig. 1. Uptake at 24 h of human IgG aggregate by spleen and liver tissues in Balb/c strain adult mice. Vertical lines show range (500, 100, 0·1 μg, two mice; 10 μg, four mice; 1 μg, eight mice).

Preliminary experiments showed that heat aggregated[6] human IgG becomes localized in germinal centres in the white pulp of the mouse spleen within hours of intravenous injection. Normal mice were then studied using heat aggregated human IgG labelled with ^{125}I. A monospecific rabbit anti-human IgG fluorescein conjugate was used to produce immunofluorescence. Intravenous injection of less than 10 μg aggregated human IgG produced a dendritic pattern of germinal centre staining in the spleen. There was no evidence of phagocytosis of this material by macrophages in the red pulp of this organ or by the Kupffer cells of the liver. With larger amounts of aggregate, extensive phagocytosis of IgG by macrophages in the liver and spleen was seen. This phagocytosed material was rapidly catabolized, as shown by loss of isotopic label and loss of fluorescence from the spleen and liver, both more or less completed by 24 h after injection. At this time there was always bright germinal centre staining of IgG.

In the guinea-pig experiments[6], this germinal centre localization persisted for at least 4 weeks. It therefore seemed that germinal centre localization of human IgG is one means of preserving molecules of this protein intact in the body. This preservation of aggregated IgG by the mouse spleen (Fig. 1) is shown by the increasing ratio of spleen/liver uptake 24 h after intravenous injection of decreasing doses of protein. Maximal incorporation of

Table 1. SPLENIC UPTAKE AND GERMINAL CENTRE LOCALIZATION OF CEL BOUND AGGREGATED HUMAN IgG ON TRANSFER TO SYNGENEIC Balb/c MIC

No. of recipients	Cell source	No. of cells transferred	Protein bound to cells (μg)	Splenic uptake at 24 h (μg)	Germinal centre staining
1	Blood leucocytes	$2{\cdot}6\times10^6$	5·7	0·016	Negative
2		20×10^6	7·29	0·023	Negative
2		40×10^6	6·21	0·028	+
2	Spleen	100×10^6	8·0	0·096	++

aggregated IgG into the germinal centre of mice spleens occurred 24 h after intravenous injection, when most of the phagocytosed material had been catabolized. Immunofluorescence a few hours after injection of aggregated IgG showed a diffuse pattern of localization in the white pulp marginal zones and after 24 h the typical dendritic pattern of distribution had developed. Mouse complement was not detected by staining in these areas in sections treated with rabbit anti-mouse complement conjugate. Because lymphocytes are the cells which characteristically migrate from the blood to the splenic white pulp[7], cells of this type might be involved in the mechanism whereby aggregated IgG reached germinal centres. Two types of experiment were done.

In the first, peripheral blood leucocytes or spleen cell suspensions from normal C57Bl or Balb/c mice were incubated *in vitro* with ^{125}I-labelled aggregated human IgG in medium 199 containing normal mouse serum at 4° C. Uptake was measured by scintillation counting. In these conditions 10–20 per cent of the radioactivity was retained after exhaustive washing of the cells. Immunofluorescence revealed that at least 40 per cent of the cells had taken up the IgG on their surface membranes in a characteristic staining pattern. These had the morphological features of lymphocytes and not macrophages. The cell membrane staining was punctate and distributed uniformly around cells like a necklace. Occasional cells showed polar staining with concentration of IgG at one or two areas on the surface of the membrane. Table 1 shows that the intravenous injection of 40×10^6 peripheral white cells or 100×10^6 splenic cells, pre-incubated in this way with aggregated IgG, caused germinal centre localization of IgG in the spleens of normal syngeneic recipients. It should be noted that less than 1 per cent of the injected IgG was localized in the spleens in this way, the rest being catabolized. Thus many of the cells carrying human IgG were either removed by the phagocytic cells of the recipients or lost their labelled IgG to them. The amount of aggregated IgG on the cells if given alone would have resulted in some germinal centre staining. It was therefore possible that some IgG was transferred in some way from the donor cells and was subsequently localized in germinal centre areas of the spleens of recipients. Further studies are required to investigate this crucial point using lymphoid cells pre-labelled with isotopically labelled precursors of DNA and RNA. Because lymphocytes were postulated to be transporting the aggregated IgG into germinal centres it was considered possible that the protein demonstrated in these areas was on lymphoid cells closely apposed to the dendritic cells[3]. When spleens which would have shown staining confined to germinal centres were teased into single cell suspensions and stained for IgG with the anti-human IgG fluorescein conjugate, many lymphocytes with a characteristic membrane fluorescence were found. It can therefore be argued that at least part of the localization of aggregated IgG in germinal centres of normal mouse spleens was due to lymphocytes with this protein on their surfaces.

If localization of aggregated human IgG occurs by a lymphocyte mediated mechanism in which circulating lymphocytes transfer it into the white pulp of the spleen where it becomes concentrated in germinal centre areas, interference with the functions of these cells should prevent this characteristic localization. Antilymphocytic serum can be considered to possess this property[8]. Mice treated with the γ-globulin prepared from a potent

Table 2. EFFECT OF ANTILYMPHOCYTIC IgG ON SPLENIC UPTAKE OF AGGREGATED ^{125}I-HUMAN IgG 24 H AFTER INTRAVENOUS INJECTION INTO Balb/c MICE

No. of mice	Treatment	Dose of human IgG (μg)	Mean splenic uptake*	Germinal centre staining
3	ALG, 2·5 mg ×2†	10	28	Negative
3	ALG, 2·5 mg ×2	1	41	Negative
3	Normal rabbit IgG, 2·5 mg ×2	10	100	+ +
3	Normal rabbit IgG, 2·5 mg ×2	1	100	—
2	Nil	10	100	+ +
2	Nil	1	100	Not done

* Expressed as percentage of uptake in untreated or normal rabbit IgG treated mice.

† Doses spaced 24 h apart; human IgG given 3 h after second dose.

antilymphocytic serum were found to be unable to localize aggregated human IgG in the germinal centre areas of their splenic white pulp.

Table 2 shows the results of such an experiment. Antilymphocytic IgG given in two spaced injections reduced the splenic retention of aggregated ^{125}I human IgG 24 h after injection to 28 per cent of that in mice given either human IgG alone or human IgG and the same quantity of normal rabbit IgG instead of antilymphocytic IgG. This reduction of uptake was correlated with negative immunofluorescence. Another experiment (Table 3) showed that this effect of antilymphocytic IgG was dose dependent. Results not shown here indicated that significant prevention of localization of aggregated human IgG in germinal centres lasted for about 48 h after administration of 5 mg of antilymphocytic IgG.

Table 3. SPLENIC UPTAKE AND GERMINAL CENTRE LOCALIZATION OF AGGREGATED HUMAN IgG* IN PAIRS OF MICE PRE-TREATED WITH ALG

Antilymphocytic IgG dose (mg)	Splenic uptake†	Germinal centre staining
5	5·49 (4·96, 6·03)	Negative
2·5 ×2‡	5·09 (4·47, 5·71)	Negative
2·5	6·07 (5·43, 6·72)	±
1·0 ×2	8·65 (8·63, 8·68)	+
1·0	11·68 (11·58, 11·78)	+ +
0·1 ×2	11·80 (10·31, 13·30)	+ +
0·1	16·07 (15·50, 16·64)	+ + +
Nil	15·54 (15·24, 15·84)	+ + +

* 50 μg injected intravenously 3 h after last antilymphocytic IgG injection.
† μg protein ×10^{-2}.
‡ Two intravenous doses spaced 24 h apart.

These results indicate that a large proportion of lymphocytes in the blood and spleens of normal mice can take up aggregated human IgG on to their surface membranes. The *in vitro* uptake is unlikely to involve binding of C1q because complement was not present in the medium. Presumably these cells possess specific receptors for altered gamma globulin. The nature of this receptor and the functional significance of these cells is being studied. Lymphocytes of normal mouse spleen possess receptors for specific antigen[9,12] and the present studies indicate that a large number of lymphocytes apparently possess receptors for altered human γ-globulin. It is therefore postulated that for every lymphoid cell possessing receptors specific for any given antigen there are many more with receptors for altered antibody, and it is possible that all antigen reactive lymphocytes possess receptors for altered γ-globulin. If this were so, a recruiting mechanism for induction of the immune response to an antigen would exist. Only a few antigen reacting cells need respond to their specific antigens by producing specific antibody. These antibodies would form complexes with their antigen which would in turn be the means of recruiting other cells carrying immunoglobulin receptors for altered γ-globulin. Lymphocytes carrying these complexes would become localized in the white pulp of the spleen or the cortex of lymph nodes, where they could migrate to the germinal centres. This would explain the mechanism of localization of autologous immunoglobulin in germinal centres. Thus antigen would be brought to areas where it could play a crucial, direct part in regulating the production of new cells capable of producing specific antibodies during their migration from germinal centres to the red pulp of the spleen and medullary areas of lymph nodes. The suppression of localization of aggregated human IgG by antilymphocytic IgG in these studies suggests that the cells involved form part of the recirculating pool of lymphocytes, and are either of thymic origin or are dependent on the presence of an intact thymus[13]. Such a population of cells functioning in the immune response to antigen in the manner suggested could provide a basis both for the mechanism of action of early antibody in enhancing the response to sheep erythrocytes[14] and also for the suppression of the response by antibody[15,16]. The importance of these cells in immune responsiveness to antigens is being evaluated.

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Antibody Response to Tumour Xenografts in Mice treated with Antilymphocyte Serum

Antilymphocyte serum (ALS) has been shown to suppress the cell mediated response to tissue grafts[1], but its effect on the humoral response is less marked[2]. A system for assessing the effect of ALS on the humoral response to xenografts is provided by the human tumour line HeLa grown in mice treated with ALS[3]. We have therefore studied the antibody response to HeLa cells in mice treated with ALS and bearing HeLa cell tumours, using a fluorescent antibody technique.

ALS was prepared in rabbits and assessed as previously described[3]. Sera of adequate potency were pooled and stored at −20° C. Swiss mice were injected subcutaneously with 0·5 ml. of ALS three days before tumour transplantation, and with 0·25 ml. on the day of transplantation and on alternate days thereafter. HeLa cells were maintained *in vitro* as previously described[3]. Mice were injected subcutaneously in the ventral midline with 0·1 ml. of cell suspension, each mouse receiving approximately 5×10^6 cells. Tumour-bearing mice were bled at approximately 5 day intervals from day −3 to day 30; the sera were separated and stored individually at −20° C. Mice were killed on day 30, the tumours excised, snap

Table 1. FREE SERUM ANTIBODIES: FLUORESCENT INDICES

Experiment 1

Day	Regressed tumours Average maximum volume 20 mm: regressed by day 10	Progressively growing tumours Average maximum volume 1,000 mm³; day 30
6	0·1	—
11	—	0·2
17	—	0·2
24	—	0·2
30	—	0·01

Experiment 2: tumours regressing by day 30

Day	Average maximum volume 20 mm³; day 20
6	0·46
10	0·36
17	0·36
24	0·33
30	0·30

frozen, and stored at −20° C until they were examined for antibodies.

Antibodies to pooled normal mouse globulins were prepared in New Zealand white rabbits, and conjugated with fluorescein isothiocyanate[4]. Sections (5 μm) of excised tumour were cut on a cryostat and incubated with rabbit anti-mouse conjugate for 15 min at room temperature. Control sections were incubated with unconjugated rabbit anti-mouse γ-globulin before incubation with the conjugate.

Free serum antibodies were demonstrated using a method adapted from that of Moller[5]. Equal aliquots of viable HeLa cells from *in vitro* cultures were incubated with measured amounts of serum from tumour-bearing mice for 15 min at room temperature. The cell suspensions were washed with balanced saline solution (BSS), incubated with conjugate and washed three times with BSS to remove free conjugate. The number of cells in a total of 500 showing membrane (ring) fluorescence was then estimated for each serum, and the fluorescent index was calculated[6] as the ratio

$$\frac{\text{Percentage unstained cells after incubation with normal serum} - \text{percentage unstained with immune serum}}{\text{Percentage unstained cells after incubation with normal serum}}$$

Control cell suspensions were incubated with serum from tumour-bearing mice and unconjugated anti-mouse serum before incubation with conjugate, with normal mouse serum before incubation with conjugate, or with conjugate alone.

Bound antibodies were apparent on all excised tumours. Cells in the central necrotic areas of the tumours exhibited the diffuse fluorescence characteristic of dead cells, but particulate staining of globulin on the cell membrane was also apparent on these cells and on viable cells at the periphery. Particulate membrane staining on control sections was reduced or absent. Approximately 15 per cent of cells incubated with sera from untreated mice before injection with tumour and ALS gave fluorescent staining. Positive fluorescent indices were obtained with sera from all mice bearing progressively growing tumours; positive fluorescent indices were not, however, obtained with either of the controls. It remains to be determined whether the apparent decrease in free serum antibody titre during tumour growth is the result of an increase in the rate of absorption of antibodies by the tumour, or of the induction of tolerance by ALS, and whether tumour enhancement is occurring.

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Retrogenetic Expression: the Reappearance of Embryonal Antigens in Cancer Cells

VARIOUS reports of embryonal antigens in extracts of tumour tissues have appeared since 1932 (refs. 1–9). Such antigens, absent from homologous normal tissues, have been detected in colonic[6], hepatic[7] and lung cancers[8]. These findings stimulated a study of the possible universal appearance of embryonic antigens in cancer tissues.

The Swiss white mouse was chosen for the investigation for the following reasons: (1) many well characterized tumours are readily available; (2) chemical, viral and radiation induced cancerous lines are easily produced; (3) embryonal extracts from known developmental stages can be prepared with facility; (4) maximum heterogeneity of antigens may be expected in a random-bred strain; (5) test material may be obtained easily and in large quantity.

Antisera have been prepared in the rabbit, using whole mouse embryo extracts as immunizing antigen. Sera were screened by Ouchterlony agar diffusion and immuno-electrophoresis methods[10,11] against normal embryonal, neonatal and adult antigens extracted from the respective tissues, and against seventy-two extracts of malignant cells or tissues from cancerous animals.

Embryos were obtained free of maternal tissue from random bred Swiss white mice (Millerton Research Farms) after various periods of gestation. These were minced, washed in phosphate-buffered saline and homogenized in a VirTis "45" at 35,000 r.p.m. for 2 min. Tumour and normal adult tissues were similarly extracted in three times the wet cell volumes of sal-P (0·15 M NaCl, 10^{-4} M PO_4, *p*H 7·2). The homogenates were centrifuged at 1,500*g* for 10 min and the supernatants were dialysed against three changes of 500 volumes of sal-P and lyophilized. All operations were performed at 4° C.

For immunization the lyophilized embryonal material was resuspended in sterile distilled water at 15 mg/ml., and emulsified with equal volumes of Freund's adjuvant. Subcutaneous injections of 2 ml. each were administered weekly for 3 weeks, using complete adjuvant. Booster injections of 2 ml. were prepared with incomplete adjuvant and administered intramuscularly one month following the first injection. Blood was collected from the marginal ear vein weekly for 4 weeks, starting 10 days after the booster.

The cancer tissue extracts were obtained from seventy-two specimens originating in eighteen different mouse strains. The cancers arose in twelve diverse tissues following induction by viral agents in twenty-one instances (Bittner, Gross, Rauscher and Friend), and by chemical agents in twenty instances (methylcholanthrene, dimethylbenzanthracene, benzpyrene, urethane and stilboestrol); two arose following X-irradiation and twenty-nine of "spontaneous" origin were obtained from various laboratories[12–20].

Rabbit antisera to mouse embryo extracts prepared on the ninth to the nineteenth day of gestation all produced double-diffusion precipitin lines in agar with extracts of normal adult tissues as well as with extracts of tumour tissues. A composite extract of five organs (heart, lung, spleen, liver and kidney) from two female and two male adult mice was used to absorb the sera. The composite extract-absorbed serum from a rabbit immunized with 19 day embryo extract, designated AS-19, reacted to produce precipitin lines with the extracts of all seventy-two cancers, but failed to react with extracts of mouse L-cells

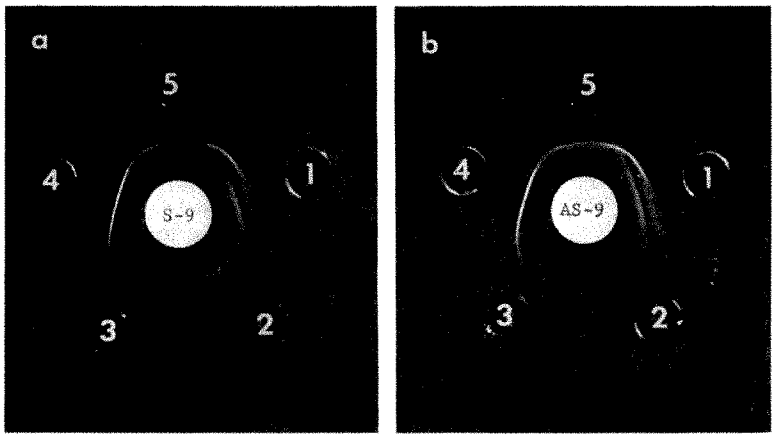


Fig. 1. Double diffusion precipitin reactions in agar gels. Immune sera in centre wells, antigen extracts in peripheral wells. 1, Embryo, 19 days; 2, spleen, normal Swiss white; 3, liver, DBA/2, solid tumour-bearing animal; 4, spleen, Swiss white leukaemic mouse (Friend); 5, skin, normal Swiss white. Wells of Micro-Ouchterlony plates (7 mm well spacings, Hyland Labs.) each received 0·005 ml. Antigens were adjusted to 10 mg protein/ml. for the tests. *a*, Unabsorbed anti-embryo immune serum; *b*, immune serum absorbed with adult extract.

and with any of the normal adult tissue extracts tested, except skin, small intestine and regenerating liver. Because the 19 day embryo contains a higher proportion of epithelial tissue than does the 9 day embryo, this would be reflected in the extracts. Accordingly, serum S-9, from a rabbit immunized with 9 day embryo extracts, was used in subsequent work. After absorption with adult antigen (composite extract) this antiserum (AS-9) was found to interact only with extracts of adult skin, the seventy-two cancer extracts, and with embryonal and neonatal extracts (Fig. 1).

Embryo-specific murine antigens reappear in adult cells following malignant conversion. These antigens were not detected in normal cells with one exception, but have been demonstrated in a variety of murine strains in which malignant transformation had been induced by viruses, chemicals or radiation, or by some unknown "spontaneous" event. These results suggest that, at least in the mouse, the reappearance and the persistence of embryonal antigens in malignant cells are universal phenomena.

Embryo-specific gene products have been identified in various tissues of cancer patients[1–8,21,22] and in polyoma virus transformed cells[23]. Embryonal polymerase activity has been described in tumours of the adult chicken[9]. Antibodies to embryonal antigens have recently been demonstrated in the sera of cancer patients[24]. These and other similar findings indicate disturbances of gene control which may be considered examples of retrogenetic expression. This is manifested by the acquisition of properties abnormal for the adult cell, such as the appearance of embryonal gene products and unrestrained growth.

Assuming the totipotency[25] of differentiated adult cells which contain an intact nucleus[26], a random disturbance in the established gene constellation may activate some dormant foetal function. This will occur with increasing probability as the ratio of embryonic to adult genes increases. If this ratio is of the order of 10^4 in mammalian cells it would be difficult to identify an unbalanced cell that lacked embryonic antigens. That no such cell line was identified among the seventy-two specimens examined in the present study is consistent with the earlier conclusion that one consequence of oncogenesis is the reappearance of embryo-specific gene products.

The completion of embryogenesis and regeneration, both proliferative phenomena, are marked by the repression of certain gene functions. The syntheses of the antigenic gene products common to both embryo and tumour tissues are each handled differently; it appears that the tumour has acquired the high proliferative activity of the embryo without its shut-off mechanisms.

The most serious dilemma concerns the occurrence of embryonal antigens in tumours and in normal skin. It may be that the tumour and embryonal antigens are not entirely identical and may yet be distinguished from those in the skin extracts when analytical procedures of greater resolution are used. Insight may be gained when the antigens involved are identified biochemically, for they may be products of derepression or they may themselves have derepressive activity.

It cannot be determined from the present investigation whether retrogenetic expression is a cause or an effect of cancerigenesis. The oncogenic event disturbs the existing controls and establishes an aberrant arrangement of activated genes characteristic of the malignant cell. What constitutes the control factors, and what controls these, remains a speculative issue.

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Investigation of Leucocytes from Chickens with Marek's Disease

THERE is evidence that the aetiological agent of Marek's disease is a herpes-type virus[1–4], which has been propagated successfully in chicken embryo fibroblasts (CEF), duck embryo fibroblasts (DEF) and chick-kidney cells (CKC)[1,5,6]. Virus-like particles have been found in tumours, peripheral leucocytes and plasma pellets from birds with Marek's disease[7,8]. We have made electron microscopic, fluorescent antibody and bioassay studies of leucocytes from chickens with Marek's disease.

Blood (30 ml.) collected from chickens infected with the Conn-A[9], Conn-B[10] and JM[11] strains of Marek's disease and showing clinical signs of Marek's disease was placed in centrifuge tubes containing 1,000 U of heparin. The same quantity of blood was collected from uninfected birds, identified as such by histopathological examination of brain, sciatic nerve and spinal cord. Leucocytes were separated either by leaving the blood for 2 h at 4° C, after which the plasma leucocyte suspension was removed with a pipette, or by the method of Vallee *et al.*[12]. Cells were washed twice in Tris-buffered saline and then resuspended in Eagle's basic medium with 20 per cent calf serum and 10 per cent chicken serum free of Marek's disease antibodies as determined by the agar gel precipitin test[13].

Between 3 and 4×10^6 cells/ml. in 60 ml. of medium of infected cells were placed in one side of a double sidearm spinner flask (membrane type) (Bellco Glass Inc., Vineland, New Jersey), separated by a 45 μm filter, while the other side received the same number of uninfected cells. A separate flask with uninfected cells served as a control. The spinner flasks were placed on a multi-stir magnetic stirrer adjusted to 90 r.p.m. and kept at 37° C. Infected and uninfected cells were bioassayed before incubation by inoculating 4 day old COFAL negative chicks with 0·05 ml. of cell suspension intracerebrally. Chicks were isolated for 2 weeks, then killed and examined for Marek's disease lesions as described before[5]. Subsequent samples were taken after 12, 24 and 48 h from both sides of the spinner flask for further bioassay in 1 day old chicks, fluorescent antibody studies and electron microscopic examination. To test the integrity of the separating filter, *Salmonella typhimurium* was added to one side of the spinner flask at the end of the experiment and a bacteriological examination was made on each side of the flask 12, 24 and 48 h later.

Antisera used in fluorescent antibody studies were prepared in rabbits against plasma pellets from heparinized infected whole blood prepared as described earlier[8]. Rabbits received eight injections, four intraperitoneally with complete Freund's adjuvant; two intravenously, and two intramuscularly with no adjuvant. They were bled 12 days after the last inoculation. Rabbit serum was absorbed twice for 12 h at 4° C with leucocytes from birds free of Marek's disease. Different batches of cells of the same origin were used each time. Serum was then centrifuged at 10,000*g* for 20 min and used in the indirect staining method. Culture medium from the spinner flask was centrifuged at 400*g* for 5 min.

Cells were washed twice with saline, resuspended in fresh buffer to a concentration of $1{\cdot}0 \times 10^6$ cells/ml., smeared on slides, allowed to dry, and fixed in acetone for 8 min. A drop of rabbit serum diluted 1 : 10 was placed on the smear, allowed to react for 30 min at 37° C, washed and reacted to fluorescein-tagged anti-rabbit globulin. The number of stained cells out of approximately 500 cells was recorded and the percentage of positive cells calculated.

For electron microscopic studies, cell pellets were covered with 1 per cent glutaraldehyde, dehydrated in alcohol and embedded in 'Epon'. Thick sections (0·5 μm) were cut, stained with methylene blue, and observed under the light microscope, while thin sections were examined under the electron microscope.

In vitro infected cells had a faster metabolic rate, indicated by changes in *p*H, but counts revealed no significant multiplication. Infected cells also tended to clump after 12 h in culture, and so did uninfected cells after about 24 h in the two-sided spinner flask, but not in the single sidearm flask.

Table 1. INFECTIVITY OF WHITE BLOOD CELLS FROM MAREK'S DISEASE AND NORMAL CHICKENS FOR 1 DAY OLD CHICKS AFTER BEING CULTURED IN SPINNER FLASKS SEPARATED BY A 45 μm FILTER

Experiment	Marek's disease isolate	White blood cells	h of postcultivation 0	12	24	48
(1)	Conn-A	MD	4/4*	NT†	4/4	2/4
		Normal	0/4	NT	3/4	2/4
(2)	JM	MD	3/4	NT	2/4	1/4
		Normal	0/4	NT	2/4	0/4
(3)	Conn-B	MD	4/4	NT	4/4	4/4
		Normal	0/4	NT	4/4	3/3
(4)	Conn-B	MD	4/4	4/4	NT	NT
		Normal	0/4	4/4	NT	NT

* Number of chicks with lesions/total necropsied.
† Not tested.

Four sets of results for the bioassay of the double sidearm spinner flask cultures are shown in Table 1. Marek's disease agent apparently crossed the filter and infected the normal white blood cells. Fluorescence revealed that the number of immunofluorescent-positive cells increased during culture and that the percentage of positive cells continued to change for 48 h. When cells that had clumped were isolated and stained, 95 per cent of them were immunofluorescent-positive. Only 1–2 per cent of uninfected cells were weakly fluorescent before culture, increasing to 30 per cent at the end of 48 h, while never more than 1 or 2 per cent of cells kept in separate flasks were positive. The results of fluorescent antibody tests are summarized in Fig. 1.

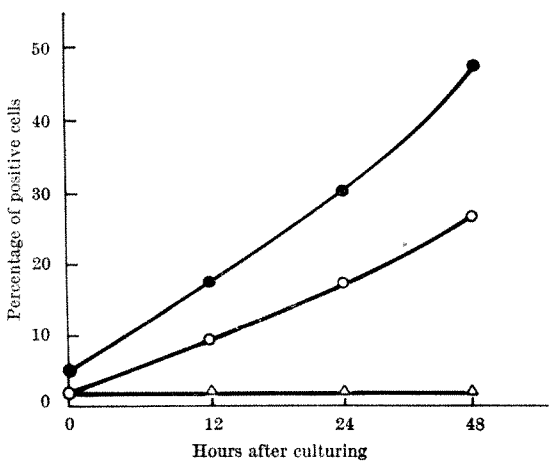


Fig. 1. Proportion of immunofluorescent-positive cells during the first 48 h in culture. ○, Side of flask with infected cells; ●, side of flask with uninfected cells; △, uninfected cells in a separate flask.

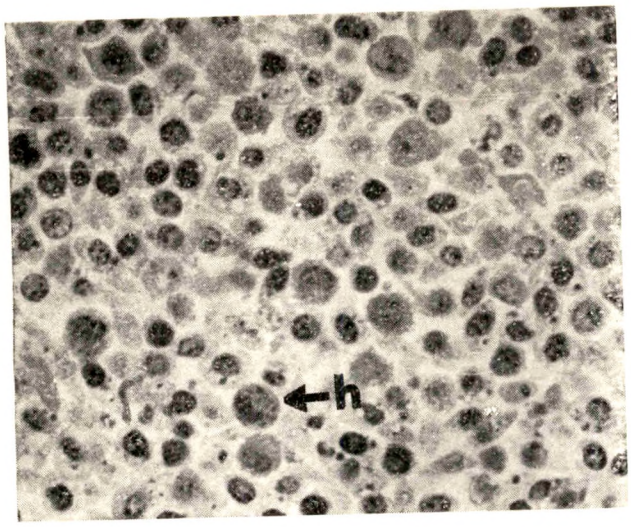


Fig. 2. Light micrograph showing a thick section from infected suspension culture. Note the number of heterophiles (h). (×160.)

When thick sections were viewed under the light microscope, most cultured cells looked similar (Fig. 2). Fresh leucocytes had no special morphological features and only after being put in culture did they change. Most cells were rounded, with round and irregular nuclei, with no cytoplasmic or nuclear inclusions.

Under the electron microscope, infected cells cultivated *in vitro* were undifferentiated with irregular nuclei, enlarged mitochondria or variable forms, sometimes containing filaments. These were cells of the heterophile type, characterized by large cytoplasmic granules and containing unidentified particles 120 to 150 nm across (Fig. 3).

Marek's disease agent has been reputed to be closely associated with cells[1], but in this study the agent seemed to cross the filter membrane and infect normal leucocytes. It is possible that all previous attempts to transmit Marek's disease with cell-free material have failed because of the methods used.

Again freshly isolated cells had no special morphological features and only changed when in culture. Infected cells clumped after at least 12 h in culture. This property has been observed in abnormal human leukaemia cells[14].

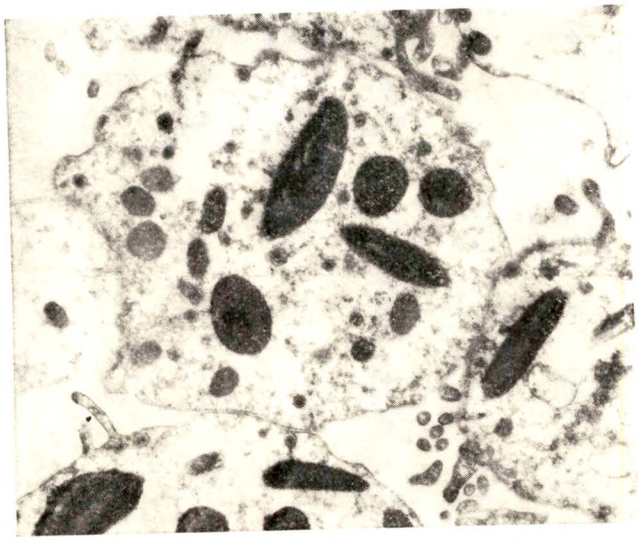


Fig. 3. Thin section of heterophile cells showing unidentified particles and long cytoplasmic projections. Infected cell culture 24 h in suspension. (×11,000.)

DeHarven[15] noted that cells that formed clumps were characterized by tight interdigitation of cytoplasmic processes. Our findings suggest no explanation for this, but because it is only an affinity displayed by infected cells, infectivity must be a contributory factor. The increase in fluorescent cells was also observed in normal leucocytes cultivated with infected cells separated by the membrane. The antigen demonstrated by immunofluorescence seemed to correlate with infection. Similar observations have been reported with Burkitt's lymphoma[16]. Work with human leukaemia cells has indicated the presence of virus particles in degenerating cells[17]. Herpes-type virus has also been suggested in frog tumours with and without outer envelopes with uncoated particles predominating[18–20]. It is interesting to note the similarity in the behaviour of the infected cultures *in vitro* (clumping, fast metabolic rate and increase in immunofluorescent-positive cells) to that described for human leukaemia cells. Further studies with chicken leucocytes infected with Marek's disease could be of great importance in understanding lymphoproliferative disorders in other species.

A most striking observation was the presence of particles exclusively to heterophile cells. Dhingra and co-workers[21] described the heterophile of birds and reptiles as the equivalent of the neutrophile in mammals. Approximately 200 thin sections of infected leucocytes were studied and only a few lymphocytes were observed to contain these particles.

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Effects of an Imprinting Procedure on RNA Polymerase Activity in the Chick Brain

Exposure to an imprinting stimulus has been shown to be associated with an increase in incorporation rates of lysine into protein and uracil into RNA in the forebrain roof in one-day-old chicks' brains[1,2]. A maximum increase in RNA incorporation is seen at 76 min after the start of

the imprinting procedure and in protein incorporation at 120 min. Studies of the changes occurring in other protein synthesizing systems in response to external stimuli such as hormones have demonstrated a similar type of temporal sequence for the protein and RNA changes. In hormonal systems one of the events preceding both of these is an increase in RNA polymerase activity[3]. It seemed reasonable to test whether such an effect also occurs during imprinting in the chick. We present here preliminary results of an enquiry into the effects of the imprinting stimulus on the activity of RNA polymerase in three regions of the chick's brain.

Three batches of 'Chunky chicks' were hatched and kept in a dark incubator until they were 21–27 h old. In each batch six chicks were separately exposed for 30 min to a flashing light previously found to be highly effective as an imprinting stimulus[4]. Meanwhile six other chicks were kept in dark pens for the same length of time and at the same temperature as the experimental birds (28°–31° C). Immediately after exposure to experimental or dark control conditions the responses to the flashing light were measured for 2 min in each chick. It was then decapitated, its brain removed and dissected into three regions (forebrain roof, forebrain base and midbrain) which were frozen separately in pots on solid CO_2 and kept at −20° C until assay. The dark control birds were likewise tested and killed, their brain sections being frozen and kept for analysis.

The nuclei were removed and collected from each sample by a modification of the method of Mertelsmann[5]. The samples were homogenized separately in 4·5 ml. of medium (HM) containing 0·41 M sucrose, 1 mM $MgCl_2$, 0·4 mM KH_2PO_4, 0·4 mM K_2HPO_4, 5 mM mercaptoethanol (*p*H 6·5) for 45 s at 0–4° C, using an MSE top-drive macerator. The homogenates were filtered through two layers of 110 gauge nylon bolting cloth and the filtrate centrifuged at 2,000*g* for 10 min at 0° C. The pellets were resuspended in 4·5 ml. of HM containing 0·5 per cent 'Triton X-100' by means of a glass hand homogenizer fitted with a loose Teflon pestle. These suspensions were then centrifuged at 2,000 *g* for 10 min, the crude nuclear pellet was resuspended in 10 mM Tris buffer (*p*H 7·8), 10 mM $(NH_4)_2SO_4$, 6 mM $MgCl_2$, 1 mM disodium EDTA, 5 mM mercaptoethanol and 33 per cent of glycerol (buffer A), and the suspension centrifuged at 2,000*g* for 10 min. The final nuclear pellets were resuspended in 0·5 ml. buffer A and checked for purity under the microscope. The assays of RNA polymerase activity were performed within 3 h of the final suspension being prepared to avoid "ageing" the nuclei.

All the assays of enzymic activity were carried out in triplicate. 100 μl. aliquots of the nuclear suspensions of each sample were incubated at 37° C for 5 min in 400 μl. of assay medium containing 0·5 mM of each of ATP, CTP, GTP and UTP, and 0·167 μCi 8-^{14}C-ATP; 300 mM $(NH_4)_2SO_4$, 5 mM $MgCl_2$, 5 mM mercaptoethanol; 100 mM Tris buffer (*p*H 8·5), as used by Kato and Kurokawa[6]. Control experiments showed that, first, the reaction was linear beyond 5 min incubation time; second, the amount of radioactivity incorporated was directly proportional to the quantity of nuclei present as measured by DNA; third, the substrates were not rate-limiting. The reactions were stopped by the addition to each tube of 0·5 ml., 10 per cent trichloroacetic acid (TCA) containing 2 M sodium pyrophosphate at 0° C and the precipitates filtered onto Whatman GF/C glass fibre filters. These were washed successively with 5 per cent TCA, chloroform–ethanol (3:1) and ether, and the filters counted in a butyl-PBD–toluene–methoxyethanol scintillant. A 100 μl. aliquot was taken from each nuclear suspension and its DNA content determined[7]. The incorporation of radioactive nucleotide into the recovered RNA was expressed as the average of d.p.m. incorporated per μg of DNA present in the assay.

To eliminate batch differences between experiments the samples from one dark control brain were processed in parallel with those from the brain of an experimental bird. The individual values for incorporation from each sample within such a dark-experimental pair were then calculated as percentages of the mean derived from all the results of that pair. These values were pooled and their means, with standard errors, calculated. The brain regions of the experimental group were then compared statistically (Student's *t* test) with the equivalent regions in the dark control chicks (Table 1).

Table 1. RNA POLYMERASE LEVELS IN BRAIN REGIONS OF EXPERIMENTAL AND CONTROL BIRDS

Condition	Forebrain roof (per cent)	Forebrain base (per cent)	Midbrain (per cent)
Experimental	110·3±7·6 (n=15)	124·3±8·6 (n=17)	109·6±8·6 (n=17)
Dark	82·4±7·9 (n=17)	102·4±9·3 (n=17)	97·6±7·3 (n=17)
$e/d \times 100$	133·9	121·3	112·3
$P \leq$	0·02	0·09	0·30

A marked increase in RNA polymerase activity was observed in the forebrain roof of the experimental animals over that of the dark controls after 30 min exposure to the imprinting stimulus. The effect was significant in the same region of the brain, the forebrain roof, as were the increases in RNA and protein[1,2]. It was observed after an exposure to the flashing light briefer than that previously found to be necessary to obtain detectable increases in incorporation into RNA (76 min). Compared with the 34 per cent increase in the forebrain roof, there were increases of 21 and 12 per cent in the forebrain base and midbrain, respectively. This enhancement of RNA polymerase activity is reminiscent of the sequence of events which follow the stimulation of tissues by hormones[3] and is compatible with the model of intraneuronal events consequent on stimulation by an environmental "trigger" recently proposed by one of us[8]. We believe that this is the first report of an increase in RNA polymerase activity after behavioural stimulation. Because exposure to a static overhead light eventually leads to an increase in RNA synthesis in the chick brain[2]—although taking longer to do so than a flashing light—the change recorded here may be the result of non-specific visual stimulation. Further work is necessary to elucidate the details both of the specificity and the time course of the enhancement of enzyme activity.

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Motion of Spermatozoa in Fluid Streams

It is generally accepted that spermatozoa constrained to move in a fine tube swim preferentially upstream (they exhibit positive rheotaxis) when a current is established in the tube[1]. Rothschild[2] has shown that the orientation of dead sperm depends on the direction of the local velocity gradient (Fig. 1); because the head of the spermatozoon sinks more rapidly than the tail under gravity, organisms in the bottom half of a horizontal tube point head upstream while those in the top half point head downstream. Thus it was not at all clear why live spermatozoa should tend on average to swim upstream[2].

Any mechanism which could cause live spermatozoa to migrate to the lower region in a velocity gradient could account for their behaviour in fluid streams. I have therefore investigated the motion of bull sperm in horizontal glass tubes, 200–300 μm in diameter, with a microscope arranged with its axis horizontal so that motion in the vertical plane could be examined. Fresh bull semen was diluted (1 : 100) with Rothschild's medium[3]. Marked positive rheotaxis was observed for velocity gradients of 2–15 s^{-1}. Most organisms swimming upstream were found in the bottom half of the tube, although a few were observed swimming upstream in the upper half. Spermatozoa swimming downstream in the upper half could be seen sinking slowly and turning round when they reached the bottom half. Some active mechanism is therefore producing a downward migration of spermatozoa in the presence of velocity gradients.

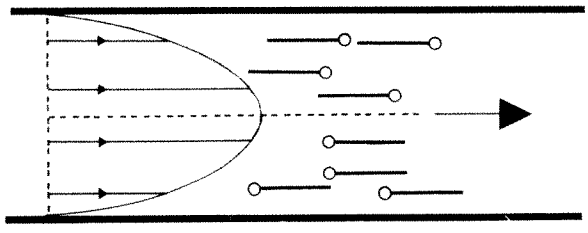


Fig. 1. The steady-state orientation of dead bull sperm when a current is established in the medium. Spermatozoa in the lower half of the tube align themselves head upstream.

Although it has been shown that spermatozoa tend to move preferentially downward (they exhibit positive geotaxis)[4,5] no estimate has been made of the magnitude of the effect. I have investigated the distribution of non-interacting randomly moving aquatic microorganisms under gravity[6], and shown that the vertical distribution in an undisturbed medium is in general exponential, with a scale height, δ, given approximately by

$$\delta = u/(2\beta) \qquad (1)$$

where u is the mean swimming velocity and β is a measure of the rate at which an immobilized organism, falling under gravity, reaches its equilibrium position (in spermatozoa, head downwards). β is defined by the relation

$$d\theta/dt = -\beta \sin\theta \qquad (2)$$

where θ is the angle between the long axis of the organism and the vertical. I have found that β for bull sperm in the diluted medium used in these experiments lies in the range 0·02–0·03 rad s^{-1} at 22° C. Assuming an average swimming velocity of 50–100 μm s^{-1} the scale height of a sperm suspension should be about 1–2 mm, that is, about 63 per cent of the sperm should be found in the bottom layer of this thickness. In practice no sedimentation is observed in dense suspensions of active sperm; because the average distance between one sperm and its nearest neighbour may be as small as 10 μm, compared with 50 μm for the sperm length, direct interactions between organisms are obviously important, and will increase the effective scale height of the distribution many times. Thus it is unlikely that positive geotaxis can account for the number differences over a height of 200–300 μm.

When a velocity gradient is established in the medium, spermatozoa will be influenced both by the gravitational torque (tending to align it vertically downwards) and the flow-orientation torque (tending to align it in the direction of the streamlines). If the velocity gradient is sufficiently large, the motion of the spermatozoa will no longer be random, and they will tend to swim in the direction of the resultant of the two torques. For a horizontal tube the downward velocity component, w, is given by

$$w = (u\beta)/\beta_v$$

where β_v is the flow-induced orientation constant defined by an equation similar to equation (2). Data[2] suggest that β_v for bull sperm is about 0·3 rad s^{-1} per unit velocity gradient. Thus in a velocity gradient of 3 s^{-1} the downward velocity is in the region 1–3 μm s^{-1}, which is an order of magnitude greater than the downward falling velocity of immobilized spermatozoa. Thus most sperm in a 300 μm diameter tube will have migrated to the lower region in about 1 min. If the velocity gradient is increased the downward migration velocity decreases, and rheotactic behaviour becomes less marked. If the velocity gradient is decreased, the spermatozoa tend to regain their normal random movement, and the downward migration again ceases. The explanation of positive sperm rheotaxis in a thin tube is that most spermatozoa which exhibit this phenomenon are in the lower half of the tube where the sense of the velocity gradient imposes this type of behaviour on them.

It is not certain whether this mechanism plays any part in directing mammalian spermatozoa along the female

reproductive tract. Vandemark and Moeller[7] found that immobilized spermatozoa appeared in the ovarian region of the bovine oviduct less than 5 min after insemination, suggesting that other mechanisms may be involved in sperm transport. Belve and McDonald[8] have shown that the net amount of fluid flow in the oviduct of the ewe is generally away from the uterus, which may explain the passage of non-motile sperm up the tube. If the cilia of the oviduct are responsible for transporting the ovum to the uterus, fluid velocity gradients will be set up at the walls which could initiate rheotaxis up the tube even if the net fluid flow is in the same direction. Because the ovum might also be expected to travel along the bottom of the oviduct in its passage to the uterus, the downward migration of spermatozoa in velocity gradients may be a mechanism by which the ovum and motile spermatozoa are brought together by purely mechanical means. In weightless conditions this guiding mechanism is inoperative, and it should be possible to assess its importance by conducting experiments in these conditions.

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Mechanism of Action of Luteinizing Hormone

The mechanism by which luteinizing hormone stimulates the synthesis of ovarian steroid hormones is the subject of controversy. McKerns[1] has proposed that a primary action of this gonadotrophin in rat lutein and bovine corpus luteum cells is to raise the rate of production of reduced nicotinamide–adenine dinucleotide phosphate (NADPH) by a direct stimulation of glucose-6-phosphate dehydrogenase. Savard *et al.*[2] have concluded that luteinizing hormone stimulates steroid production by an effect on protein synthesis following an increase in the intracellular concentration of cyclic-3′,5′-adenosine monophosphate. If luteinizing hormone acts by increasing the rate of production of NADPH by glucose-6-phosphate dehydrogenase (or some other cytoplasmic NADP-linked dehydrogenase) it should be possible to demonstrate a rise in the tissue concentration of NADPH when steroid synthesis is stimulated. We have made parallel measurements of the concentrations of oxidized and reduced pyridine nucleotides, and the rates of steroid synthesis in lutein tissue taken from rats before and after treatment with luteinizing hormone.

Slices of the luteinized ovaries from immature Wistar rats treated with pregnant mare serum gonadotrophin and human chorionic gonadotrophin[3,4] were incubated for 1 h at 37° C in Krebs–Ringer bicarbonate buffer containing ^{3}H-water and $^{14}C_6$-sorbitol with or without glucose (1 mg/ml.). Luteinizing hormone (NIH-LH-SII) was administered intraperitoneally (20 μg/ 100 g body weight) in saline 2 h before autopsy; control animals received saline only. Net *in vitro* synthesis of progesterone and 20α-hydroxy-pregn-4-en-3-one was determined as described previously[5], the mass of total Δ^4,3-oxo-steroids being measured on the basis of their ability to form thiosemicarbazides with high extinction coefficients at 302 nm. Oxidized[6] and reduced[7,8] pyridine nucleotides were assayed fluorimetrically in acid and alkaline extracts of tissue slices after incubation. The slices were frozen for extraction in liquid nitrogen and extracts were made in Kontes tissue grinders (Kontes Glass Co., Vineland, New Jersey). Intracellular water spaces were calculated from the ^{3}H-water and $^{14}C_6$-sorbitol contents of the tissue slice extracts as described previously; the values obtained have been used in calculating the intracellular concentrations of the pyridine nucleotides. Previous investigations have indicated that many parameters of both glucose metabolism and lipid metabolism proceed at steady state rates in the incubation conditions used in this study[4,5].

Table 1. EFFECTS OF GLUCOSE *in vitro* AND LUTEINIZING HORMONE *in vivo* ON THE CONCENTRATIONS OF PYRIDINE NUCLEOTIDES IN, AND RATES OF STEROID SYNTHESIS BY, SLICES OF RAT LUTEIN TISSUE

Luteinizing hormone *in vivo:*	−	−	+
Glucose *in vitro:*	−	+	+
NADPH*	68·4 ± 6·8 (4)	112·8 ± 8·6‡ (8)	113·2 ± 3·3 (4)
NADP+	72·9 ± 6·8 (4)	26·9 ± 2·9‡ (8)	28·8 ± 1·5 (4)
NADH	29·1 ± 2·9 (4)	85·2 ± 10·6‡ (8)	83·2 ± 4·7 (4)
NAD+	411·8 ± 25·9 (4)	424·4 ± 16·6 (8)	377·2 ± 20·8 (4)
Rate of steroid synthesis†	0·21 ± 0·02 (4)	0·20 ± 0·01 (4)	0·39 ± 0·03§ (4)

* Intracellular concentrations of pyridine nucleotides are expressed as μM (means ± s.e. for number of determinations given in parentheses).
† Rates of steroid synthesis are μmoles/g/h (means ± s.e. for number of determinations given in parentheses).
‡ $P < 0.005$ for effects of glucose *in vitro*; for other changes, $P > 0.1$.
§ $P < 0.005$ for effects of luteinizing hormone *in vivo*; for other changes, $P > 0.1$.

The pyridine nucleotide concentrations in tissue slices and the corresponding steroid release rates are shown in Table 1. Glucose *in vitro* raised the intracellular concentrations of NADPH and NADH, and lowered that of NADP+, but had no effect on the rate of steroid synthesis. No change was detected in the relatively high concentration of NAD+. Incubation with glucose raised the intracellular concentration ratios NADPH/NADP+ and NADH/NAD+ by factors of 4·4 and 2·9 respectively. By contrast with the effect of glucose *in vitro*, pretreatment of the tissue with luteinizing hormone *in vivo* significantly increased the rate of steroid synthesis but had no effect on the concentrations of the pyridine nucleotides. Thus trophic stimulation of steroid synthesis can occur in the absence of any change in the intracellular concentrations of oxidized and reduced pyridine nucleotides, and a stimulation of steroid synthesis does not always follow an increased availability of NADPH.

It has been suggested[9] that the NADPH produced in response to gonadotrophic stimulation may be localized in a compartment occupying a small fraction of the total cell volume, and that local changes in NADPH concentration may not be detectable on extraction of the whole tissue. This now seems unlikely, for if the ultimate source of mitochondrial NADPH is glucose-6-phosphate dehydrogenase (a cytoplasmic enzyme[10]) a rise in the mitochondrial NADPH concentration would involve a rise in the concentration of NADPH in the cytoplasm. Mitochondrial NADPH is required because the enzyme system responsible for converting cholesterol to steroids (the rate limiting step in steroid synthesis) is in the mitochondria[11]. We therefore conclude that a change in the concentration of cytoplasmic NADPH is not involved in the primary steroidogenic response of rat lutein tissue to luteinizing hormone. A similar conclusion was reached regarding the effect of adrenocorticotrophin on the adrenal cortex from measurements of the levels of oxidized and reduced pyridine nucleotides in adrenals from intact and hypophysectomized rats[12]. It also seems unlikely that such a change is involved in the other stimulatory effects of

luteinizing hormone observed in rat lutein tissue, such as those on the incorporation of acetate into sterol and the metabolism of glucose[5].

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Separation of a Hyaluronate-free Lubricating Fraction from Synovial Fluid

THE lubricating qualities of synovial fluid from animal joints have been ascribed to the presence of large hyaluronate or hyaluronate–protein complexes[1], although it has been questioned whether the protein was in fact an integral component of these macromolecules[2]. It is also uncertain whether the high relative viscosity of synovial fluid is important in building up a boundary layer of lubricant on the cartilage surface[3]. Some investigators suggest that the boundary layer is chemically bound, and that the viscosity of the lubricant is of little importance in the lubrication at the cartilage to cartilage interface[4,5].

The finding that mild hyaluronidase digestion, which reduces the viscosity of the joint fluid to that of serum, does not destroy the lubricating qualities of the synovial fluid in joints[6] favours the hypothesis that viscosity is not a determining factor in the production of a boundary lubricating layer. Although in these experiments mild hyaluronidase digestion did not destroy joint fluids lubricating advantage over buffer, severe and complete digestion with this enzyme ('HSE', Worthington, Freehold, New Jersey) did. Furthermore, tryptic digestion, which left the hyaluronate and therefore the viscosity of the fluid intact, also totally destroyed the lubricating qualities of the synovia[6], suggesting that protein might be essential in the lubricating action of joint fluid.

The implications of the hyaluronidase digestions were, however, unclear and the specificity of the hyaluronidase preparation was questioned. Worthington HSE has displayed some inconstant proteolytic activity. We therefore repeated this experiment with a purer form of the same enzyme, Worthington HSEP (6,000 USP units/mg) and also hyaluronidase obtained from Leo (Hälsongborg, Sweden, 20,000 IU/mg), in the same conditions as initially reported. These experiments showed that although 90 per cent of the hexuronic acid[7] was dialysable after treatment with enzyme, the protein content[8] and lubricating quality[9,10] of the synovial fluid were the same after digestion. In an earlier study, low molecular weight hyaluronate solutions were markedly inferior to synovial fluid as joint lubricants[11]; this was now confirmed using a high molecular weight hyaluronate[12] (molecular weight 20 million).

To test whether a protein fraction could be the active lubricant in joint fluid, the protein from the hyaluronate of joint fluid had to be separated without disrupting the physicochemical characteristics of the component macromolecules, for harsh separation would destroy all the lubricating qualities of the constituents. Density gradient sedimentation equilibration[2] was therefore carried out on cell-free bovine synovial fluid using a caesium chloride density gradient centrifugation at an initial density of 1·65 g cm^{-3}. The solution was centrifuged in a Spinco model L preparative ultracentrifuge at 46,000 r.p.m. for 64 h in a number 50 rotor. Then 1 ml. fractions were collected from each tube through a hole pierced in their bottoms. The corresponding fractions from each tube were combined and aliquots analysed for density, hyaluronic acid[7] and protein content[8]. Typical results are shown in Fig. 1. All the hyaluronate banded in the middle of the gradient at a density of 1·63 g cm^{-3}, compared with 1·66 g cm^{-3} reported by Silpananta *et al.*[2]. Most of the protein was present in the top layer of the gradient.

These fractions were combined to obtain three test lubricants as indicated in Fig. 1. Friction tests were carried out as described[9,10], after the three test lubricant solutions had been extensively dialysed against 0·15 veronal buffer (*p*H 7·2). Test lubricant A contained no hyaluronate; B contained all the hyaluronate present in the original joint fluid; C contained most of the protein present in the original synovial fluid and was hyaluronate-free. Test lubricants A and B showed no lubricating advantage over buffer; C, at the equivalent dilution, had the same lubricating qualities as whole synovial fluid (Table 1). Although the frictional values are consistent within each joint, they vary from joint to joint so that these data must be considered relative rather than absolute.

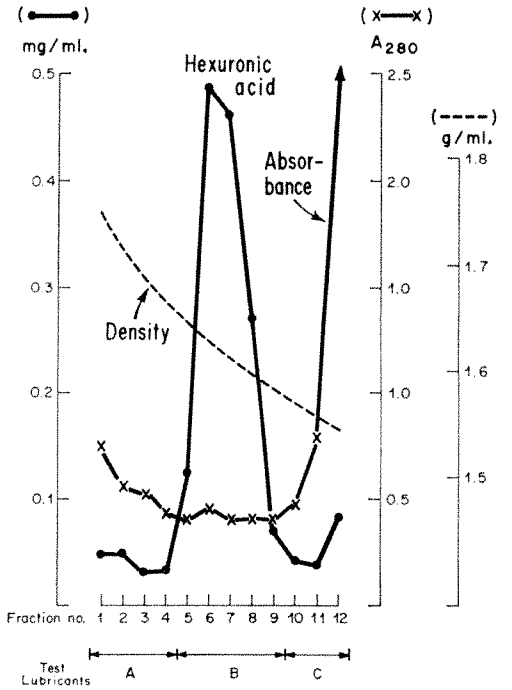


Fig. 1. Results of a density gradient sedimentation equilibrium done in cell-free bovine synovial fluid. The composition of the test lubricants is shown.

Table 1. COEFFICIENTS OF FRICTION OBTAINED WITH TEST LUBRICANTS IN BOVINE METATARSAL-PHALANGEAL JOINTS

	Joint 1	Joint 2	Joint 3
Synovial fluid	0·0021	0·0014	0·0039
Buffer	0·0046	0·0031	0·0079
Test lubricant A	0·0045	0·0033	0·0077
Test lubricant B	0·0046	0·0030	0·0077
Test lubricant C	0·0023	0·0015	0·0039

Conditions were 40 cycles/min; 250 lb load; 23° C.

The joint lubricating fraction of bovine synovial fluid is therefore a protein or glycoprotein which is free of hyaluronate. Because bovine serum fails to display similar lubricating abilities[11], it is assumed that this protein is specific to synovial fluid. Further characterization of this fraction is under way.

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Branchial Reduction and Renal Stimulation of (Na^+, K^+)-ATPase by Prolactin in Hypophysectomized Killifish in Fresh Water

THE discovery, in 1959, that prolactin enables hypophysectomized killifish (*Fundulus heteroclitus*) to live in fresh water[1,2] stimulated much investigation of the osmoregulatory functions of this hormone in euryhaline teleosts[3]. The presence of prolactin, or its teleostean equivalent (paralactin), is necessary for freshwater survival in many euryhaline cyprinodonts and in some unrelated species. In others, such as the eel (*Anguilla anguilla*), prolactin participates less in electrolyte homeostasis, and hypophysectomized fish of such species can withstand fresh water. Maetz and his associates[4–6] have shown that a primary osmoregulatory function of prolactin is the reduction of extrarenal sodium outflux which is uncontrolled, or poorly controlled, after removal of the hypophysis. This becomes critical when a prolactin-dependent species, such as *F. heteroclitus*, is transferred to fresh water.

Recent investigations have implicated Na^+, K^+-activated ATPase in the osmoregulatory functions of euryhaline teleosts[7–11]. Adrenal cortical function contributes to the regulation of this enzyme[12] but, because cortisol cannot maintain freshwater survival in hypophysectomized *F. heteroclitus*[1], its osmoregulatory function in these circumstances, and in this species, is of secondary importance. We have therefore studied the effects of prolactin.

Male *F. heteroclitus*, maintained in salt water at about 15° C, were hypophysectomized in November 1969, and autopsied in February 1970. Because hypophysectomized *F. heteroclitus* fail after 5–7 days in fresh water[13], and are extremely sensitive to the stress of handling, careful planning was imperative. Control fish were transferred to dilute salt water (about 1·5 per cent salinity) for 24 h, and then to dechlorinated city water, changed daily, for three days. They were not injected with solvent, in case of early death from stress. The experimental group received five daily injections of ovine prolactin (NIH-P-S4, a preparation successfully used in an earlier investigation[2]); the dose was 5 μg (= 105 mU) per g per injection, in a volume of 0·01 ml. of 0·6 per cent NaCl. The first injection was given while the fish were in salt water, the second in diluted salt water, and the last three in fresh water. Experience has shown that prolonged pretreatment in salt water is no advantage in promoting freshwater survival. All fish were fed daily, except on the day of autopsy. Both control and experimental groups were staggered so that not more than sixteen fish were autopsied on any one day, which enabled us to do enzyme assays within a few hours.

At autopsy a small volume of blood was taken in a heparinized haematocrit tube. The remainder, in non-heparinized tubes, was allowed to clot and the serum was used for the determination of selected inorganic ions. Here we are reporting the data for Na^+ and K^+ (5 μl. samples, IL flame photometer), and for Cl^- (5 μl. samples, Aminco-Cotlove chloride titrator). Pooled homogenates of gill filaments, kidneys or intestinal mucosa were prepared from two or three fish per sample, and assayed for Na^+, K^+ and residual Mg^{3+}-activated ATPase by methods described previously[9–11].

Table 1. SERUM SODIUM, POTASSIUM AND CHLORIDE FROM CONTROL AND PROLACTIN-TREATED HYPOPHYSECTOMIZED *Fundulus heteroclitus* AFTER 3 DAYS IN FRESH WATER

Group	N	Mequiv/l. (mean ±s.e.) Sodium	Potassium	Chloride
Controls	28	130·1±3·1	4·18±0·43	77·5±3·5
Prolactin-treated	20	* 165·5±2·5	4·75±0·44	* 122·3±4·1
† Mean levels for intact fish				
In FW		172·5±4·1	3·04±0·91	126·4±3·8
In SW		182·9±6·3	4·76±1·19	145·8±1·8

* Significantly different from the controls ($P < 0·001$).
† Mean of the means for five experiments; data from Pickford, Grant and Umminger (Table 3) (ref. 22) (s.e. added).

The principal serum electrolytes of the prolactin-treated fish were in the normal range for intact *F. heteroclitus* adapted to fresh water (Table 1). These fish appeared active and healthy, and were eating well. The control fish, after only three days in fresh water, were already showing signs of lethargy and loss of appetite, although they had not yet developed overt asthenia. The onset of these symptoms, in *F. heteroclitus*, is known to be associated with hypochloraemia[13] and a lowering of serum osmolarity[14]. In another cyprinodont, *Poecilia latipinna*, there is a fall in serum Na^+ (ref. 14) which has been suspected but not hitherto demonstrated in *F. heteroclitus*. Our results agree with these expectations.

Enzyme assays for Na^+, K^+-ATPase are summarized in Table 2. The ability of hypophysectomized *F. heteroclitus* to maintain normal levels of serum Na^+ in fresh water is correlated with a highly significant decrease in Na^+, K^+-ATPase activity in the gills, the main site of sodium

Table 2. Na^+, K^+ AND RESIDUAL Mg^{2+}-ATPASE IN GILLS, KIDNEYS AND INTESTINAL MUCOSA OF HYPOPHYSECTOMIZED *Fundulus heteroclitus* AFTER 3 DAYS IN FRESH WATER

Tissue	Enzyme activity (μmoles Pi/mg protein/h; mean ±s.e.*) Na^+, K^+-ATPase Controls	Prolactin	Residual Mg^{2+}-ATPase Controls	Prolactin
Gills	9·00± 0·60 (11)	† 3·11± 0·46 (8)	22·68± 1·38 (11)	† 14·75± 1·02 (8)
Percentage decrease	—	−65·4	—	−35·0
Kidneys	12·24± 1·05 (11)	† 19·80± 1·32 (9)	32·11± 2·82 (11)	35·98± 2·71 (9)
Percentage increase	—	61·8	—	12·1
Intestinal mucosa	5·93± 0·48 (9)	5·78± 0·39 (9)	19·65± 1·38 (9)	18·28± 1·41 (9)

* Pooled samples from two or three fish; number of samples in parentheses.
† Significantly different from the controls ($P < 0·001$).

excretion. This decrease is even greater (−65·4 per cent) than that observed previously in intact fish adapted to fresh water (−38·6 per cent)[10]. In contrast, untreated controls, near failure in fresh water, have an enzyme activity similar to that of hypophysectomized *F. heteroclitus* in salt water[9,12].

The reverse effect was observed in homogenates of kidney tissue, implying the need for increased sodium retention in fresh water. Na^+, K^+-ATPase was significantly more active in the kidneys of prolactin-treated fish. A similar increase was observed before when intact *F. heteroclitus* were adapted to fresh water[10]. There is strong support for the role of ouabain-sensitive ATPase in the regulation of sodium reabsorption in the teleostean nephron[16,17].

No change was seen in enzyme activity in the intestinal mucosa. Freshwater fish drink little[18,19], if at all[20], and intestinal sodium uptake is minimal, in contrast to the increased uptake that is correlated with drinking in salt water. The low levels of intestinal Na^+, K^+-activated ATPase observed in hypophysectomized *F. heteroclitus* in fresh water may be compared with the higher levels reported previously for such fish in salt water[12]. Adrenal corticosteroids, rather than prolactin, seem to regulate the increased enzyme activity in the intestine of salt-water-adapted *Anguilla japonica*[21] or *F. heteroclitus*[12].

Thus prolactin, which permits survival of hypophysectomized *F. heteroclitus* in fresh water, decreases the activity of the sodium transport enzyme Na^+, K^+-ATPase in the gills while reducing branchial outflux, and increases the activity of this enzyme in the kidney, so increasing sodium retention.

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Evidence for a Second Haemoglobin α-Locus Duplication in *Macaca irus*

IN a study[1] of the haemoglobins of *Macaca irus* obtained from Thailand and Vietnam two α-chain variants, electrophoretically distinguishable from the normal type ($Hb\text{-}A^{mi}$), were found to be frequent. One, $Hb\text{-}Q^{mi}$, was anodal to $Hb\text{-}A^{mi}$ on starch gel at *p*H 8·6 and varied considerably in relative concentration when present with the latter. The fingerprint pattern of the soluble tryptic peptides was, however, indistinguishable from that of $Hb\text{-}A^{mi}$ and the difference is presumably in the insoluble peptide core. The other variant, $Hb\text{-}P^{mi}$, had the same electrophoretic mobility as $Hb\text{-}Q^{mi}$ in very fresh haemolysates but formed aggregates with ageing so that it then appeared in starch gels as a trail extending cathodally from the $Hb\text{-}A^{mi}$ position. The fingerprint of $Hb\text{-}P^{mi}$ was distinct from that of $Hb\text{-}A^{mi}$, αT3 (tryptophan positive) and αT4 (histidine positive) being absent and a new tryptophan and histidine positive peptide appearing in the neutral region. A single amino-acid substitution in α15 or 16 would probably account for this. The phenotype frequencies of these two variants in the population were consistent with their being determined by alleles at the α-locus. In addition, about 20 per cent of the animals had a minor haemoglobin component. The distinctive chain of this component was shown[2,3] to differ from the normal α-chain in at least four substitutions. In some animals the minor component was present together with $Hb\text{-}A^{mi}$ and either $Hb\text{-}P^{mi}$ or $Hb\text{-}Q^{mi}$ and it was suggested that the characteristic chain arose by duplication of the α-locus and mutational divergence.

In a collection of 231 blood specimens of *M. irus* from Malaya (provided by Dr A. C. Laursen, Glaxo Research Laboratories, Buckinghamshire, and by Dr J. Moor-Jankowsky and Mr J. Davis, Lemsip Primate Center, New York), the AQ phenotype was remarkably frequent; 184 (80 per cent) were initially scored as AQ, 39 (17 per cent) as A and 8 (3 per cent) as AP. No animals with $Hb\text{-}Q^{mi}$ alone were found. There was statistically significant heterogeneity of phenotype frequencies between the seven batches of blood, but the frequency of the AQ type was above 75 per cent in all but one. This curious frequency pattern can scarcely be due to selective elimination of homozygotes, for this would require an improbably high mortality, and it was therefore suspected that a duplication might be present. Oliver and Kitchen[4] also suggested a duplication to explain the fact that all animals in a sample of *M. speciosa* had two haemoglobins.

In one of the seven batches of material it was noticed that three specimens scored as AQ and one scored as A had a blurred band at the $Hb\text{-}A^{mi}$ position and when the haptoglobins were examined on starch gel it was seen that these four had a 2–1 m-like pattern which has been shown[1] to occur only when $Hb\text{-}P^{mi}$ is present. It there-

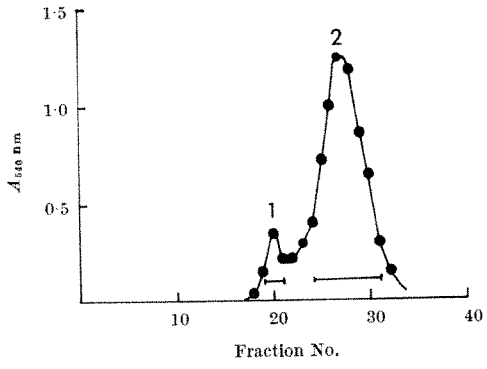


Fig. 1. Separation of *M. irus* (BX 181) haemolysate on 'Sephadex G-100'. Two fractions (1 and 2) are present.

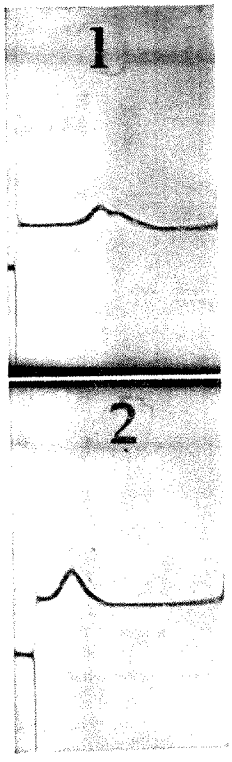


Fig. 2. Ultracentrifuge schlieren patterns of fractions 1 and 2 from 'Sephadex G-100' fractionation of BX 181 haemolysate.

fore seemed that three of these animals might each have three major haemoglobins, Hb-A^{mi}, Hb-Q^{mi} and Hb-P^{mi}.

The haemolysate from one of them (BX 181) was examined by gel-filtration on 'Sephadex G-100' at *p*H 7·0. About 10 per cent of the haemoglobin eluted with the void volume (fraction 1, Fig. 1). Ultracentrifugation of material from this fraction in 0·05 M phosphate-EDTA at *p*H 7·0 showed three peaks (Fig. 2) and the component with the smallest size had an $s_{20,w}$, of 10S, corresponding to an aggregate of at least four haemoglobin molecules. Fraction 2 showed a single symmetrical peak with an $s_{20,w}$ of 4·5S which is the same as that of human Hb-A in this solvent. On starch gel at *p*H 8·6 (Tris-EDTA–borate) material from fraction 1 appeared as a trail with its leading edge in the Hb-A^{mi} position and three sharp bands (Fig. 3*a*, *b*). Fraction 2 showed two components, one at the Hb-A^{mi} position and a stronger, more anodal one at the position of Hb-Q^{mi} (Fig. 3*c*, *d*). When fraction 2 was stored for several days at 0°–4° C and again examined on starch gel, it was found to have developed a cathodal trail suggesting that more polymer had been formed (Fig. 3*c*, *d*). It was therefore subjected to two further gel-filtrations over the course of a month and more polymerized material was recovered each time. After the third gel-filtration an aliquot of fraction 2 was left at 25° C for 2 days and then examined on starch gel. There was no cathodal trail and it was concluded that polymerization was no longer occurring. The two components of fraction 2 were then separated on a starch block at *p*H 8·6. The proportions of the haemoglobin in the A^{mi} and Q^{mi} positions were 36 and 64 per cent. The pooled polymerized material (fraction 1) comprised 40 per cent of the total haemoglobin. The three components were then fingerprinted. The polymerized material showed the changes characteristic of Hb-P^{mi} while the two components of fraction 2 were both indistinguishable from Hb-A^{mi}. These two components were also hybridized with canine haemoglobin[5]. This showed that the α and β-chain charges of the more cathodal one were the same as those of Hb-A^{mi} but that the α-chain of the more anodal one had a higher negative charge like that of Hb-Q^{mi}. This anodal component can therefore be identified provisionally as Hb-Q^{mi} on the grounds that (1) it has the same electrophoretic mobility as this variant; (2) its charge difference from Hb-A^{mi} is in the α-chain; and (3) the fingerprint pattern of the soluble tryptic peptides is indistinguishable from that of Hb-A^{mi}. It seems therefore that this animal had three major haemoglobins in the proportions 22 per cent Hb-A^{mi}, 38 per cent Hb-Q^{mi} and 40 per cent Hb-P^{mi}. The simplest explanation is that it was heterozygous for a gene producing Hb-P^{mi} and a duplication producing Hb-A^{mi} and Hb-Q^{mi}. Another specimen showing this phenotype was run on starch block and the more anodal of the two components was shown by fingerprinting to contain some Hb-P^{mi}. The fourth specimen, with a single blurred band at the Hb-A^{mi} position, was examined by gel-filtration and two fractions were obtained; the first showed the characteristic trailing pattern of Hb-P^{mi} on starch gel and the second showed only a single band at the Hb-A^{mi} position. This specimen apparently contained only Hb-P^{mi} and Hb-A^{mi}. In our earlier studies on animals from Thailand and Vietnam, polymerization of Hb-P^{mi} was virtually complete after storing the haemolysates at 0°–4° C for a few days. It is not clear why polymerization was slower in the present material.

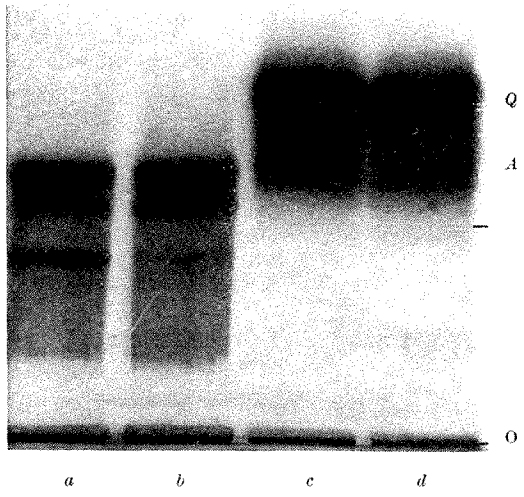


Fig. 3. 'Sephadex G-100' fractions on starch gel (Tris–EDTA–borate, *p*H 8·6). *a*, *b*, Fraction 1 showing cathodal trail extending from Hb-A^{mi} position (*A*) and three sharp bands. *c*, *d*, Fraction 2 showing Hb-Q^{mi} (*Q*) and Hb-A^{mi} components (*A*) and a slight trail of polymerized material (*t*). Specimens *b* and *d* were incubated at 25° C for 1 h.

If our interpretations are correct (and family data are desirable), *M. irus* has two different duplications. The one described here is associated with the production of Hb-A^{mi} and Hb-Q^{mi} in substantial amounts, but in the other case a minor component occurs which comprises less than 5 per cent of the total haemoglobin. The reason for this quantitative difference is obscure. The presence of a duplication may have a bearing on the striking variations in the relative concentration of Hb-Q^{mi} in the AQ phenotype, ranging from about 35 to 70 per cent of Hb-Q^{mi}. If the duplicate produces about 35 per cent of Hb-A^{mi} and 65 per cent of Hb-Q^{mi} the genotype A/AQ would be expected to produce about 32·5 per cent of Hb-Q^{mi} and the genotype AQ/AQ about 65 per cent of this haemoglobin which is in accord with these figures. If we assume Hardy–Weinberg equilibrium for the batch of forty-nine animals in which the four with Hb-P^{mi} occurred, however, and calculate the expected numbers of the genotypes we find $AQ/AQ = 10·5$ and $A/AQ = 22·5$; but nineteen were judged to have more Hb-Q^{mi} than HB-A^{mi} and fourteen less. The discrepancy is quite large, though barely significant for this small sample, and does not favour the idea that the quantitative variation

of Hb-Q^{mi} can be entirely explained by the presence of a duplication, together with two alleles producing Hb-A^{mi} and Hb-P^{mi} respectively. The assumption of Hardy–Weinberg equilibrium is, of course, open to question, because neither the genetic structure of wild macaque populations nor the sampling procedure involved in trapping is known. It should also be noted that there were wide variations in the relative concentration of Hb-Q^{mi} in the Thailand and Vietnam material in which the phenotype frequencies were consistent with equilibrium values for three simple alleles.

If the high frequencies of haemoglobin variants in *M. irus* are maintained by heterosis, like sickle-trait in man, the presence of a duplication might be an advantage since a high frequency of the AQ phenotype could then occur without entailing the production of the Q homozygote which may perhaps have lowered fitness. There is no definite evidence that malaria plays a part in maintaining haemoglobin polymorphism in certain macaque species, but it is interesting that *M. irus* from Malaya is often found to be infected with several species of simian malaria parasite whereas this is not the case in *M. mulatta* in which haemoglobin variants seem to be rare or absent.

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Hibernation and the Production of Glycerol in the Ichneumonidae

The Ichneumonidae (Hymenoptera) are a family of parasitic wasps (15,000 species in the world[1]) which deposit their eggs in larvae or pupae of other insects. Twelve genera can overwinter as free adults, and individuals of these genera may be found hibernating in rotten forest logs, between rocks and under moss carpets. It seems likely that species that survive the winter possess some physiological mechanism absent in non-hibernating species. Glycerol and sorbitol accumulate in a limited number of unrelated diapausing and non-diapausing insects subjected to temperatures resembling overwintering conditions[2]. This study was undertaken to see if (*a*) the overwintering ability of a group of closely related species is manifested by a common observable phenomenon, namely polyol accumulation, and (*b*) this phenomenon is restricted to those species which hibernate.

Specimens were collected from Massachusetts and Ohio during all seasons. Following a period at room temperature in the laboratory all specimens were cold stressed at 4° C for 3 weeks. Glycerol was extracted from individual weighed specimens with a methanol–water solution (3:1). The filtered extracts were evaporated to dryness, acetylated and analysed quantitatively by gas chromatography for glycerol triacetate[3]. Free glycerol was also identified in deacetylated extracts by paper chromatography.

Table 1. GLYCEROL CONTENT OF HIBERNATING AND NON-HIBERNATING SPECIES OF ICHNEUMON WASPS (♀)

	Glycerol (per cent fresh body wt)	
Hibernators		
Aoplus confirmotus Cress.	0·07	
Hoplismenus m. moralus Say.	0·12	0·46*
Ichneumon centrator Say.	0·08	0·35*
Ichneumon fuscifrons Cress.	0·12	
Ichneumon grandisops Hein.	0·03	
Ichneumon heterocampae Cush.	0·02	
Orgichneumon calcatorius Thun.	0·05	
Ichneumon sp.		0·2†
Non-hibernators		
Megarhyssa macrurus Linn., *Megarhyssa atrata* Fab., *Habronyx* sp., *Euceros* sp., *Arotes* sp.	0	

* −1° C for 1 month.
† 0° C for 1 week (male).

Results are shown in Table 1. Only hibernating species are capable of low temperature induced glycerol accumulation. All values are corrected for traces of glycerol in control specimens not subjected to low temperatures. While glycerol accumulation during low temperature stress or diapause is well documented[4] its relationship to cold stress or frost hardiness (the ability of the insect to withstand freezing) is unclear. Glycerol in larvae of *Loxostage sticticalis* (L.) and adult *Camponotus* sp. does not confer frost resistance[5]. *Pyrausta nubildis* larvae, however, accumulate glycerol and can survive freezing. The formation of polyols is not essential for all insects to withstand cold stress[4].

If the ability to accumulate glycerol has survival value, it must have a physiological function. It has been shown that glycerol and other polyols help stabilize certain cold sensitive enzymes[6,7], so it is possible that glycerol protects such enzymes from inactivation in somatic cells. Another possible use for glycerol is suggested by the reproductive behaviour of these insects. Hibernating species mate in the autumn and the overwintering fertilized female stores spermatozoa in the spermatheca until spring when she leaves her hibernaculum to parasitize pupae[8]. It is believed that at this point spermatozoa are released from the spermatheca and fertilize the eggs before oviposition. Glycerol may help protect sperm during the overwintering period. (It is a well known cryoprotective agent for long term storage of bovine spermatozoa at sub-zero temperatures[9].)

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Validity of the Selective Coefficient of a Behaviour Trait in Hawkmoth Larvae

In Jamaica the fifth instar larvae of the New World hawkmoth, *Erinnyis ello*, exhibit four basic colours: green, blue, green-grey and brown. The green, blue and green-grey larvae feed and rest on the leaves of their commonest food plant (*Poinsettia pulcherrima*). The brown larvae also feed on these leaves, but they rest for many hours during the day on the base of the trunk, sometimes several yards below where they feed[1]. All the larvae are preyed on during the day by wasps (*Polistes crinitus*) and to a very small extent by an anole (*Anolis lineatopus*). Because the wasps search for their prey only in the foliage of the *Poinsettia* bushes, the three forms staying constantly on its leaves suffer heavily from predation, as selection experiments in the wild have demonstrated[1]. By contrast, all the larvae which rest from time to time on the trunk of the food plant enjoy an almost complete freedom from predators.

From mortality data obtained on the campus of the University of the West Indies in Mona, Kingston, and in neighbouring gardens, a relative selective coefficient of the habit to rest on the trunk was calculated. (I take relative selective coefficient as a quantitative measure for differential survival of different larvae at one particular instar). Because the data showed a vast selective advantage of the order of $V_t = 88$ per cent (see Table 1 and refs. 2, 3, 8) and because V_t represents the first quantitative assessment of such a value for a behaviour pattern, I decided to check its validity.

Table 1. SELECTIVE COEFFICIENT V_t OF THE TRUNK RESTING HABIT IN FIFTH INSTAR LARVAE OF THE HAWKMOTH *Erinnyis ello* BY THREE DIFFERENT METHODS

Determination of m or m'	m_f or m_f'	m_t or m_t'	Determination of E or E'	$V_t = 1 - E_f/E_t$ or $V_t = 1 - E_f'/E_t'$	Refs.
(1) $m = 1 - N_a \cdot e^{-R}$	0·055	0·007	$E = 1/-\ln(1-m)$ or $E = 1/\lvert R \rvert$	0·88	4, 5, 6
(2) $m = 1 - N_a \cdot e^{-R}$	0·055	0·007	$E = 1/m$	0·87	7
(3) $m' = N_m/\mathrm{Tet}$	0·250	0·071*	$E' = 1/m'$	0·72	This paper

m, m' = mortality; E_f and E_t = life expectancy of larvae in the foliage and on the trunk of the food plant; R = risk of predation; N_a = number of larvae initially present; N_m = larvae which died. Tet = total exposure time (h).

* Initially there were 131 larvae on the trunk of *Poinsettia* bushes compared with 916 larvae in the foliage; original $m_t' = 0{\cdot}010$ was therefore weighted for equal initial numbers N_a by multiplication with 916/131.

Three different methods were used which are based on two different determinations of the mortality of the larvae per hour of daylight. Fifth instar larvae were placed singly on twigs of *Poinsettia* bushes in groups from one to forty-six per bush, the average being 6·7 per bush. Altogether 916 individuals of all four colour forms were counted in the foliage, and 131 were found on the trunk (these last had moved down from the foliage). The survivors in each group released were checked after 6·2 h ($n = 162$) and after 4·9 h ($n = 61$), respectively. (For details of the census technique and of the calculation of the mortality rate, see ref. 1.) The methods used to check the original estimate of V_t were as follows:

(1) After it had been ascertained that the mortality rate m was density independent, a constant death rate among the larvae was assumed. The basic method for the evaluation of m allowed for the variance of the group size and of the time interval between censuses. Because the mortality rates per hour differed only by chance between the four colour forms, the data for all four groups were pooled to give an overall mortality rate of $m_f = 0{\cdot}055$ and of $m_t = 0{\cdot}007$ (Table 1, 1), which differ significantly from each other ($P < 10^{-6}$) (the group on the trunk contained a small percentage of green, blue and green-grey larvae as well). The similarity of the mortality rates of the four colour forms in each respective place would be expected if the predator does not hunt by sight which is, in fact, true for the wasps. The resulting relative selective coefficient V_t of the habit to rest on the trunk was 88 per cent checked by method 2.

(2) m was calculated as in method (1), the only difference in calculating V_t being in the assessment of E which is probably less accurate[6,9]. But the ensuing selective advantage V_t of the trunk group of 87 per cent agrees closely with the value determined by (1) (Table 1, 2).

(3) While the first two methods are independent only with regard to the calculation of the respective life expectancies, the following rather crude estimation of V_t is based on another piece of information from the mortality data. Because of algebraic reasons explained elsewhere[1], twenty-seven counts from the foliage larvae with the total loss of a sample ($N_a = N_m$) have so far had to be omitted from the analysis. They can now be included in the grand total of N_m if the number of larvae per hour of daylight killed in each of the two localities is considered (Table 1, 3). Because the initial numbers N_a were different in each case, a weighting to compensate for this seemed advisable (see footnote to Table 1). The resulting selective value of 72 per cent is considerably lower than the ones obtained previously but it is based on the simplified assumption that the death rate is a linear function of time. From this it follows that the number of larvae killed after a given time is smaller than if the death rate were constant, that is, it would follow a logarithmic function as has been shown. The deviation from this function of the (simplifying) "linearity model" is negligible if m is very small, as, for example, in the trunk group; accordingly, it must be larger, where m is higher, as, for example, in the foliage group. Thus the difference between m_t' and m_f' obtained through the linearity model in this method is smaller than in the more realistic method used before. As a consequence, method (3) must lead to too low a selection coefficient which is confirmed by a comparison of the three methods in Table 1. Moreover, this V_t is especially liable to sampling error; if one assumes, for instance, that, in spite of careful searching, I overlooked only one out of the three caterpillars which disappeared from the trunk "population" the V_t value would rise to 81 per cent. Thus method 3 actually leads to an estimate of V_t which is reasonably close to the two former methods.

Consequently, the selective disadvantage of the larvae inhabiting the foliage seems so high that some strong advantage should compensate for it. This advantage might lie in the feeding behaviour of an anole. It hunts all over except in the foliage; surprisingly, it preys on non-brown larvae and rejects the brown ones[10].

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Incorporation of Metabolic CO_2 into Coral Skeleton

Two possible sources for the carbonate in the calcium carbonate coral skeleton are (*a*) soluble carbonates from seawater and (*b*) carbon dioxide produced in metabolism by the living coral tissue. Although radioisotopic tracer experiments have established that carbonate from seawater is incorporated into the skeleton by many corals[1–3], evidence for incorporation of metabolic CO_2 into the skeleton is much less direct. Goreau[1] found that calcification rates measured by incorporation of radioactive carbonate from seawater were usually lower than those measured by using radioactive calcium; he proposed that the labelled carbonate might be diluted by unlabelled carbonate in coral tissue. In addition, findings that the ratios of ^{13}C to ^{12}C and of ^{18}O to ^{16}O in seawater differ from those in coral skeleton suggest that not all of the skeletal carbonate originates directly from carbonate in seawater[4–7]. To examine further the possibility that some skeletal carbonate originates from metabolic CO_2, I fed ^{14}C-labelled mouse tissue to small individuals of the coral *Fungia scutaria*, a solitary polyp containing symbiotic algae.

Table 1. ^{14}C ACTIVITY IN ORGANIC AND CARBONATE FRACTIONS OF CORAL

Days fasted after ^{14}C feeding	Per cent activity in organic carbon	Per cent activity in carbonate
4–6 (3)*	95·7	4·3
13 (4)*	91·1	8·9

* No. of corals.

To obtain labelled mouse tissue, we injected a laboratory mouse intraperitoneally with 0·5 mCi of ^{14}C-labelled protein hydrolysate in 0·1 ml. physiological saline. The mouse was killed 24 h later. Pieces of liver, kidney and intestine were fed to small corals (90–240 mm diameter, 150–1,650 mg dry weight), over a period of several days. The animals were then fasted, one group for 4–6 days, another for 13 days. At the end of the period of fasting, the tissue and skeletal components were separated as follows: each coral was placed in a small beaker and covered with concentrated ammonium hydroxide (58 per cent). The beakers were heated in a water-bath at 60°–70° C for about 1 h. The loosened tissue was easily washed off the skeleton with jets of ammonium hydroxide solution. The tissue in ammonium hydroxide was homogenized with a 'Teflon' pestle and brought to known volume. Aliquots were removed to planchets, acidified with HCl, dried, and counted on a Nuclear-Chicago gas-flow detector model 470. The skeletons were washed under a strong jet of water to remove any remaining fragments of tissue, dried in an oven at 100° C, and weighed. The clean skeletons were dissolved in 6 M HCl in evacuated flasks, and the CO_2 evolved was recovered in a small beaker containing 5 M KOH on the bottom of each flask[8]. After the skeletons had dissolved, 1 h was allowed for the CO_2 to become more completely absorbed; the flasks were then opened, and aliquots of the KOH solution were removed for counting. Approximately 95 per cent of the ^{14}C from skeletal carbonate was recovered. The acid-insoluble residue, which included the organic matrix of the skeleton, was collected on a 'Millipore' filter, dried and counted. The acid filtrate was also counted, but did not contain measurable amounts of ^{14}C. Separate control experiments showed that no measurable amount of $^{14}CO_2$ was released from tissue treated with acid. The data are presented in Table 1. Values for tissue and organic matrix were combined to give a single value for total organic ^{14}C; the matrix value was never more than 2 per cent of the total.

A portion of the activity was consistently found in the carbonate of the skeleton, offering direct evidence that metabolic CO_2 was incorporated into the skeletal carbonate. Also, the distribution of the labelled carbon in the two groups of corals was significantly different ($P < 0.05$). Among the corals fasted for a longer period (13 days), the percentage of radioactivity in skeletal carbonate was twice that of the first group (fasted 4 days), while the percentage of activity in the tissue decreased. For this difference to reflect only metabolic loss of labelled organic carbon would require that the total ^{14}C of the second group be reduced almost by half, while in fact there is no significant difference between the means for total ^{14}C in the two groups ($P = 0.33$). Alternatively, the apparent shift in distribution of the labelled carbon may be real, that is, the animals may have continued to calcify during the fasting period and, in doing so, fixed some of their metabolic $^{14}CO_2$ into skeletal carbonate. Also, some of the metabolic CO_2 may have been photosynthetically fixed by the symbiotic algae in the tissues of the coral. These two mechanisms could account for the negligible turnover of total ^{14}C in this experiment and may make possible very efficient conservation of organic carbon taken in food by the corals.

My evidence that metabolic CO_2 is deposited in coral skeleton as carbonate supports Goreau's interpretation of his experiments in which he found that calcification rates calculated from ^{14}C in skeletal carbonate were lower than those calculated from skeletal ^{45}Ca. He interpreted the lower carbonate values as being the result of isotopic dilution of the exogenous carbonate pool with unlabelled CO_2 in the coral tissue. It is important to note, however, that the relative contributions of carbonate from seawater and from metabolism are still unknown. The sources of carbonate for mineral deposition at any one instant might be expected to vary with such factors as the degree of illumination and the nutritional state of the animal.

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Skeletons of *Lumbricus terrestris* L. and *Arenicola marina* (L.)

FOR the theoretical investigation of their locomotion, worm-like animals have conveniently been treated as thin walled, liquid-filled cylinders[1–3]. Observation of living animals, however, suggests that rigidity of the body wall, both inherent and developed by contraction of the muscular layers, represents a more or less important component of the total force exerted on the environment.

Some references to the rigidity and elasticity of the body wall in worms appear in the literature[4–9], and Clark[10] discussed radial forces exerted by worms in relation to the presence and absence of septa; but no quantitative

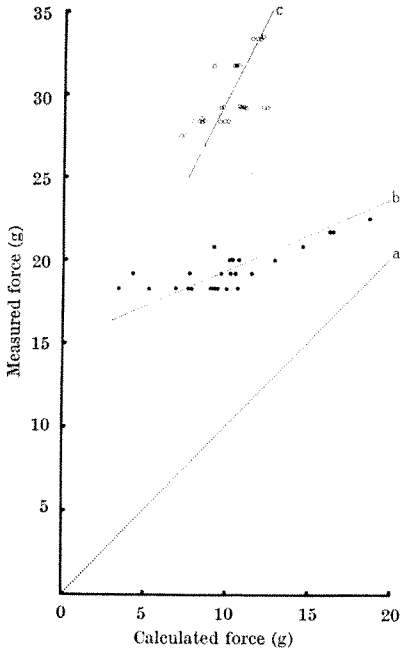


Fig. 1. Graph showing the relationship of measured forces exerted by *Lumbricus terrestris* against a movable bridge to expected forces calculated as the product of internal pressure and measured area of the worm in contact with the bridge. If the body wall had neither inherent rigidity nor internal frictional losses, all points would lie on line *a*, for measured forces would equal calculated forces. Lines *b* and *c* are for two worms (5 g and 6·3 g weight respectively) showing the range of forces encountered. Regression $P < 0.02$.

estimate of the relative importance of the internal hydrostatic and intrinsic body wall skeletal functions has been published.

In an attempt to resolve the prevailing uncertainty about the skeletal role of the body wall in worm-like invertebrates, simultaneous measurements have been made of internal pressure, externally exerted force and the area over which the force was exerted for two annelids: the terrestrial, septate earthworm *Lumbricus terrestris* L., which burrows in the earth, and the sand-burrowing, marine, effectively non-septate lugworm *Arenicola marina* (L.). The results obtained will be discussed more fully elsewhere.

Worms moving beneath a narrow 'Perspex' bridge of known area displaced it against the tension of a light myograph transducer. A Statham P23BB transducer monitored coelomic pressure in the segment central under the bridge through a fixed stainless steel cannula. Transducer output was displayed on a multichannel inkwriting recorder. From synchronized photographs of the area, A, of worm surface touching the bridge, and the recorded internal pressure, P, a force value ($P \times A$) was calculated and compared with the corresponding force value directly recorded by the myograph.

In an internally pressurized liquid-filled cylinder of which the wall has no inherent rigidity, the force against an external object flattening the wall equals internal pressure multiplied by the area of flattening. Thus measured force exerted by the cylinder in the bridge would equal calculated force, ($P \times A$), at the same instant; and measured force plotted against calculated force (Figs. 1 and 2) would yield a straight line passing through zero (lines *a*). For a real worm, however, such a plot yields lines *b* and *c* (Fig. 1) and line *b* (Fig. 2); actual forces against the bridge exceed those which could be produced by internal pressure alone. This "excess lifting force" is developed directly by contraction of the body wall musculature, and its magnitude is a measure of the "intrinsic rigidity"[8] of the muscle layers.

Excess lifting force is expressed as the percentage by which mean measured force exceeds mean calculated force. Values of excess lifting force found for *Lumbricus* and *Arenicola* are 145 and 85 per cent respectively. For *Lumbricus*, measured force and area vary reciprocally; as increased force is applied to the bridge an increase in curvature of the worm from its flattened state occurs.

Complete intersegmental septa are present in *Lumbricus* and muscle fibres within them are kept taut to resist bulging of the septa under differential pressures in locomotion[4]. Such tension must aid the circular muscles in the development of lifting force, by restricting lateral spread of the body wall in a bridge or crevice situation. Also, the thicker, and therefore shorter, is a given region of earthworm, the more septa are present in that region and so the greater is the total resistance to deviation from a circular cross-section when the worm is in the bridge apparatus, or is burrowing through and enlarging a crevice in the earth.

In contrast to *Lumbricus*, for *Arenicola*, where the trunk is without septa, the greater the force exerted against the bridge the greater the area over which it is exerted. This means that, during lifting, the body wall remains more flattened in *Arenicola* than in *Lumbricus*, and that the circumference is less reduced. Thus less stiffness is developed; the circular muscles alone, without the aid of septal elasticity and musculature, can produce relatively less excess lifting force on contraction than can the cooperating circular muscles and septa of *Lumbricus*.

Although in *Lumbricus*, lifting segments must be fully shortened to undergo their greatest dilation (because watertight septa are present and segmental volume is constant[4]), in the aseptate trunk of *Arenicola*, dilated lifting segments are usually also elongated. In this situation the dilation is achieved by longitudinal and circular contraction elsewhere in the body wall surrounding the large coelom. Such a configuration is seen during the dilation anchorage sequence of burrowing in *Arenicola*[9,11,12] wherein posterior trunk segments are short and thin and anterior trunk segments are elongated and dilated. Such simultaneous elongation and dilation minimize body wall thickness and therefore its inherent rigidity in that region. Thus *Arenicola* is far less able to develop that unidimensional lifting force necessitated by a crevice situation than is *Lumbricus*; it is, however, ideally suited for all-round enlargement of a more homogeneous substratum. Attainment of the considerable dilation required by the situation and obtainable by the system would clearly be hindered

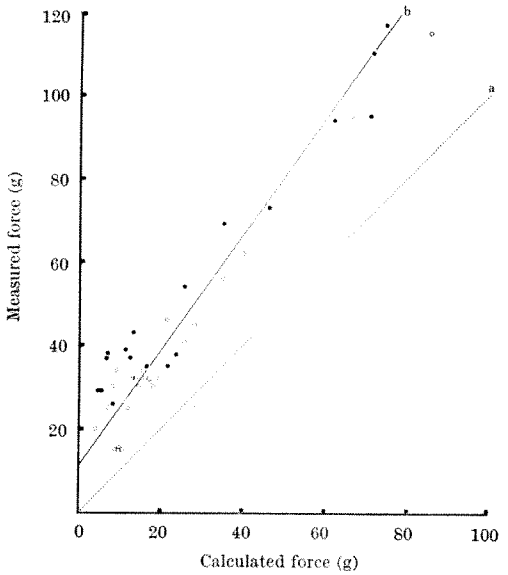


Fig. 2. Graph prepared and to be interpreted as Fig. 1, for two specimens of *Arenicola marina* (9·7 g (●) and 12·4 g (○)). Regression $P < 0.001$.

by septa limiting dilation and preventing the necessary transfer of fluid and of coelomic pressure from the posterior trunk.

Two types of penetration of the substratum can be distinguished. First, crevice burrowing, which makes use of a layer of the substratum penetrated with relative ease between two harder layers; for example, an air space in earth or an earth or sand layer between stones or roots. The body is first flattened and then exerts a lifting force at right angles to the plane of flattening (Figs. 1–6 of ref. 13). This force anchors part of the worm against elongation of the more anterior region into the crevice[9], and also enlarges the crevice. Limitation of lateral spread of the body is necessary and is much enhanced if muscular septa are present. Second, homogeneous burrowing: in a homogeneous substratum, for example sand or mud, there is complete circumferential contact with the substratum and relatively greater dilation of the burrowing animal radially in all directions. The development of excess lifting force and prevention of lateral body wall spread are inappropriate.

Thus it can be seen that the septate earthworm is better suited to, and much more likely to perform, crevice burrowing than is *Arenicola* with its open coelom; whereas *Arenicola* is well adapted for homogeneous burrowing.

I thank Professors P. G. 'Espinasse and J. G. Phillips for facilities, and Professors G. P. Wells and E. R. Trueman for reading and criticizing the manuscript. This work was carried out during tenure of an SRC research studentship at the University of Hull.

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Structure of the Nuclear Pore in Higher Plants

Two recent models which have been proposed to describe the structure of the nuclear pore[1,2] have given conflicting interpretations of the structural relationship of the various pore components in animal cells. No model has satisfactorily incorporated the evidence obtained from vertical sections of a pore, and no convincing model has been proposed for the nuclear pores in plant cells. In view of the obvious significance of nuclear pores for nuclear-cytoplasmic transport, and considering the current confusion about their structure, we have investigated the three dimensional picture of the pore complex in higher plants.

We wish to describe the pore structures observed in suspension culture cells of sycamore (*Acer pseudoplatanus*) and bean (*Phaseolus vulgaris*), crown gall tumour cells of *Parthenocissus tricuspidata* and root tips of sycamore and pea (*Pisum sativum*). (A more complete report will be published elsewhere.) Tissues were processed for electron microscopy[3] and examined in either a calibrated AEI EM6B or a Philips EM 300 equipped with a tilting stage. Pea roots were examined using the freeze etch technique[4].

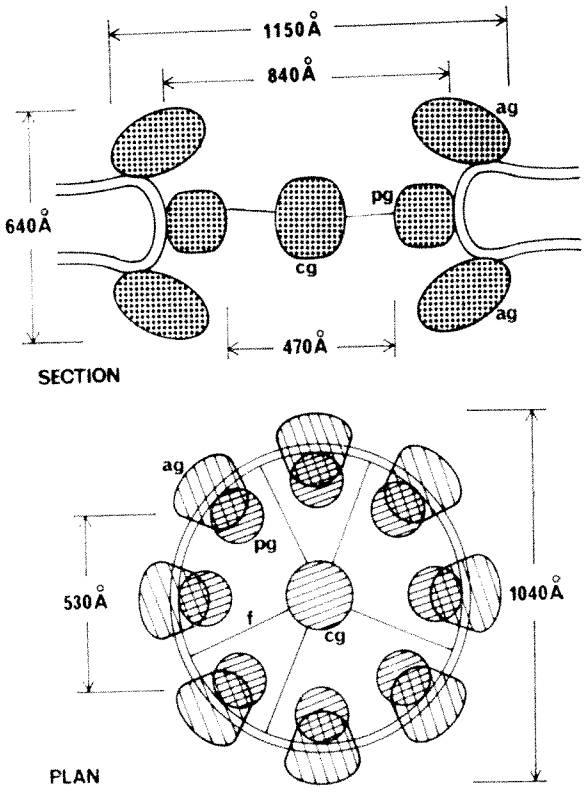


Fig. 1. Schematic diagram, drawn to scale, of the model of the nuclear pore showing its average dimensions. ag, Granular subunits of the annulus; cg, central granule; pg, peripheral granules; f, filaments.

Sectioned material revealed the following components common to all specimens (the measurements refer to the sycamore callus cells).

(*a*) The membrane boundary of the pore proper is circular. We agree with the report[2] that the octagonal appearance of the membrane seen in some negatively stained preparations[5] is a result of distortion during dehydration and spreading. The pores appear circular in the freeze etch studies. The diameter of the inside of the pore margin is 836 ± 88 Å.

(*b*) The peripheral granules, usually eight in number (visual inspection and rotation analysis), are spherical electron dense granules with a diameter of about 180 Å and a centre to centre spacing of about 250 Å. In vertical section the channel formed by the ring of granules is 472 ± 104 Å in diameter and in horizontal section is 527 ± 96 Å.

(*c*) The granular subunits of the annulus comprise two rings of eight granules, situated on the nuclear and cytoplasmic sides of the pore respectively, and attached to the membrane. Each granule appears as a flattened ovoid and together they form rings with an outer diameter of 1147 ± 83 Å in vertical section and 1037 ± 74 Å in horizontal section. The full depth of the pore complex so formed is 639 ± 71 Å.

(*d*) The central granule is present in many of the pores examined (Figs. 3 and 4). It is a large electron dense

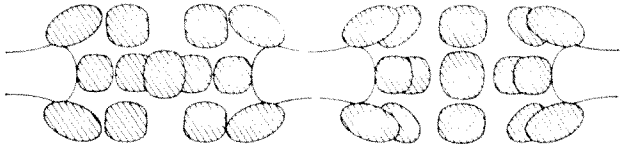


Fig. 2. Theoretical prediction of two possible projections to be seen in vertical section of a nuclear pore, assuming a section thickness of ~600 Å (silver/grey). Compare these with the actual sections in Fig. 3.

granule about 200 Å in diameter and 250 Å long, sometimes made up of subunits.

(*e*) Thin filaments (~30 Å) similar to those described in animal cells[2] can sometimes be seen in horizontal sections, connecting the central granule and the pore margin. Their number varies.

(*f*) Pore complex amorphous material fills the remainder of the area within the pore. It has an increased electron density, which suggests the presence of material acting at a barrier to free diffusion.

Taken together these features give the model shown in Fig. 1, and from this several points arise. In order to correlate the horizontal and vertical sections it must be remembered that the image obtained in the microscope is a projection of the electron density within the thickness of a section. The width of the pore complex seen in horizontal section is explicable in terms of the superposition of the peripheral granules and the granules of the two annuli above and below it. The measured widths support this argument. Similarly the variety of images obtained in vertical section can be interpreted if the thickness of the section is taken into account. A gold or silver section (600–1000 Å) will include at least five, if not all eight, sets of granules. This, depending on the line of projection, will lead to the images derived in Fig. 2, which are observed in Fig. 3. This explanation would clarify some of the pictures obtained before[1,2]. It would need a very thin grey section (~300 Å) to observe the simplified vertical section shown in Fig. 1. The correlation of the two images (vertical and horizontal section) and a check on the various measurements were made possible by examining several pores through a full 90° tilt (Fig. 4). This reveals the same pore both in horizontal and vertical section and confirms the predicted images (Fig. 2). In freeze etch pictures of the outer layer of the envelope two sorts of image are obtained, one having a "capped" appearance and one being a circular depression (Fig. 4). The former is on average 1·2 times greater in diameter than the latter, and this is explicable in terms of the fracture plane, which can either pass over the whole pore complex, including the granules of the annulus, giving the "capped" formation, or remove these (sometimes together with the peripheral granules) to give the smaller picture. This adds confirmation to the model and to the existence of the granules of the annulus. These various lines of reasoning lead to the model of Fig. 1, which in some respects resembles that of Franke and Scheer[2]. It differs in the definite presence of peripheral granules, the shape of the granules of the annulus and some of the measurements involved. Also, we only rarely see the 30 Å fibrils and associated granules. We find no evidence to support the model of Abelson and Smith[1] involving minitubules, central tubules and asymmetrically placed granules, although we support in general their view of the dynamic existence and character of the pore. What the various granules represent, beyond the knowledge that they are ribonucleoprotein[6], or how the pore operates in transport mechanisms remains obscure.

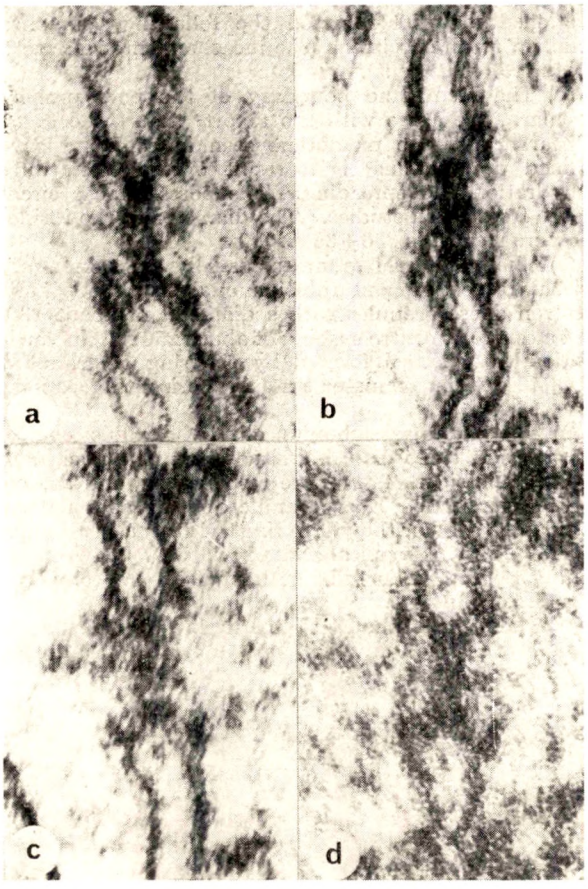


Fig. 3. *a–d*, Four different nuclear pores (sycamore) in vertical section (×160,000).

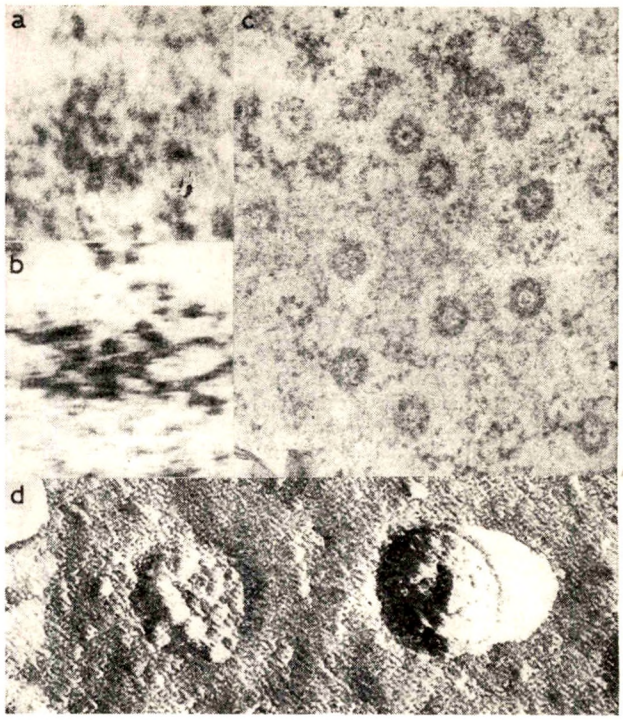


Fig. 4. The same nuclear pore (sycamore) in vertical (*a*) and horizontal (*b*) section by tilting it through 90° in the microscope (×120,000). (*c*) Horizontal sections of nuclear pores (sycamore). Most possess a central granule (×60,000). (*d*) Freeze etch picture of pea root nuclear pores, showing the two images "capped" and "hollow".

We thank Mr Thurley for the use of the EM 300 in the Department of Anatomy, Cambridge. K. R. acknowledges the receipt of an SRC research grant.

Note added in proof. A recent publication of W. W. Franke (*Z. Zellforsch*, **105**, 405; 1970) is substantially in agreement with the conclusions presented here.

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Book Reviews

SCIENTISTS AND SOCIETY

Reason Awake

Science for Man. By René Dubos. Pp. xviii + 280. (Columbia University: New York and London, July 1970.) 63*s* ($6.95); 27*s* paper.

If the 1950s can be characterized by the growth, at least in the developed countries of the West, of the scientific estate, the 1960s can be viewed as the beginning of widespread social discontent about science. We are, according to the author, living in the age of the "new pessimism" brought on by disillusionment with the wonders of science. The initial expectations of those who in the nineteenth century looked forward to a new society firmly rooted in scientific understanding stand in sharp contrast to the actual state of society that more than one hundred years of commitment to scientific and technical progress has produced. Lack of attention to the social implications of science and technology has transformed our environment beyond all recognition—so deep has been the change that the ecological balance of nature and the future of man are now seriously threatened.

The first four chapters of this book are concerned with drawing out the implications, both for science and society, of the unbridled development of technology. Thus, the central theme of the book, which derives from a set of lectures given by Professor Dubos at the Institute for the Study of Science in Human Affairs at Columbia University, is "not science *per se* but rather the penetration of science into all aspects of human life". Although the author's theme is not a new one, it is amply illustrated by references to the biological sciences rather than to the physical sciences as has usually been the case. But Dubos's chief concern is not simply to face his readers once again with a pessimistic picture of the ills of our technological society. As the central theme suggests, his concern is with the penetration of science into all aspects of human life; and this, for Dubos, means the effect of scientific technology on the intimate relationship between man and his environment. The ecological balance, according to Dubos, has been evolving over centuries. So, too, has man; his evolution, both mental and physical, is deeply involved with the development of his physical surroundings. Now, with the aid of science and technology, man has so profoundly altered the environment that his physical and mental make-up may no longer be able to cope with the rapid rate of change. But one of the principal characteristics of man is his adaptability. Throughout his evolution it has been necessary for man to adapt in order to survive biologically, but "paradoxically the greatest threat to the quality of human life is that the human species is so immensely adaptable that it can survive even the most objectionable conditions. . . . There is much evidence that tolerance of undesirable conditions is achieved at the cost of physical and mental disabilities in later life. To a large extent the so-called diseases of civilization are the delayed consequences of biological and mental stresses to which the organism has made adaptive responses that appear effective at the time but are inadequate in the long run". In chapters five and six, Dubos illustrates this thesis by drawing extensively on recent research in psychology, microbiology and the neurosciences.

Although the case for the ecological relationship between man and his environment is well made, the solution offered is less compelling. The answer to an ecological problem is an ecological solution. Man, the author argues, having grasped that his survival depends on a very delicate balance between myriads of conflicting forces must somehow attempt to "will" or plan the future so that the appropriate ecological balance is maintained or at least constrained to change more slowly.

It is refreshing, nonetheless, to see a distinguished scientist repudiate as inadequate the technological scenarios of the Hudson Institute but, alas, there is much work to be done before the ecological thinking of Dubos permeates the intellectual structures of Western society and becomes an effective alternative to the one dimensional "technological fixes" that society has so far provided to solve its problems. In a society committed to technological and economic growth as ends in themselves the battle of technology versus counter-technology must lead to the destruction both of the environment and man himself. Dubos urges us to abandon as quickly as possible the "philosophy of endless quantitative growth and opt for a social and economic 'steady state' ". Many persons may feel that to opt for such an alternative is to condone stagnation and eventually decadence. Dubos counters that ". . . a steady state is compatible with creative changes. In fact, change within a closed system will probably offer intellectual possibilities much more challenging than those offered by the kind of rampant growth that prevails at present". Accordingly, when man truly enters the age of science he will abandon his crude and destructive attempts to conquer nature by technological force. "He will instead learn to insert himself into the environment in such a manner that his ways of life and technologies make him once more at harmony with nature."

This is a thought-provoking series of essays which will be of interest to both the general reader and to the specialists who seek to understand the paradox of current disillusionment with science in a civilization dedicated to the advancement of knowledge.

Michael Gibbons

SURVEYS OF OVERCROWDING

Population Control

Edited by Anthony Allison. Pp. 240. (Penguin: Harmondsworth, Middlesex, June 1970.) 7*s*.

This collection of twelve essays is rather like a basic biology textbook; it moves from an initial discussion of protozoa, through plant, bird and animal populations to the second half which is devoted to human populations. As the editor freely admits in his introduction, such a wide ranging survey of populations will "have varying degrees of relevance to the human situation". This relevance could have been increased if there were more explicit discussion in the first half of the book, of the implications of plant and animal population fluctuations on human activity and vice versa. For example, in the paper by Leslie Brown on large mammals, it would have been interesting to know the likely ecological implications of proposals to exploit wild herds of mammals in Africa for human food consumption.

The second half of the book opens with a cogent summary of past and likely future growth of the world's human population by W. Brass, in which he notes how past predictions of population trends have not been incorrect but simply overtaken by changes, chiefly in fertility patterns which the projectors could not have been expected to foresee. There follows a discussion of the linkages between population growth and food supplies and then three papers on population regulation in the context of primitive societies, family planning programmes

in several developing countries, and contraceptive methods currently available. The final paper, by Colin Clark, is somewhat disappointing in a book which, to judge from the cover, sets out to examine population growth as "the major biological problem overshadowing the second half of the twentieth century", for about half the paper is concerned with basic factors affecting family size in past and present day societies. The emphasis, one feels, should have been on the economic and social implications of likely future developments.

The most remarkable essay is by John B. Calhoun. It links the two halves of the book physically and is a remarkable *tour de force* in that he casts his attention from the behaviour of rats in experimental habitats, through calculations of the population of western Guatemala in the sixteenth century to estimates of the optimum population of the world of 7,500 million. These are, he says, speculations which "the scientist often engages in but normally does not make public until several years later after the conclusion of studies generated by such speculation".

These speculations are based on the discovery that aggressive tendencies in rats developed at higher population densities the larger the initial size of the colony. He then goes on to argue that the totality of space that an individual is aware of around himself consists of physical space and "conceptual" space, that is, the "breadth of our insights and concerns in the arts, humanities, sciences and on the political and social scene". He sees extensions of conceptual space as compensating for reductions of physical space, but fears that there is currently occurring a reduction of both components. Overlapping conceptual space may lead to conflict even where there is no overlap of physical space. This was demonstrated by the behaviour of rats, but the sociologist might think of examples where human behaviour could tentatively be explained in such terms, such as the hostility by long standing residents to immigrants settling in their area. Although it has been shown that overcrowding existed long before new settlers arrived, nevertheless, the creation of overcrowding is frequently quoted as the justification for hostility. As Calhoun says, however, such speculations should be backed by several years of studies.

In all, this is a bitty book. If it does give, as it claims, a "biological perspective of man's predicament", this merely confirms that such a perspective is too myopic. The problems posed by population growth demand social and political perspectives and require social and political solutions.

D. R. COPE

THEORY OF POPULATIONS

Theoretical Population Biology

An international journal. Edited by J. F. Crow, N. Keyfitz, S. Karlin and R. MacArthur. Three issues per annum. (Academic: London and New York, 1970.) Private subscription $10.00; institutions $16.00.

"POPULATION" has a good chance of going to the top of the trendy protest charts. There is nothing trendy in the pejorative sense about this new journal, which aims to bring together papers on theoretical aspects of epidemiology, demography, population ecology and population genetics, although its appearance is opportune in view of the need for such a synthesis. Problems to be solved in this way include that perennial teaser, the genetic load, and the suggestion made recently in the *Lancet* (ii, 1249; 1968) that birth control will do genetic damage to the human population. There must also be rich gains in studying the relationships of genetic, evolutionary changes to the increasing exploitation of the environment, and their general effects on population dynamics or their influence on epidemics. The urgent practical implications of this kind of theory for resource conservation, animal breeding and population control are not far to seek.

A distinguished international team of editors and associate editors ensures a high standard for the journal, although in the first issue they have not succeeded in making all the papers comply with the journal's format for abstracts and summaries. Some of the contributors are a bit happy-go-lucky about explaining the meaning of their mathematical symbols to the uninitiated (later editions please note); other papers are models of clarity. Misprints are of the minor kind that will not worry anybody familiar enough with the material to be using the journal in the first place.

The reader can gain some idea of the potential scope of the journal from the contents of the first issue: the definition of a function which can be used to predict the number of competing species in a habitat at equilibrium; the building of a probabilistic model to describe a population with individuals who grow older (some theories assume instantaneous birth and death); a study of the equilibria at two genetic loci under natural selection; a solution of the rate of fixation of two linked loci in a population of finite size, and another of the effect of migration between populations on the rate of fixation at a single locus; a discussion of the evolutionary usefulness of sex, and one of the optimum strategy for animal breeding programmes.

I feel that the papers which communicate best are those which give numerical examples; indeed, it seems likely that the necessary and difficult line of communication between mathematicians and biologists will increasingly be kept open by the high-speed computer, presenting results intelligible to the non-mathematician, especially as graduates of the more worthwhile biological science courses now have some training in programming.

This deserves to become an important journal, bridging the gap between several disciplines.

JOHN R. G. TURNER

INTERNATIONAL HEALTH

Building the Health Bridge

Selections from the works of Fred L. Soper. Edited by J. Austin Kerr. Pp. xxxvii+567. (Indiana University: Bloomington and London, July 1970.) $17.50; 167*s*.

ANY book on the history of medicine that may be written at the end of this century is bound to say that one of the principal achievements of the present generation was the degree of international collaboration in the field of public health. The national responsibility for detection, notification and control of communicable diseases is now being gradually extended to international bodies. In the age of jet planes and mass travel such an approach is more suitable than the legalistic and restrictive attitude of the early phase of quarantine regulations.

This book contains forty-five selected papers from the writings of one of the great American epidemiologists and medical administrators, but it gives more than the outline of the work of Dr Fred L. Soper, formerly of the International Health Division of the Rockefellor Foundation and of the US Public Health Service, the retired director of the Pan-American Sanitary Bureau, WHO Regional Office for the Americas. The volume covers one of the most exciting, fruitful and significant periods in the recent history of international health.

After an introduction and a brief biography of Dr Soper, it opens with the story of the survey and control of hookworm in Brazil, Paraguay and other South American countries. It then deals with the eradication of yellow fever from Brazil, through the control of the peri-domestic mosquito, *Aedes aegypti*. The need for more accurate reporting of the disease led to the setting up of the

"viscerotomy" service, which collected specimens of liver tissue from all persons dying of a disease that might have been yellow fever; serological studies on large groups of inhabitants and on captured monkeys were also of immense value for the study of the distribution of the disease. The information thus provided gradually unfolded the picture of "jungle yellow fever" carried by elusive forest mosquitoes, while howler monkeys of Brazil and Panama were the sources of the virus. This fascinating story of epidemiological detection was well matched by the outstanding success of the 17D yellow fever vaccine, for prevention of the disease.

Another chapter gives the dramatic description of the invasion of Brazil and of lower Egypt by the African *Anopheles gambiae*, the subsequent epidemics of malaria and the eventual eradication of the vector from these territories. This was the origin of the notion of global eradication of malaria—a concept taken up by the World Health Organization in the following years.

The last chapters are devoted to the assertion of the belief in the feasibility of eradication of malaria, yaws, tuberculosis and smallpox from the face of the Earth. While stressing the present achievements, the book reflects Dr Soper's profession of faith that the ultimate goal of elimination of many communicable diseases can be attained by a determined and specific attack on the cycle of transmission of these infections. He feels that there is no compelling need for a previous build-up of general health infrastructure and that much can be done by medical science, quite independently of the preliminary social and economic development of underprivileged tropical countries afflicted by all the well known symptoms of the vicious circle of poverty, ignorance and disease.

Many experienced specialists of tropical public health will disagree with this oversimplified persuasion so forcibly expressed. The present consensus of opinion is that the integrated improvement of all aspects of community life offers the best chances for permanent advance of developing countries. The pursuit of some well defined health aspects should be seen not as a goal itself but as a part of the general process of orderly growth of the system of national health determined by socio-economic priorities. This should not be interpreted that economics are the key to any progress. Such an extreme view, reflected by the recent Pearson report on aid to developing countries, virtually ignored the health problems of tropical areas and created some concern among those who are aware of the present situation in the "third world".

Nobody who reads this anthology from the writings of an inspiring, courageous and often ruthless pioneer of preventive medicine on an intercontinental scale will fail to be impressed by the scope of his life-work, by his perseverance and by his vision. Those who know and admire Dr Soper not only as a foremost scientist and medical administrator but also as one of the "most unforgettable characters they have met", may regret the absence of a full page plate with his likeness; the small photograph on the back page of the blurb will not stand much wear in a book that will certainly go through many hands and will appeal to the specialist and to a general reader interested in modern trends of global epidemiology.

L. J. BRUCE-CHWATT

CHIRAL BIOLOGY

Molecular Asymmetry in Biology

By Ronald Bentley. Vol. 2. Pp. xiii + 566. (Molecular Biology: an International Series of Monographs and Textbooks.) (Academic: New York and London, June 1970.) 257*s*.

THIS second volume of *Molecular Asymmetry in Biology* is as good as the first (see review in *Nature*, **226**, 566; 1970). It deals with two major topics: stereospecificity in some important biological processes (four chapters, 344 pp.), and the determination of the configurations of some biologically important compounds (two chapters, 182 pp.). To treat either topic exhaustively would be to cover a large part of natural product chemistry and biochemistry; the particular groups considered in each case are well chosen and thoroughly discussed.

The four chapters on stereospecificity in biological processes cover dehydrogenations, reduction and related reactions; condensations with acyl-coenzyme A derivatives and hydro-lyase reactions; a variety of other reactions important in intermediary metabolism; and biosynthesis and utilization of isoprenoid compounds.

The two chapters on determination of absolute configuration deal with general types of biologically important compounds (carbohydrates, amino-acids, hydroxy-acids, lipids, nucleosides, vitamins, and the like), and a wide variety of isoprenoid and related compounds.

The book is illustrated with many good formulae and diagrams, as was the first volume, and the references are thorough. In a useful preliminary note on nomenclature, the author draws attention to some important unpublished proposals by Hanson and Hirschmann, and corrects an error in stereochemical designation in the first volume (pp. 56 and 214, [4-^{2}H]-mevalonic acid).

The two volumes of Dr Bentley's work together form an admirable introduction to steric aspects of biochemistry, and will point the way to much further interesting work.

W. KLYNE

HANDLE WITH CARE

Infections and Immunosuppression in Subhuman Primates

By H. Balner and W. I. B. Beveridge. (Proceedings of the International Symposium, Rijswijk, December 1969, organized jointly by WHO and the Radiobiological Institute, TNO, Netherlands.) Pp. 258. (Munksgaard: Copenhagen, 1970.) 120 D.Kr.

THE increasing use of subhuman primates in medical and biological research has inevitably led to problems, which were admirably summarized in 1966 by L. V. Meléndez and R. D. Hunt: "... the ecological niches of man and animal cross with increasing frequency and this undoubtedly will create or uncover new disease problems which are also important ... this is a potential threat both for human and animal health". Eleven months ago a timely international symposium was held at Rijswijk, The Netherlands, to discuss the problem and control of communicable diseases in subhuman primates and the prevention of the transfer of disease between man and animals and between animals in captivity. The lowering of resistance of the host by immunosuppressive therapy and the limitations and complications of immunosuppression were also considered in a series of authoritative papers.

It is an onerous task to single out particular contributions from this collection of uniformly excellent papers, which include reviews of tuberculosis, pathogenic enteric bacteria, group B arboviruses, simian pox viruses and more exotic infections such as green monkey disease (Marburg virus), monkey B virus, the outbreak of "monkeypox" in the Rotterdam zoo and descriptions of the epizootics caused by simian haemorrhagic fever virus. The problem of "spontaneous" infections in cell cultures derived from simian tissues, stress and latent pathogens, parasitic opportunism, infections with herpes viruses and the increase in their virulence when infecting species other than their natural host, such as the oncogenicity of *H. saimiri* and squirrel monkey heart isolate (SMHI), are important topics which are also discussed. A masterly account of spontaneous or induced hepatitis in subhuman primates is provided by F. Deinhardt, whose studies of hepatitis in marmosets form one of the

landmarks in recent research in this field. The paper on the handling of monkeys by F. T. Perkins and E. G. Hartley is an outstanding example of clarity in scientific writing, and the reader is guided step by step through the procedures which should be adopted on the reception of animals to the recapture of an escaped simian and the action to be taken if scratched or bitten. G. Mahouy and J. C. Friedmann outline the architectural and technical principles of different barrier systems for the prevention of infection from the outside and measures to reduce the possible spread of infection within a primate unit. The final chapters include superb accounts of the hazards of immunosuppression by D. W. van Bekkum, some observations of hepatic allografts and xenografts by R. Y. Calne and his associates; and R. Cortesini and his colleagues review heterotransplantation in primates.

The many photomicrographs and the photographs of the orang-utans Peek, Barbara and Nina affected by monkeypox are excellent in quality and detail. The line diagrams are clear and helpful and the tables are comprehensive.

The book is strongly recommended to all who are responsible for the care and study of non-human primates in the laboratory, in zoological gardens and animal reserves. Laboratory workers, pathologists, veterinary surgeons, microbiologists, animal house and laboratory designers, administrators responsible for the health of man and his animals and, indeed, anybody interested in the biomedical sciences will find this book an indispensable companion and a mineful of information.

A. J. ZUCKERMAN

SAVING A SHIPYARD

Fairfields

A Study of Industrial Change. By K. J. W. Alexander and C. L. Jenkins. Pp. 286. (Allen Lane (The Penguin Press): London, September 1970.) 50*s*.

IN 1965 a financial crisis hit the Fairfields shipyards in Govan on the Clyde in Scotland. The appointment of a receiver came about two-thirds of the way through the working life of the Shipbuilding Enquiry Committee which had been set up by the Board of Trade to investigate the acute crisis which faced the British shipbuilding industry. The shop stewards at Fairfields launched a vigorous and unrelenting campaign to keep the yard open. The government, as exemplified by the setting up of the shipbuilding enquiry, was interested in saving the shipbuilding industry and in stabilizing employment on the Clyde. But it was unsure—the committee not yet having reported—how to set about doing that.

The catalyst in the situation was Mr (now Sir) Iain Stewart, a Scottish businessman who had been campaigning for "new look" industrial relations on the Clyde, based upon security of earnings and a considerable extension of retraining facilities. His reaction to the threatened closure of Fairfields was that a mass pay-off affecting 2,500 workpeople would destroy any possibility of building better industrial relations in the area. The scene was now set for the unique industrial experiment that this book describes and analyses.

As George Brown said at the time in the House of Commons: "(This is) a quite new partnership between government, private enterprise and the trade unions, the motive being not just to save a recently modernized Scottish shipyard from extinction, but in addition to provide a proving-ground for new relations in the shipbuilding industry which could change the whole image of our country". Here was a unique combination of government, trade union and private enterprise capital; it was to be an industrial proving ground for new ideas and techniques in management and industrial relations.

The authors of this book were both observers and participants in the experiment at the time, and one of them was a member of the Fairfields board. This involvement itself was a unique experiment in academic research. They describe the course of events throughout the structure, at the level of union management and at that of union management within the yard. A detailed study of changing relations within the yard is made. The authors analyse salary and wage patterns, they show the impact of productivity assessments on wage negotiations, and they evaluate the overtime system and deal with problems of absenteeism. Finally, they attempt to assess the successes and failures of the experiment.

In the area of assessment they are faced with two great difficulties. The first is that the aims of the experiment were never set out clearly and comprehensively, so that criteria for judgment may themselves be disputed. But then they were dealing with a real business/work situation and not a laboratory test, and imperfections had to be accepted. The second problem is that the life of the experiment was foreshortened by the merger with other shipyards on the Upper Clyde.

Nonetheless, even if one may wish to quarrel with a particular assessment or query the basis of the judgment, one is left with the conviction that this detailed study of a unique experiment is an important academic event in itself, and that the experiment at Fairfields was worthwhile and ought to be attempted in other industries in different parts of the country in the interests of good industrial relations and cooperation, and for improved industrial efficiency.

I. KUCZYNSKI

ANOTHER REVIEW JOURNAL

Essays in Physics

Edited by G. K. T. Conn and G. N. Fowler. Vol. 1. Pp. ix+164. (Academic: London and New York, June 1970.) 30*s*; $4.50.

"WITH this issue yet another periodical in physics makes its appearance. There are already so many that some explanation, or indeed some defence, is necessary." So begins the preface to the first volume of this new publishing venture and the editors are right to be hesitant. They feel that because of the proliferation of journals in all branches of physics yet another is needed in order "to bring together straightforward accounts of ideas, methods and developments in such a way that these are accessible to as large a number of physicists as possible".

The periodical situation is really becoming intolerable, and what is needed is not a further journal explaining to us what is in the other journals, but a rationalization of the total journal system. This, one hopes, is one of the issues that the European Physical Society is tackling with vigour. In any case, surely we have enough journals of the type "Reviews of . . . ", "Progress of . . . ", "Reports on Progress in . . . ", "Advances in . . . ", "Annals of . . . ", "Comments on . . . ", not to mention "Contemporary . . . " without adding a further "Essays in . . . ".

This having been said, do the articles in this volume provide something new and of a type which does not appear in the established review and survey journals? The answer is clearly no. There are four of them: "Strong Interactions of Elementary Particles", by R. J. Eden; "Electronic Phase Transitions", by David Adler; "Optical Diffraction Methods in Crystal-structure Determinations", by H. Lipson; and "Meteorites", by G. Turner. Each article has value, and to one not well versed in the fields the last two mentioned are particularly fascinating. The point is, however, that there already exists more than a sufficiency of natural homes for each of these articles and to provide another is doing the physics community no service whatsoever.

R. J. BLIN-STOYLE

PHYSICS AND RELATIVITY

Relativity

Edited by Moshe Carmeli, Stuart I. Fickler and Louis Witten. (Proceedings of the Relativity Conference in the Midwest, Cincinnati, Ohio, June 2–6, 1969.) Pp. xii + 381. (Plenum: New York and London, 1970.) $20; 180*s*.

In 1963 R. K. Sachs said in a telling phrase, "General relativity is a legitimate, though minor, part of present fundamental physics . . . because at present it has few interactions with the remainder of physics". In this way he set the stage for a decade of development of the subject and provided a yardstick against which one can judge the steady stream of volumes of conference proceedings and collected papers which shows no sign of diminishing. The present volume reports on a national conference, but the diversity of activity in North America is sufficient to provide a view that reflects most of the recent developments in the subject.

Among others the quantization programme is reviewed by Komar and quantum gravity pursued by Klauder and Misner. A development which promises more relations with the rest of physics is foreshadowed in the Boltzmann theory paper of Anderson and in a note on the Einstein–Liouville equations by Berezdivin and Sachs. The experimental side is looked after by a report of Weber on his detection of radiation and a very valuable survey of the use of low temperature techniques by Everitt, Fairbank and Hamilton. Singularities are particularly well dealt with by Geroch, and the reader who wishes to whet his teeth may be directed towards Geroch's solution of twenty problems which he believes to be fairly difficult, and all unsolved, on pages 287–9. The fairly new topic of superspace is discussed firstly by Wheeler in a very characteristic paper called "Particles and Geometry", giving one of the usual wide-ranging views of possibilities in the subject, and in more detail by Fischer and DeWitt. Perhaps the most unusual paper is that entitled "Contribution to the History of Einstein's Geometry as a Branch of Physics" by Eugene Guth, which can be read with the greatest of interest by any physicist, including those who consider themselves experts in general relativity.

To what extent then has the subject become related with the rest of physics? The answer provided by this volume is, I think, that the process has definitely begun, but it is likely to be long and painful.

C. W. Kilmister

STELLAR SPECTROSCOPY

The Theory of Stellar Spectra

By Charles R. Cowley. (Topics in Astrophysics and Space Physics.) Pp. xi + 260. (Gordon and Breach: New York and London, May 1970.) 195*s* ($23.40) boards; 70*s* ($8.50) paper.

For approximately 100 years, astronomers have been attempting to deduce detailed information about conditions in stellar atmospheres and the abundances of the chemical elements from the appearance of stellar spectra. The physical concepts used are, on the whole, relatively simple and straightforward. For their understanding they require chiefly a knowledge of the theory of radiation of optical wavelengths and how this radiation interacts with matter in a gaseous phase. Because of the way in which the subject developed, however, the terminology of astrophysics is complex and occasionally contradictory. The result is that he who would measure and analyse stellar spectra in order to understand stars and to find clues to the origin of the chemical elements and to the history of the galaxy often has a difficult time getting into his subject.

Many of the difficulties arise because the young research worker has not had an adequate preparation in the necessary parts of the theory of radiation and of spectroscopy. This book attempts to bridge the gap between an intermediate level of college physics and the introductory stages of astrophysics. Four of the six chapters deal with relevant facets of the theory of matter and radiation, and two deal with astrophysics proper. Chapters one, three, five and six handle the theory of the line absorption coefficient, necessary parts of statistical mechanics, the semi-classical theory of line broadening, and the quantum-mechanical treatment of pressure broadening. Chapters two and four consider typical problems of spectral lines in stellar atmospheres and the classical methods of quantitative analysis of a stellar atmosphere. The book is completed with four appendices, a list of references, an extensive index of symbols used and their various definitions and a good general index.

The list of symbols used is particularly valuable because in many cases, when a particular facet of the physics of radiation is discussed, the notation of a standard textbook on the subject is maintained. This procedure leads to the same letter or symbol sometimes having two or more meanings as one proceeds through the book. Within any one section, however, the particular meaning adopted is there carefully preserved.

The author wishes his book to be a source of encouragement to the astronomer who wants to study stellar spectra without becoming too deeply involved in the non-equilibrium theory of transfer theory. This point of view is supported with statements such as "specifically we shall not concern ourselves with the details of the transfer of radiation within the spectral line" and "no one questions the use of Saha's theory of ionization in the interpretation of the spectral sequence".

I think that an introduction to astrophysics should attempt to sharpen the student's critical power by pointing out the weakness of argument underlying statements such as these and to encourage the student to think more deeply. If the student can be assumed to be prepared to accept, without preamble, derivation of the equation of transfer in general vector notation and to follow without undue struggle a discussion of theoretical line strengths (taken more or less directly from the text on *Atomic Spectra* by Condon and Shortley), he has the necessary analytical knowledge to profit by such an experience. He will then be prepared to advance towards the goal discerned by stellar spectroscopists so many years ago.

Anne B. Underhill

LIPID REVIEW

Topics in Lipid Chemistry

Vol. 1. Edited by F. D. Gunstone. Pp. ix + 451. (Logos, in association with Elek Books: London, July 1970.) 160*s*.

This volume is the first of a series intended to provide a continuing review of the chemistry, physics and technology of lipids, without omitting important biological aspects. In addition, a form of information service is offered by appending a list of relevant books and reviews, but not original papers, published in the past eight years.

Not surprisingly, in an area where the introduction of physico-chemical procedures for separation and characterization of naturally occurring lipids has initiated a vast growth of publications, the chapters tend towards either techniques themselves or the application of techniques to a particular problem. Thus there are comprehensive and authoritative reviews of the use of gas liquid chromatography for the determination of structure of fatty acid esters, of mass spectrometry, and of hydrogenation catalysts. These subjects are clearly of value on account of their generality of application and interdisciplinary

position. A chapter devoted to optically active long chain compounds brings together the techniques applicable to the study of such compounds and the information obtained. Cyclopropane and cyclopropene fatty acids are similarly reviewed in a neat and readable chapter. Milk lipids are reviewed in depth, and the large volume of analytical data accumulating in this commercially important topic makes for heavy reading. Nevertheless, even perusal of a catalogue of the milk fats of seventy-five different species can be rewarding for the general reader, if only to challenge his imagination on how to milk a black rhinoceros.

In conclusion, this volume is an excellent reference source for the specialist, and as such is to be recommended.

P. B. GARLAND

ION-MOLECULE REACTIONS

Ion Molecule Reactions

By E. W. McDaniel, V. Cermak, A. Dalgarno, E. E. Ferguson and L. Friedman. (Wiley Interscience Series in Atomic and Molecular Collisional Processes.) Pp. xiii + 374. (Wiley (Interscience): New York and London, August 1970.) 190*s*.

THE subject which this book treats has in the past few years been expanding so rapidly that a comprehensive review by notable specialists in the field is most welcome. If a verdict may be placed before the evidence, the book may at once be said to be a contribution to the literature which will be an important reference for many years, although other volumes on the same subject should be produced. Perhaps the only serious criticism which can be offered is that, after starting the project in 1964, some portions at least were probably completed by 1968, and a considerable and regrettable delay in its general availability seems to have occurred.

After a short introductory chapter, there follows in the second chapter a comprehensive review of the experimental method relevant to the study of ion-molecule reactions. The use and accurate control of electron bombardment and other similar ion sources is first described, followed by accounts of their adaptation to the measurement of rate coefficients of suitable reactions. Next follows a section on the sampling of ions from electrical discharges and afterglows. These systems, which in some cases mimic the conditions of the upper atmosphere, must be very specially controlled in order to produce reliable values of rate coefficients. These problems of control and some of the solutions adopted are fully described. The section on the "flowing afterglow" technique is of notable length and completeness.

The chapter continues with a section on drift tube techniques which includes a full treatment of the problem of diffusion in conditions of low E/P with simultaneous reaction of the ions. There is then a section describing what would now be called the earlier work of the relatively new field of ion cyclotron resonance. The chapter concludes with a lengthy and valuable review of the information obtained using beam techniques. This includes the description of classical kinematics which is needed for a proper understanding of the results.

The experimental tone of chapter two is continued in chapter four, which gives a review of existing data under the heading "Reaction Rates from Ionospheric and Airglow Data". This chapter, however, quotes only two references dated later than 1967, and those readers wishing for fully up to date information will have to search further.

The fifth chapter is headed "Ion-molecule Chemistry" and seeks to bring these reactions more fully into the gaze of chemists by discussing them in terms of heats of formation of the ions, orders of reaction and the dependence of rate coefficient on energy. A discussion of dependence of rate on system temperature is notably absent from the subject.

The compilation of "Quantitative Data" in chapter six lists all known data on cross-sections and/or rate coefficient, and for each reaction supplies also the "Langevin" rate coefficient for comparison. Tables are given for four classes of reaction. There is then a section in which graphical data on various reactions are assembled. This chapter shows several references dated 1969, plus several results "unpublished" or "to be published", and provides a very valuable collection.

The third chapter is reserved for special mention, because this treatment of the theory of ion-molecule collisions is probably the material least expected by a potential reader already well versed in the subject. Running to nearly a hundred pages, or a quarter of the book, it gives a treatment based on quantum theory which is full without being overwhelming, and is very well timed in its appearance. Improvements in experimental technique have, over several years, made the classical Langevin treatment seem inadequate. The patchwork which has thus developed may, with such a stimulus as the introduction of this chapter provides, evolve into a more coherent and generally satisfactory discussion of the phenomena.

P. F. KNEWSTUBB

FAMOUS INEQUALITIES

Finite Sections of Some Classical Inequalities

By Herbert S. Wilf. (Ergebnisse der Mathematik und ihrer Grenzgebiete, Band 52.) Pp. vii + 83. (Springer-Verlag: Berlin and New York, 1970.) 28 DM; $7.70.

THE problem here investigated may be elucidated by a simple example. For positive a_n, it is known that

$$\sum_1^\infty \{(a_1 + a_2 + \ldots + a_n)/n\}^2 \leq 4 \sum_1^\infty a_n^2$$

where the constant 4 is best possible, that is, cannot be replaced by a smaller number. If now the sums are taken not to ∞ but to an integer N, say, then we may hope to replace 4 by a constant $k(N)$, where $k(N) \to 4$ as $N \to \infty$. The examination of the character of $k(N)$ for a number of the classical inequalities brings together several analytical techniques: asymptotic methods, Toeplitz forms and integral kernels, functional analysis with operators which are not necessarily linear. Hilbert's famous inequality

$$\sum_{m,n}^\infty \{a_m a_n/(m + n)\} < \pi \sum^\infty a_m^2$$

belongs essentially to linear operator theory, and a great deal is known about its corresponding $k(N)$; for instance,

$$k(N) = \pi - \pi^5/2(\log N) + O\{(\log \log N)/(\log N)^3\}$$

On the other hand, non-linear theory is involved in Carlemann's inequality

$$\sum^\infty (a_1 a_2 \ldots a_n)^{1/n} < e \sum^\infty a_n$$

and here the first discussion of the appropriate $k(N)$ is to be found in a delicate and ingenious argument given by de Bruijn in 1963.

The author has provided a concise and lucid account of the present state of knowledge, with a useful bibliography. The young postgraduate worker interested in "hard" analysis will find here many suggestions for further research.

T. A. A. BROADBENT

Correspondence

Government Research

SIR,—Your comments on "Green Paper to Burn" (*Nature*, **228**, 1; 1970) raise again the question of the government's relations with industrial research. A fair amount has been said on the subject, but there is one form of analysis that I have not seen applied simply and directly.

This arises from categorizing research activities in the dimension that ranges from long term, fundamental and speculative work through applied research to development. Such classification can in fact be done quite straightforwardly in terms of the time expected to elapse before the work in question will be applied outside the laboratory. The time scale stretches from about one year for development that will be used very soon to the nearly pure research looking ten or more years ahead. (Work at the ultra-short end of the spectrum could hardly be called R or D.)

Generalizing for the overall national picture, I suggest that industry is looking after 1–2 years work and the universities are covering work at 5–10 years range: with the recent expansion of universities, we need not be unduly worried over shortage of long-term effort.

The serious gap occurs in the intermediate region, research looking, say, 3–6 years ahead. This is the area where spin-off from defence research was most applicable and where the old look of research associations was aimed. Only a few industrial laboratories are long-sighted enough to reach this territory, and the current tendency for research associations and the UKAEA laboratories to look for short-term income is making them also neglect this middle range of applied research.

I have myself come across two projects recently which, under the climate of a few years ago, could have been funded largely with government money. They were recognized to be promising, but because they were somewhat speculative and could only come to fruition in three or four years' time, industry, with its present tight purse-strings, was reluctant to put up the necessary finance. I suspect that these are typical of many cases formulated and unformulated.

Of course, there are honourable exceptions and, of course, diagnosis is easier than cure. But I believe that Britain's technological health will be endangered until this particular weakness in the R and D structure is recognized and attempts made to remedy it.

Yours faithfully,

B. E. NOLTINGK

"Windwhistle",
Nutcombe Lane,
Dorking, Surrey.

A Congress is a Congress is a Congress

SIR,—In view of the opinions expressed in *Nature* (**228**, 13; 1970) on international meetings, you may be interested in the "case history" of the Second International Congress of Parasitology which took place on September 6–12, 1970, in Washington DC and which involved some 1,600 participants from many parts of the world.

The organizers of this congress wanted it to be different from any previous similar gatherings by proposing a greater informality of the meetings and a full opportunity for free and ample discussion. This was attempted by parcelling up the proceedings of the congress into a large number of small "colloquia" on a well defined subject.

In addition there were eight formal meetings under the banner of "Technical Reviews" dealing with topics of wider interest (taxonomy, genetics and evolution of parasites; pathology of parasitic infections; phytonematology; pharmacology of antiparasitic agents; immunity; seroepidemiology; physiology and biochemistry of parasites; drug activity against trematodes).

In organizing the congress the selected chairmen of seventy-five colloquia approached a number of participants with requests for relevant papers. Other unsolicited contributions on the same subjects were grouped accordingly. 980 papers submitted to the congress were published in advance in three special issues of the *Journal of Parasitology* and distributed to the participants at the time of registration.

Chairmen of colloquia urged the participants not to present their papers formally but to make a single point, thus stimulating the discussion. Moreover, participants were not committed to any specific colloquium and were given free rein to follow only their personal interests. All this was done to avoid the general criticism of large international meetings that too little time is left for discussions.

The final result of this unconventional convention was hailed as a success by a majority of the participants. No doubt the new formula worked well in some colloquia and was less effective in others, especially when several chairmen were unsure how to apply it. There were complaints that a large number of colloquia and their simultaneous running were responsible for a degree of frustration in people whose interests were overlapping and who were anxious to be exposed to the greatest possible amount of cross-fertilization.

It seems that the degree of intercommunication achieved by a gathering of scientists is not proportional to the numbers assembled, and that there is a critical limit beyond which even the informal exchange of views becomes difficult. There is no uniform or infallible method applicable to a large congress intending to preserve the intimacy and the free give and take of a small meeting. The Second International Congress of Parasitology was undoubtedly a courageous and an interesting attempt to square this particular circle. The next congress will take place in Munich in 1974. *Qui vivra, verra!*

Yours faithfully

L. J. BRUCE-CHWATT

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Biological Membranes

SIR,—Your Molecular Biology Correspondent, in commenting (*Nature*, **227**, 1294; 1970) on some interesting recent model studies relevant to biological membranes, leaves the impression that it is a new idea of general importance in transport studies that the relative energies of hydration of the alkali cations may be responsible for their differing affinities for anionic or polar sites. I would like to correct this impression by drawing attention to the extensive studies by Eisenman[1] of the origins of ionic specificity which include a quantitative treatment of the dependence of ionic specificity on hydration energy. Eisenman's work during the last decade has been superbly reviewed, with special reference to its validity in biology, by Diamond and Wright[2].

Yours faithfully,

MARTIN DAVIES

Department of Biology,
University of York,
Heslington, York.

[1] Eisenman, G., in *Symposium on Membrane Transport and Metabolism* (edit. by Kleinzeller, A., and Kotyk, A.), 163 (Academic Press, New York, 1961).

[2] Diamond, J. M., and Wright, E. M., *Ann. Rev. Physiol.*, **31**, 581 (1969).

Announcements

University News

Mr J. E. L. Farradane has been appointed the first director of the Centre for Information Science of the **City University.**

Dr G. W. Bisset, National Institute for Medical Research, has been appointed to the chair of pharmacology, St Thomas's Hospital Medical School, **University of London.** The title of professor of preventive dentistry has been conferred on **Dr M. N. Naylor** in respect of his post at Guy's Hospital Medical School and that of professor of chemistry has been conferred on **Dr Mary R. Truter** in respect of her post at University College.

Dr I. A. Menzies, University of Manchester Institute of Science and Technology, has been appointed to the new chair of materials technology in **Loughborough University of Technology.**

Professor Frank Morton has been appointed to a personal chair in chemical engineering in the **University of Manchester. Mr M. C. G. Israels** has been appointed professor (part-time) of clinical haematology and **Dr S. Thomas** has been appointed to a chair of physiology.

Appointments

Mr Bryan Rofe has been appointed director of the Antarctic Division of the **Commonwealth Department of Supply, Melbourne.**

Lord Drumalbyn has been appointed to succeed **Lord Bessborough** as deputy chairman of the **Metrication Board.**

Sir Brian Windeyer, vice-chancellor of the University of London, will be chairman of the new **National Radiological Protection Board,** established to advance knowledge and provide information on protection from radiation hazards. Members of the board include **Sir Charles Cunningham,** UKAEA; **Mr J. G. Duncan,** MRC; **Professor N. Feather,** University of Edinburgh; **Mr F. W. Fenning,** AERE; **Dr E. E. Pochin,** MRC Clinical Research Department; **Dr Derek Pringle,** Nuclear Enterprises Limited; **Professor R. E. Steiner,** University of London Postgraduate Medical School.

Miscellaneous

Professor W. T. Singleton, University of Aston in Birmingham, has been made the first Distinguished Foreign Colleague of the **American Human Factors Society.**

Mr J. M. Lumley of Monash University has been awarded a **Rutherford Scholarship** for three years for work in low temperature physics at the Cavendish Laboratory, Cambridge.

Professor G. Melchers of the Max-Planck Institut für Biologie, D 74 Tübingen, Correnstr. 41, would like to hear from anybody who is engaged in the culture of haploid plants from anthers. This information would be appreciated because an EMBO workshop on this topic is planned for next year.

Applications are invited for two **Ramsay memorial fellowships,** a British (general) fellowship and a fellowship limited to candidates educated at the Universities of Glasgow or Strathclyde or who propose to work there. The fellowships are normally tenable for two years. Further information can be obtained from the Joint Honorary Secretaries, Ramsay Memorial Fellowships Trust, University College London, Gower Street, London WC1.

The **Geological Society of Uganda** was established last month at Makerere University, Kampala. The society aims to encourage the study of the earth sciences, particularly of and in Uganda, and hopes to sponsor symposia and excursions. **Mr C. E. Tamale-Ssali** is president of the society, and members of council include **Professor R. Macdonald** (hon. secretary), **Dr K. Tinkler** (hon. treasurer), **Mr J. E. N. Kagule Magambo** (hon. editor), **Mr M. D. Cruikshanks** and **Mr E. S. Balaba.** Further information can be obtained from Dr K. Tinkler, PO Box 7062, Kampala, Uganda.

ERRATUM. The electronic processing mentioned by our Materials Science Correspondent (*Nature*, **228**, 19; 1970) was developed at the Standard Telecommunications Laboratories, Harlow, and not at the Services Electronic Research Laboratory.

ERRATUM. In the review "Scavenging Cells" by F. Jacoby (*Nature*, **228**, 188; 1970) the word "macrophage" on lines 1 and 2 of the third paragraph should read "macrophages".

ERRATUM. In her written answer in the House of Commons on July 20 (*Nature*, **227**, 431; 1970), Mrs Margaret Thatcher said that the Government hopes to increase the present annual number of preclinical entry places to medical schools from 2,700 to 3,700 by 1975. This was mistakenly quoted as an increase of between 2,700 and 3,700 places.

International Meetings

January 5–7, 1971, **The Impact of New Techniques on Palaeontology,** Belfast (Dr A. D. Wright, Geology Department, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland).

January 5–7, 1971, **Solid State Physics,** Manchester (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

January 5–8, 1971, **Radiation Effects and the Mitotic Cycle,** Manchester (Dr Alma Howard, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX).

January 6, 1971, **Sorption Phenomena in Vacuum,** Liverpool (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

January 6–8, 1971, **Chemical Effects at Bearing Surfaces,** Swansea (Mr P. L. Hurricks, Swansea Tribology Centre, University College of Swansea, Singleton Park, Swansea SA2 8PP).

January 7–8, 1971, **Numerical Methods for Unconstrained Optimization,** Teddington (Secretary, Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY).

January 11–18, 1971, **Tropical Ecology emphasizing Organic Productivity,** New Delhi (Professor R. Misra, Department of Botany, Banaras Hindu University, Varanasi 5, Uttar Pradesh, India).

January 18–25, 1971, **Pathological Wilting in Plants,** Madras (Professor T. S. Sadasivan, Centre of Advanced Study in Botany, University Botany Laboratory, Madras 5, India).

January 27–February 3, 1971, **Plant Pathology,** New Delhi (S. P. Raychaudhuri, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi 12, India).

February 28–March 4, 1971, **Analytical Chemistry and Applied Spectroscopy,** Cleveland (Dr W. G. Fateley, Carnegie-Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213, USA).

February 28–March 4, 1971, **Structure and Function of Nucleic Acids,** Banff, Alberta (Dr Gordon Tener, Department of Biochemistry, University of British Columbia, Vancouver 8, Canada).

March 1–3, 1971, **Antibiotics,** Ste Marguerite, Quebec (Dr S. Rakhit, Ayerst Laboratories, PO Box 6115, Montreal, Quebec, Canada).

March 9–12, 1971, **The Exploitation of the Oceans,** Bordeaux (Secretariat du Colloque, c/o CNEXO, BP 107, Paris XVI, France).

March 16–18, 1971, **Electrical Safety in Hazardous Environments,** London (Manager, Conference Department, IEE, Savoy Place, London WC2R 0BL).

March 17, 1971, **Weather Forecasting for Agriculture and Industry,** Aberystwyth, (J. A. Taylor, Llandinam Building, University College of Wales, Penglais, Aberystwyth, Cardiganshire, Wales).

March 23–26, 1971, **Negative Ions,** Liverpool (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

March 24–26, 1971, **Structure and Growth of Thin Films,** Southampton (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

March 29–31, 1971, **Non-ionizing Radiation Safety,** Cincinnati (Laser Laboratory, Children's Hospital Research Foundation, Cincinnati, Ohio 45229, USA).

March 29–April 2, 1971, **Statistical Mechanics,** Chicago (Professor Stuart A. Rice, James Franck Institute, University of Chicago, 5640 Ellis Avenue, Chicago, Illinois 60637, USA).

March 30–31, 1971, **Electrochemical Engineering,** Newcastle upon Tyne (Professor J. D. Thornton, University of Newcastle upon Tyne, Merz Court, Claremont Road, Newcastle upon Tyne NE1 7RU).

March 31–April 1, 1971, **Meat in the Future—Problems and Solutions,** London (General Secretary, Institute of Biology, 41 Queen's Gate, London SW7).

April 1–3, 1971, **Applications of the Laser in Biology, Medicine and Dentistry,** Cincinnati (Laser Laboratory, Children's Hospital Research Foundation, Cincinnati, Ohio 45229, USA).

April 5–7, 1971, **Elementary Particle Physics,** Lancaster (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

April 5–8, 1971, **Conduction in Low Mobility Materials,** Eilat (D. S. Tannhauser, Department of Physics, Technion, Haifa, Israel).

April 14–16, 1971, **Electrical Conduction in Organic Solids,** Nottingham (Dr M. R. Willis, Chemistry Department, University of Nottingham, Nottingham NG7 2RD).

April 15–16, 1971, **Industrial Innovation through Contract Research,** Newcastle upon Tyne (R. G. Atkinson, International Research and Development Co. Ltd, Fossway, Newcastle upon Tyne NE6 2YD).

April 15–17, 1971, **Cereal Fodders in the Feeding of Domestic Animals,** Milan (Societa Italiana per il Progresso della Zootecnica, via Monte Ortigara 35, 20137 Milano, Italy).

April 15–21, 1971, **Radiationless Processes in Photochemistry,** Portmeiron, North Wales (Dr B. Brocklehurst, Department of Chemistry, The University Sheffield S3 7HF).

April 20–22, 1971, **Internal Flows,** Salford, (Administrative Assistant, University of Salford, 43 The Crescent, Salford M5 4WT).

April 21–29, 1971, **International Welding and Metal Fabrication Exhibition,** London (Industrial and Trade Fairs Ltd, Commonwealth House, New Oxford Street, London SW1).

May 3–7, 1971, **Man-made Lakes—their Problems and Environmental Effects,** Knoxville, Tennessee (Professor W. C. Ackerman, Scientific Committee on Water Research, Illinois State Water Survey, Box 232, Urbana, Illinois 61801, USA).

May 4, 1971, **Crop Protection,** Ghent (Symposium Secretary, Rijksfaculteit der Landbouwwetenschappen, Coupure Links 235, Ghent, Belgium).

May 8, 1971, **Stereochemistry,** Lucerne (Professor H. Musso, Institut für Organische Chemie der Universität, Richard Willstätter-Allee, 75 Karlsruhe 1, Germany).

May 17–20, 1971, **Australian Biochemical Society Conference,** Brisbane (Dr J. N. Mansbridge, Department of Biochemistry, University of Queensland, St Lucia, Queensland 4067, Australia).

May 17–21, 1971, **Remote Sensing of Environment,** Ann Arbor (Extension Service, Conference Department, University of Michigan, Ann Arbor, Michigan 48104, USA).

May 25–26, 1971, **Earthquake Engineering Research,** Vancouver (Dr S. Cherry, Earthquake Engineering Research Conference, Civil Engineering Department, University of British Columbia, Vancouver 8, BC, Canada).

British Diary

Tuesday, October 27

Correlation of River Flow Data for Yield Assessment (5.30 p.m. informal discussion) Institution of Civil Engineers, at Great George Street, London SW1.

Diabetes, Diet and Biochemistry (5.30 p.m.) Professor F. G. Young, FRS, Research Defence Society, in the Lecture Theatre of the Zoological Society of London, Regents Park, London NW1 (Thirty-ninth Stephen Paget Memorial Lecture).

Further Thoughts on W. T. Brande (5.30 p.m.) Mr Edward Ironmonger, Royal Institution Library Circle, at 21 Albemarle Street, London W1.

Genetic Polymorphism (5.30 p.m.) Professor Harry Harris, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Second of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

The Analysis of Malignancy by Cell Fusion (5 p.m.) Professor Henry Harris, FRS, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2. (Sixth Michael Cross Memorial Lecture.)

The Physics of a Polymer Molecule (5.30 p.m.) Professor S. F. Edwards, University of London, in Mechanical Engineering Lecture Theatre A, Imperial College, London SW7.

The Rational Society (5 p.m.) Professor S. J. Gould, University of London, at the London School of Economics and Political Science, Houghton Street, Aldwych, London WC2.

Wednesday, October 28

Lavoisier's "Opuscules Physiques et Chimiques", 1774 (1 p.m.) Mr Frank Greenaway, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Light Sources: Electrodeless Discharge Tubes *versus* Hollow Cathode Lamps (7 p.m. discussion meeting) Society for Analytical Chemistry, Atomic Spectroscopy Group, at the Leicester Lounge, Glasshouse Street, London W1.

Pollution Control (three-day course) in the Department of Chemistry, Liverpool Polytechnic, Byrom Street, Liverpool.

The Bibliography of Charles Darwin (6 p.m.) Mr Richard Freeman, Society for the Bibliography of Natural History, in the Men's Staff Common Room, University College London, Gower Street, London WC1.

The Brunels (5.30 p.m.) Lady Gladwyn, Institution of Civil Engineers, jointly with the British Section of the Société des Ingénieurs Civils de France, at Great George Street, London SW1.

The Thawing of Frozen Foodstuffs (6.15 p.m.) Mr D. W. Everington and Mr E. R. Cox, Society of Chemical Industry, Food Group—Food Engineering Panel, at 14 Belgrave Square, London SW1.

Thursday, October 29

Biological Aspects of Water Pollution (3 p.m. symposium) Society for Analytical Chemistry, Biological Methods Group, at the Wellcome Building, Euston Road, London NW1.

Crystallization: a Study in Molecular Engineering (5.30 p.m.) Professor J. W. Mullin, University of London, in the Engineering Theatre, University College London, Gower Street, London WC1 (Inaugural Lecture).

Economics of Solvent Extraction (2 p.m. symposium) Society of Chemical Industry, at 14 Belgrave Square, London SW1.

New Chrysanthemum Varieties Developed at Wye College (8.15 p.m.) Mr A. A. Jackson, University of London, at Swanley Hall, Withersdane, Wye College, near Ashford, Kent.

Paroxysmal Nocturnal Haemoglobinuria (5.30 p.m.) Professor J. V. Davie, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Third of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Some Reflection upon London's Passenger Transport (5.15 p.m.) Professor T. C. Barker, University of London, at Bedford College, Regents Park, London NW1.

The Application of Forensic Science to Archaeological Problems (5 p.m.) Professor R. G. Harrison, British Academy of Forensic Sciences at the Royal Society of Arts, John Adam Street, London WC2.

Theory *versus* Reality in Organizational Structure (5.30 p.m. informal discussion) Institution of Civil Engineers, at Great George Street, London SW1.

Friday, October 30

Air Pollution and its Effects on Man (9 p.m.) Mr P. J. Lawther, Roya Institution, at 21 Albemarle Street, London W1.

Photochemistry of Aromatic N-Oxide Compounds (1 p.m.) Dr C. Lohs Royal Institution, Photochemistry Discussion Group, at 21 Albemar Street, London W1.

Powder Coatings (7.15 p.m.) Mr G. T. Bassett, Oil and Colour Chemist Association, jointly with the Birmingham PVL Club, at the Hav thorns Hotel, Bristol.

Monday, November 2

Development, Use and Requirements of STOL Aircraft (5.30 p.r informal discussion) Institution of Civil Engineers, at Great Geor Street, London SW1.

Protective Coatings for Gas Pipe Lines and Storage Vessels (7 p.m Dr J. T. Harrison, in the Bullock Lecture Theatre, Hull College Technology, Hull.

The Chemical Industry and Government-Sponsored Corporatio (6.30 p.m.) Sir Frank Schon, Society of Chemical Industry, in t Scientific Societies Lecture Theatre, 23 Savile Row, London W1.

The Pharmacology of Neuromuscular Transmission (6 p.m.) Profess W. C. Bowman, University of London, at the Royal Postgradua Medical School, Du Cane Road, London W12.

What is the Future for Linkage Synthesis? (6 p.m. discussion) Instituti of Mechanical Engineers, Applied Mechanics Group, at 1 Birdca Walk, London SW1.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Natural Environmental Research Council. Report of the Council for period 1 April 1969–31 March 1970. Pp. vii+108. (London: HMSO, 197 13*s* (65*p*) net.

Philosophical Transactions of the Royal Society of London. B: Biologi Sciences. Vol. 259, No. 830 (10 September 1970): Marine Algae of Solomon Islands. By H. B. S. Womersley and A. Bailey. Pp. 257–35 plates 24–27. (London: The Royal Society, 1970.) 56*s*; $7.25.

Royal Observatory Annals. No. 4: Observations of Stellar Paral Vol. 3, from photographs taken at the Royal Observatory, Greenwich, the years 1924 to 1939. Prepared under the direction of the Astronor Royal. Pp. 148. (London: Science Research Council, 1970.) 50*s* net.

Little Botany, Big Botany. By Professor D. H. Jennings. Inaugu Lecture delivered 29 October, 1969. (University of Liverpool Inaugu Lecture Series.) Pp. 26. (Liverpool: Liverpool University Press, 19 7*s* 6*d*.

Other Countries

Bulletin of the Museum of Comparative Zoology, Harvard Univers Vol. 140, No. 2 (August 6, 1970): Studies on the Fossil Snake *Dinil patagonica* Woodward. Part 1: Cranial Morphology. By Richard E T. H. Frazzetta, and Ernest E. Williams. Pp. 25–74. Vol. 140. N (August 6, 1970): The Lynx Spider Genus *Hamataliwa* in Mexico and Cen America (Araneae: Oxyopidae). By Allen R. Brady. Pp. 75–128. Vol. No. 4 (August 6, 1970): The Spider Family Oecobiidae in North Ame Mexico, and the West Indies. By William A. Shear. Pp. 129–164. (C bridge, Mass.: Museum of Comparative Zoology, Harvard Univer 1970.)

US Department of the Interior: Geological Survey. Water-Supply P 1840-C: Summary of Floods in the United States During 1964. By J Rostvedt *et al.* Pp. vi+124. $0.60. Water-Supply Paper 1870-A: Fl of December 1966 in Southwestern Utah. By Elmer Butler and J Mundorff. Pp. iv+40+2 plates. (Washington, DC: Government Prir Office, 1970.)

Gamma Field Symposia No. 7: The Present State of Mutation Breedi Report of International Symposium held on August 15 to 17, 1968. Pp. 136. (Ohmiyi-machi, Ibaraki-ken, Japan: Institute of Radiation Bree Ministry of Agriculture and Forestry, 1970.)

Smithsonian Contributions to Zoology. No. 43: Revision of New W Species of Anthrax (Diptera: Bombyliidae), other than the Anthrax fasciatus Group. By Norman Marston. Pp. 148. $1.75. No. 53: Rev of Some Species Referred to Leanira Kinberg (Polychaeta: Sigalioni By Marian H. Pettibone. Pp. 25. $0·40. (Washington, DC: Smiths Institution Press, 1970. For sale by US Government Printing Office.)

Australia: Commonwealth Scientific and Industrial Research Orga tion. Annual Report of the Division of Mechanical Engineering 1968/ Pp. 67. (Melbourne: CSIRO, 1970.)

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OCTOBER 31, 1970

Ungilding the Lily

THE British Government has fired an ominous shot across the bows of the research councils in its declaration, on Tuesday this week, of the steps which are to be taken in an attempt to reduce public expenditure. Mr Anthony Barber, the Chancellor of the Exchequer, said that he was proposing to reduce the total cost of the research councils by £2 million in the year beginning next April and then to look for further savings from the recommendations of a review of the work of the research councils now being carried out by the Council for Scientific Policy (see page 400). On the face of things, at least, £2 million from one year's budget, at present running at £115 million a year, will not make much difference. It should be possible to rob Peter to pay off Paul without much trouble. At this stage, the more distant implications of Mr Barber's statement are more worrying. By all accounts, he is hoping that part of the present cost of running the research councils can be transferred from the public purse to industry, but it is exceedingly hard to see precisely what he can have in mind. Indeed, the objective of government policy in the past ten years, ever since the Trend report, has been to isolate the substantially long-term functions of the research councils from the government's more direct concern with the sponsorship of industry and of industrial innovation. It may be sensible—certainly it is consistent—for the new government to abolish the Industrial Reorganization Corporation in the belief that this is something which industry could well undertake on its own behalf. For the most part, however, the research councils are concerned with much less practical matters for which industry would be unable on strictly commercial grounds to foot the bill.

Where can Mr Barber's review body hope to find some slack? The Science Research Council is about as lean an organization as any could be. Some of its research grants are at present handed out to people with one foot in industry and another in the academic world, but this is not a large part of the council's expenditure. For the rest, the Science Research Council is a tautly organized body, chiefly preoccupied with the interests of the universities. If anything, there is a case for more and not less public support, especially with postgraduate teaching. It will be regrettable if the new government does not openly acknowledge that central government must these days assume responsibility for the cost of the research that has become a necessary part of higher education. The case of the Medical Research Council is different, for a large proportion of what it spends goes on research institutes and units the staff of which remains on the council's payroll. Very little of the research which the council supports is of such direct interest that industry could be expected to foot the bill. There may be a case for a different way of spending the money, with more research grants and fewer units, but that will not make Mr Barber's ship come home.

So are the Agricultural Research Council and the Natural Environment Research Council the chief candidates for economy? The temptation will be, of course, to wonder whether some of the Agricultural Research Council's stations—Rothamsted for example—could not raise part of their keep by means of subventions from agricultural producers to whom the research station is a blessing. The trouble, of course, is that the government's interest is to ensure that work of this kind is supported whether or not the commercial interests are willing to pay the cost continuously. To be sure, there are other parts of the Agricultural Research Council's work that may seem more vulnerable—that is something for them to worry about. Even the Natural Environment Research Council, however, has very little fat on it in spite of its ambitions to embark on ocean engineering and other vaguely industrial projects. To be sure, there might be a case for looking again at the need that the Natural Environment Research Council should exist separately from the other research councils—its arrival on the scene has not been a conspicuous success. But even such a radical reversal of the present government's plans is unlikely to save much money.

Now that Mr Barber has thrown down his gauntlet, it is important that he and his colleagues should be made to appreciate the role of public expenditure on research councils. The first objective, and a sufficient justification by itself, is the stiffening of higher education with a good backing of research. The second is to support research of a kind likely to keep the British scientific community alive and on its toes. The third is to provide British industry with a sufficiently rich source of innovation for economic growth to be sustained. None of these objectives can be neglected without serious damage, possibly in the long run. No amount of urgency to save money in the financial years immediately ahead can justify such profligacy.

Benn's House Dismantled

THE new British Government has been swift in dismantling one of the pieces of apparatus of the previous government most offensive to Conservative doctrine—the Ministry of Technology. The White Paper on the reorganization of central government, published last week (HMSO, 2*s* 6*d*), is chiefly concerned with the dismemberment of the ministry along lines which have been openly hinted at for the past six months, but particularly since the accession of Mr John Davies to the Ministry of Technology in July. First of all, the

functions of the Ministry of Technology in the making of industrial policy are to be united with the functions of the old Board of Trade in a new Department of Trade and Industry. Second, the responsibility of the old Ministry of Technology for the aerospace industry has been transferred to a new organization, the Ministry of Aviation Supply. This, however, is intended to be simply a temporary measure, for the White Paper says that the government is looking for some way to integrate all defence research development and procurement within a single organization, most probably within the Department of Defence. In the long run, this development is likely to be of crucial importance not merely for the defence laboratories with which the Labour Government never quite managed to grapple but also for the new government's policy—or method of making policy—on civil aviation. A third innovation, at least of nomenclature, is the setting up of a Department of the Environment to unite the old Ministries of Housing and Local Government, Public Building and Works, and Transport. A fourth development of some importance is the decision that in future the Ministry of Overseas Development should become part of the Department of Foreign and Commonwealth Affairs.

These changes are important not merely for what they imply for the immediate future but also because they may throw light on the way in which the new government will manage its affairs. The linking together of the industrial responsibilities of the old Ministry of Technology and the old Board of Trade is, however, a sensible step—the tension between the Board of Trade and the Ministry of Technology in Mr Wedgwood Benn's days was something of a legend. The most serious cause for anxiety under the new arrangement is that there will be a tendency for the scientific work of the government laboratories, and particularly that of the National Physical Laboratory, to be undervalued. What does the new government plan to do with this part of its inheritance? And what, in any case, is to happen with the laboratories of the Atomic Energy Authority, now presumably more unpopular than ever? Will Aldermaston, for example, finish up with the Ministry of Defence? And will Mr John Davies be happy to let Dr Walter Marshall pursue the ambition of making Harwell into a more or less self-supporting institution? No amount of paper reorganization can avoid the need to make decisions on these points.

The plan to carry out a thorough analysis of the functioning of the defence laboratories is also to be welcomed, although there is no obvious reason why at this stage the government should have assumed that defence research must ultimately be united with the Ministry of Defence. What of laboratories such as Farnborough and Malvern, now both with the Ministry of Aviation Supply? Is there not a reason for thinking that each of these laboratories has a valuable part to play in the development of civil industry in Britain? And should not each of them be edged in that direction? It is to be hoped that in the few months set aside for this examination, the government will give serious attention to the danger that its proposals may make less than full use of these important facilities for research. In the long run, there may be some value in retaining an independent organization such as the Ministry of Aviation Procurement as a device for undertaking all kinds of research and development in the semi-public sector. Telecommunications are a crying need.

The Department of the Environment is the most dubious of the new proposals, if only because it may be nothing more than an attempt to ride the bandwagon of the moment without changing anything important. To be sure, there is a need that responsibility for city planning, previously with the Ministry of Housing and Local Government, should be more closely linked with responsibility for transport planning, previously with the Ministry of Transport. It is also important that policies for the economic development of regions of the British Isles should be coordinated with those for the development of new cities. In that sense, the new arrangement may have something to contribute towards better government. But will the creation of a super-ministry make it any easier for the new government to decide on a better policy for the encouragement of economic growth outside the London region than that on which the fancy of the Labour Government fell? This is a case in which the reorganization may be taken as a sign that urgent problems have been solved or at least have been made to disappear.

The proof of the pudding will be in the eating and this is where it may be crucial to know how faithfully the government follows its own precept, spelled out in the White Paper, that there must be a better method than in the past for ordering the priorities of central government. There is, for example, to be a central policy review staff at the Cabinet Office, working not merely for the Prime Minister but for the other departments of government. There is to be a better or at least more selfconscious procedure for the review of public expenditure—one of the parts of the previous system for which the new White Paper has a few good words to say. The trouble, of course, is that no amount of good resolutions can make up for a lack of will to operate the machine efficiently. This, in the long run, is where the new government's battle will be won or lost.

Palace and Pollution

For three days of the week just past, 850 people have thronged Guildhall in London as delegates to the third conference of the organization which has for the past seven years been known as "The Countryside in 1970". To the extent that many of the participants are people whose work would normally have led to them to erect new factories, build new roads and commission new types of aircraft, the conference may indeed have done something to abate the nuisances which it has held up for public complaint. Whether the occasion, or the activities which have preceded it, will have a more lasting effect, only time will tell. On the face of things, however, the outlook is not bright. The Countryside in

1970 has been a splendid theme for harnessing the energy and the emotion of the Duke of Edinburgh to a public cause, but he must already be wondering whether he has backed the right horse.

The conference this week seems to have been a strange pantechnicon for allowing all kinds of organizations to stand up in public and declare their fondness for the proper management of the environment. Not merely the National Union of Students but the Greater London Council have been able to publish some cheerful platitudes about their special interest in the environment—the GLC, for example, busy with its gargantuan and controversial scheme for building urban motorways in London, is nevertheless able to pat itself on the back with statements such as "the GLC is taking practical measures to ensure that Londoners are given the opportunity to enjoy life to the full in a satisfactory environment" and "the problems of traffic noise, mentioned elsewhere in this paper, must also be solved". The chairman of the Natural Environment Research Council, Lord Wynne Edwards, seems to have devised the term technosphere (the complement of biosphere) as part of the jargon of the new ecology. Urban development has been widely berated, and the continuing increase of the population of Britain has been considered as an ominous threat to the amenities of British life. In spite of the practical character of many of the reports which appeared as working papers at the conference, there has been an awkward sense that conservation is next to godliness and that, in any case, the management of the environment needs some central mechanism for keeping the wayward tendencies of ordinary people within bounds. And in spite of the protests of some of the delegates, the conference has been an occasion for resurrecting old concepts such as the view that there is nothing wrong with the environment that would not be cured if people were abolished.

In discussions like these, it is customary to balance the potential benefits of some course of action against the disadvantages—pesticides kill insects but also damage (or may damage) more desirable species, for example. Roads permit movement but create noise. Plastic bottles are a convenience until they are emptied and then become a nuisance. The conference this week seems to have fought shy of the most serious problem of all—the ineradicable conflict between the wish of most people to become more prosperous and the likelihood that, if they do so, they will help to damage the environment. This, certainly, is why the solicitude of prosperous populations such as that of the United States for the environment sounds hollow to people in developing countries (and it is significant if no doubt illogical that the ban on the major uses of DDT which has been imposed in the United States does not prevent American companies from exporting this supposedly global pollutant to countries where the need for effective pesticides is as urgent as it has ever been). Within nations such as Britain, the cry for a better environment and for more care in preserving natural surroundings makes more sense to the well-to-do than to those still hoping that they may yet be able to imitate their betters by owning a car and driving it about the countryside at the weekend. This week's conference has been most seriously deficient in the way in which this essentially political issue was carefully glossed over. But what are the steps that should be taken to ensure that proper preservation of the environment does not become an obstacle to the economic advancement of those communities or sections of communities which at present are disadvantaged ?

The issue is splendidly if poignantly illustrated by the problem of the national parks, which in Britain and the United States tend nowadays to be crowded with visitors whenever there is a fine day in the summer. In both countries, schemes for restricting access to some places like these are being talked about in a desultory fashion. There is a great danger, however, that in seeking more vigorously to preserve the character of the environment as it is at present, the conservationists may be alienating those who moderately look forward to pursuits that were respectable in the thirties or even the fifties. In this sense as in many others, the conservation movement has yet to rid itself of the taint of eclecticism.

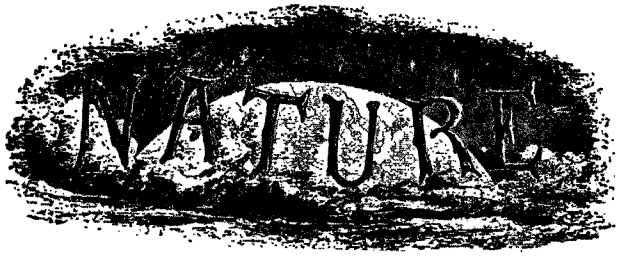
The equitable resolution of these and other dilemmas is unlikely to be acceptable to all conservationists. Because of the way in which the population of Britain has grown to more than fifty million people, and because it would be unrealistic as well as unjust to expect to contain the population within seventy million, it may for example be impossible to preserve large parts of south-east England in what might be thought of as a more or less natural condition. Isolated pockets of greenery may remain, but already there are good reasons to think that the Green Belt which the old managers of London threw about the city in the thirties is now as much a source of discontent as an indispensable physical asset. Nobody at the conference this week set out to calculate the economic cost of the city planning policies which at present restrict the development of industry and commerce within the London Green Belt and which preserve that institution intact. But may not this entail such a large economic handicap for the country as a whole that the lives of people living elsewhere are less prosperous than they might be ?

To raise these and other awkward questions does not of course imply that many of the things now being done in the name of conservation are entirely beside the point. A better understanding of what happens within the environment is entirely to be welcomed, especially when divorced from the view that "ecology is a state of mind". The view that it may also be necessary to manage the natural environment of densely populated countries such as Britain so as to permit the fullest use of the countryside for leisure rather than for agricultural production is also something to be grappled with, the National Farmers' Union permitting. But it is by no means obvious that conferences like that held this week will stimulate the hard-headed thinking necessary if methods are to be found of allowing all kinds of people to lead the lives they want to lead, within cities and outside them.

Two opposing tendencies are disquieting. There is

a risk that conservationists may be found to have embraced unnecessarily authoritarian views of what should be done to preserve the natural environment. Another possibility is that conferences like that at Guildhall this week will have allowed many people to pretend that they are on the side of the angels while caring nothing for the better management of the environment. What circumstances may urgently require is that there should be a more flexible view of the interaction between modern life and the problem of conservation. Is it not important that jet aircraft, much reviled, have nevertheless made it possible for thousands of Englishmen to spend their summer holidays on other people's less crowded beaches? And is it not important that industrial technology has provided them with the prosperity with which to pay their fare? In the long run, the narrowness of last week's conference will be its undoing. Next time round, it is to be hoped that the Duke of Edinburgh will not lend his support to the supposed conflict between technology and the environment but rather to the view that both of them can be exploited to everybody's benefit.

100 Years Ago



In a word, henceforward there must be a portion of every day taken up with teaching children by objects, specimens, or experiments, the nature of the great laws by which the universe is governed. We cannot argue here on the necessity for this knowledge. Look at that great German army, recently spoken of as the most wonderful military engine ever seen on the face of the earth. What makes it so? The intelligence of each individual of which it is composed. It is the same with wheels and pistons, spindles, hammers, chisels, and ploughs, as with guns and bayonets: the more intelligent the man is who wields or superintends them, the more successfully and prosperously will they do their work. Ten years ago Mr. Whitworth astonished the Manchester manufacturers with the account of the machines he had seen in America. "Why should we not have such machines here?" said the Manchester men. "Because," said Mr. Whitworth, "you have not intelligent hands to work them." And for these long ten years we have gone on talking about educating our working classes, and allowing priceless treasures to pass out of our hands. Every portion of Europe, as well as the United States of America, is stealing something of our rightful wealth and increasing our pauperism, because of our stolid indifference to the introduction of those branches of human knowledge which alone can properly develop the powers of industry and application, of which the English people are so wonderfully capable.

From Nature, **2**, 509, *October* 27, 1870.

OLD WORLD

PUBLIC EXPENDITURE

Cuts in Research Grants

THE cuts in public expenditure announced last Tuesday by Mr Barber, Chancellor of the Exchequer, will be received by the research councils, the Industrial Reorganization Corporation and some parts of the social services with dismay. The budget for the research councils, now about £110 million a year, will be cut by £2 million next year, rising to about £5 million by 1974–75. The Industrial Reorganization Corporation is to be wound up completely, and many subsidies will be abolished and charges increased in the social services.

These are casualties of the government's desire to see public expenditure substantially reduced to pave the way for cuts in taxation. Mr Barber's axe has been calculated to remove £1,100 from public expenditure by 1974–75, and to reduce the annual rate of growth in public expenditure to 2·8 per cent a year, compared with a rate of 3·5 per cent which was implied by previous plans.

Although a cut of £2 million from the combined budgets of the research councils does not seem too harsh, the long-term implications are more worrying. Sir Brian Flowers, chairman of the Science Research Council, said last month, for example (*Nature*, **227**, 1284; 1970) that the growth rate of the SRC's budget is already too small for the council to support many important areas of research. It also seems less likely now that the government will be prepared to sanction British participation in the CERN project, on which the SRC has set its heart. Coupled with the announcement of cuts in the budgets of the research councils is the indication that "this field of expenditure is now being critically reviewed to establish whether activities are being carried on which might more appropriately be financed by industry, and whether there is duplication of effort". The review is being carried out by the Council for Scientific Policy, which will report to the Secretary of State for Education and Science.

It is no surprise that the government is to wind up the Industrial Reorganization Corporation. Such a measure is in line with the policy, outlined in the Queen's speech and repeatedly at the Conservative Party conference earlier this month, to liberate industry from government interference. The Conservative Party has always opposed the IRC's powers to influence industrial mergers, and winding up the corporation will result in savings next year of between £20 and £30 million.

As far as education is concerned, Mr Barber had little to say, and no mention was made of the costs of higher education. Charges for school meals will, of course, be increased, and supply of free milk to pupils over the age of seven will be discontinued, both measures being part of Mr Barber's thesis that "where the user can afford it, he should bear more of the cost and the taxpayer less". The same principle guides the government's decision to institute charges for admission to national galleries and museums. Mr Barber did, however, commit the government to spending an additional £110 million during the next four years on the social services, chiefly for new hospital facilities.

SELECT COMMITTEES

Science and Technology Reprieved

MR WILLIAM WHITELAW, Leader of the House of Commons, seems to have had a slight change of heart during the Summer recess. Although his Green Paper on the select committees of the House of Commons (HMSO, 3*s*) which was published last week reflects many of the intentions he announced last July (see *Nature*, **227**, 217; 1970), he has spared some of the specialist select committees—including the Select Committee on Science and Technology—at least for the present session.

Guided by a desire to reduce demands on members' time, while at the same time maintaining an effective machinery for scrutinizing the work of the executive, Mr Whitelaw has taken up many of the recommendations of the Select Committee on Procedure. The most important is that the Estimates Committee should be restyled into an Expenditure Committee with a membership of about 45. The new committee would "focus its attention on public expenditure rather than on the Supply Estimates and examine a wider selection of issues arising in this field".

Mr Whitelaw parts company with the Select Committee on Procedure in his proposals for the specialist select committees, however. The Green Paper suggests that the Select Committee on Nationalized Industry should be retained and that it should continue to have general oversight of the whole range of activities of the nationalized industries. Similarly, it is suggested that the Select Committee on Science and Technology should continue with its work. The Procedure Committee suggested that these committees should be scrapped, and that the proposed Expenditure Committee should take over some of their functions, but Mr Whitelaw believes that a dual system in which the three exist side by side would provide a more effective instrument for scrutinizing the work of public corporations. Also to be retained are the select committees on Race Relations and Immigration and on Scottish Affairs. But the Select Committee on Education and Science will not be reappointed; the evidence on teacher training which the committee took last session will be made available to the proposed Committee of Enquiry into the Training of Teachers.

The retention of the Select Committee on Science and Technology is something of a triumph for the pressure placed on Mr Whitelaw by some of his own backbenchers as well as by some of the opposition members of the committee. But what will happen to the enquiries on which it was engaged before the election is open to question. It seems likely, however, that the enquiry into the computer industry and into the British space research programme will be continued, while the investigation of the consequences of population growth in Britain may be scrapped or postponed.

The membership of the Select Committee on Science and Technology will also be somewhat different from that which sat before the election. Five members of the old committee failed to be re-elected last June, and the change of government means that the chairmanship of the committee will go to a Conservative member. Mr Airey Neave seems to be the most likely candidate, and Mr Arthur Palmer, chairman of the old committee, will probably be given the chairmanship of one of the subcommittees.

OIL STRIKES

Dividends from the North Sea

BRITISH PETROLEUM'S oil strike just inside the British sector of the North Sea confirms that the discovery last June of a large field in the Norwegian sector by the Philips consortium was not just an isolated freak. The strikes suggest that there is a wide distribution of oil under the North Sea. The BP strike lies about 100 miles north-west of the Philips Ekofisk field, along a line which passes through the Ekofisk field itself and several minor traces of oil found by BP and other groups. This places it in 350 feet of water (rather deeper than the Philips find) and more than 100 miles east of Aberdeen, so that many technical problems remain to be solved before Britain will feel the benefits of this discovery in terms of hard cash.

That the eventual fruits of this discovery will go in large measure to the government is beyond question —under the present taxation system, 12½ per cent of the well-head value of the oil is taken in tax, while a further 45 per cent of any eventual profits is subject to corporation tax. Moreover, 49 per cent of resulting dividends go to the government as the major shareholder in BP, and two of the blocks adjacent to BP's find are licensed by groups in which the National Coal Board is a chief partner.

In spite of the difficulties involved in bringing oil ashore compared with natural gas, the financial incentives are attractive. Oil will sell at commercial rates, whereas gas must be sold to the Gas Council, which has used its monopoly to keep the price for North Sea gas down to 2·87 pence per therm. BP is also already trying out the underwater construction of wells and has formed a partnership with Westinghouse Electric and the French groups CFP and Group Deep to design equipment suitable for oil production in deep waters.

The problem of financing a pipeline remains, however, and although BP claims that the cost should be about £25 million (equivalent to two new supertankers) this is almost certainly overoptimistic. But there will undoubtedly be other groups interested in forming a partnership comparable with the BP/Sohio deal which is providing the capital for the development of BP's Alaskan wells. There is also the possibility that the company could sell some of its Middle East interests. It seems likely that North Sea oil can be brought ashore for about two dollars a barrel, making it highly competitive with Middle East and African supplies.

Earlier claims that North Sea oil might eventually provide most of Europe's requirements seem to have been rather extravagant. The new BP field will do well simply to keep pace with domestic requirements, which are increasing by nearly 6 per cent a year. The oil could start coming ashore in commercial quantities in 1972, when it should provide about 10 per cent of Britain's requirements.

OIL POLLUTION

Inglorious Pacific Glory

THE precarious position of the tanker Pacific Glory on a shingle bank off the southern tip of the Isle of Wight raises once again the spectre of the Torrey Canyon and a country ill equipped to deal with widespread pollution. But fortunately a cogent series of emer-

gency measures have been devised, and the picture is now one of being prepared for the worst.

Responsibility for pollution at sea divides itself into two parts; outside a one mile limit it belongs to the Department of Trade and Industry, but closer to the coast it is very much in the hands of the local authorities and therefore of the Department of the Environment. The report of a committee set up under Sir Solly Zuckerman after the disastrous spill of Easter 1967 advocated the setting up of a coordinated chain of command along the coast with responsibility for anti-pollution measures. In the case of the Pacific Glory, for example, responsibility is vested in the Marine Superintendent at the Marine Survey offices in Southampton and the overall situation, as it affects the coastline and beaches, will be overseen from an Operations Room at the Department of the Environment.

The local centres of command have been equipped with a device designed at the Warren Springs Laboratory, Stevenage, which can be fitted to an ocean-going tug. As the tug passes through the water covered with oil a wooden arrangement, which has been likened to a five barred gate, stirs up the sea and oil while detergent is sprayed from a boom on the tug. The oil is much more easily disposed of naturally by microorganisms when emulsified in fairly small drops.

Sir Solly Zuckerman's committee also recommended that a group of experts should be available in such emergencies to give advice of a scientific nature. The Central Scientific Unit set up in April at the then Ministry of Housing and Local Government under Dr Martin Holdgate is expected to provide the expertise if it should be needed.

The hope is, of course, that the Pacific Glory's oil burden can be removed without her back breaking, but the incident raises the question of the safety of large tankers, especially with the advent of the 250,000 ton giants. Internationally, however, the situation is complicated because both the Pacific Glory and the Torrey Canyon sailed under the Liberian flag of convenience with its attendant tax advantages. There was criticism of the thoroughness of the Liberian government enquiry into the Torrey Canyon disaster and the Department of Trade and Industry has now announced that a preliminary enquiry will be held into the causes of the latest incident. The powers of a possible full British Board of Enquiry are rather unclear, however, because the collision took place outside territorial waters.

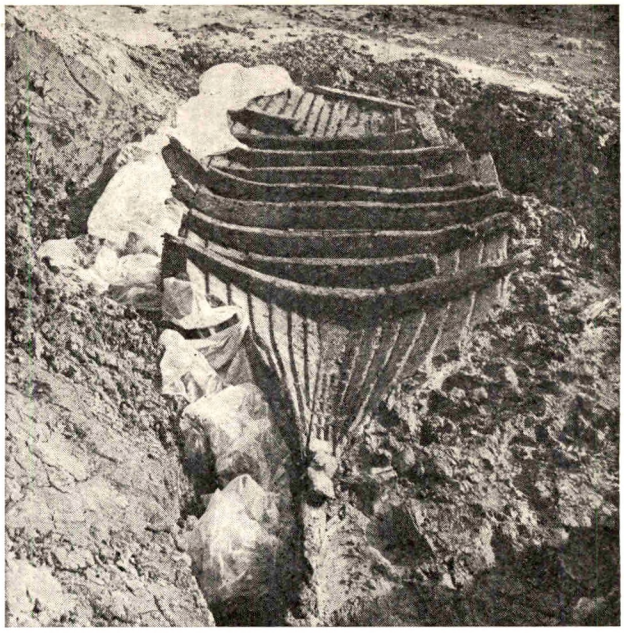
After two maritime misfortunes which it seems should never have happened, it is questionable whether large tankers can any longer be allowed to ply busy seaways such as the English Channel without the kind of supervision which is now accepted in air corridors. The days when individual crews navigate their own lethal loads are surely almost past.

ARCHAEOLOGY

Oldest English Ship Unearthed

from a Correspondent

WHAT may be the oldest English ship yet traced has been unearthed during drainage works in the Seasalter marshes of Kent. It has since been measured, excavated and raised in a joint operation of the National Maritime Museum, Greenwich, and the British Museum. The boat is lodged in thick clay some 10 feet from the surface in the bank of the channel. Local archaeologists called in experts from Greenwich and the team from the British Museum that recently re-assessed the Sutton Hoo 7th century ship burial site, and part of a new ship gallery under construction at the Greenwich museum, has been set aside for the find when it has been conserved and reconstructed.



Reproduced by permission of the National Maritime Museum.

The vessel was originally about 40 feet long, of which 32 feet has survived, and 8 feet in the beam. It is clinker built in the Northern European or Viking style, and has a long curving stem which was probably repeated at the end which is lost. The robust build and size indicate that it could have carried cargo in the open sea. It was presumably rowed—there is no sign of a mast step though a jury mast might have been rigged for running before the wind.

Estimates of its age so far rest chiefly on the style in which it was built, resembling in some aspects the Viking ship tradition of the period from AD 600 to 1100 in which the Sutton Hoo ship lies. The Seasalter vessel is of generally "Northern European" construction, but it is heavier and more roughly finished than was typical. It is built from native oak. This may suggest that it was built before the Sutton Hoo ship, but alternatively it may belong to the period when Viking tradition had begun to fade or was being modified by Mediterranean influence. The date may not be fixed until carbon-14 evidence becomes available.

ENVIRONMENT

Countryside Seven Years After

As European Conservation Year reached a frenzied climax of talk, the Countryside in 1970 conference opened in London on October 26. A large concourse of people, gathered in the Guildhall, had before them an array of reports on the environment which had been in preparation since the first conference on this topic in 1963. Although some bold speakers attempted to raise debate on the reports, most seemed to feel obliged to use their few minutes to air their own, all too familiar, points of view.

The proceedings were opened by the Duke of Edinburgh who struck a political note by welcoming the recent government reorganization which created the Department of the Environment. He also advocated yet more administrative coordination, but noted that the real bar to environmental progress is likely to be the allocation and spending of the necessary funds.

Urbanization and industry dominated the first day's proceedings, and so perhaps it was to be expected that population and economic growth should have had their usual airing. The naturalist Mr Richard Fitter propounded a basic equation of conservation: $q=r/p^2$, where q is the quality of life, r represents available resources and p is the population. If r cannot be increased, Mr Fitter said, then to maintain q, the only thing to do is to keep p steady. He was supported by a call for the conference to ask the government to provide everybody in Britain with free contraceptives. There was applause for a demand for a national population policy and an end to the striving for economic growth at all costs.

The industrialists, not surprisingly, leapt to their own defence, pointing out, in the person of Mr A. E. Higginson, of the Institute of Public Cleansing, that in the manufacturing industries it is often cheaper to use fresh raw materials than to recycle wastes. In a very competitive world, he said, to the scorn of many a conservationist, the price and quality of goods are paramount. The industrialists were, of course, also quick to point out how much they have done for conservation. The Sand and Gravel Association, for example, is to give an award for the best example of restored sand and gravel workings.

But an applause meter would have shown that many of the delegates were not impressed by such industrial largesse. For once, the National Union of Students received a wide measure of support. Mr G. Searle, a member of the NUS executive, called for recycling of wastes and a firm hand on companies seeking to build factories on sites of natural beauty. Mr Searle also lamented the lack of debate in the proceedings, but doubtless it is difficult to bring new arguments to bear at this stage in the environmental crisis. It is time for action now.

FOOT AND MOUTH

Mystery Outbreak at Pirbright

Six cattle in a special holding pen outside the experimental compound, but within the security perimeter, of the Animal Virus Research Institute at Pirbright, Surrey, recently developed symptoms of foot and mouth disease. Within a few hours three more animals had contracted the infection, and shortly afterwards the entire group of eighteen animals had to be destroyed. There has been considerable speculation about the cause of the outbreak, but the source of infection is still not known, and, apart from long term projects whose progress could not be jeopardized, research has been suspended while investigations are in progress.

There seem to be three ways in which the outbreak could have arisen. First, the virus may have been brought in from outside the institute, but this frightening possibility is fortunately remote, because no other outbreaks have been reported in the area. Alternatively, the disease may have been carried physically from the experimental compound to the holding pens; again this seems unlikely because the Pirbright administration are fully aware of this danger and their security precautions are rigorous. Finally, a few virus particles may have escaped through a temporary breakdown in the filters which clean the outgoing air from the experimental compound. These filters were checked and found to be working perfectly as soon as the outbreak was confirmed, but it is well known that no biological filter can be more than 99·9 per cent efficient, and this seems the most likely source of the infection.

The chance of such an escape is a danger that all disease research stations live with, but in ten years there have been only two such incidents at Pirbright and both have been confined within the perimeter of the institute.

The role that may be played by the wind in spreading viral infections is still a matter of contention (see, for example, *Nature*, **227**, 860; 1970) and so the possibility that viral particles may be able to defeat the highly sophisticated filters developed at Pirbright is a cause for concern. But the risk to livestock on the farms surrounding Pirbright and elsewhere seems minimal. Dr J. B. Brooksby, director of the institute, pointed out last week that for 35 years the institute has carried out a full research programme, using the distinctive African variety of virus, without air filtration and with no greater incidence of infection than in the rest of the country.

PESTICIDES

More Research Required

The overwhelming impression left by the third report of the Research Committee on Toxic Chemicals (Agricultural Research Council, available from HMSO, 5*s*) is that a great deal more information will have to be collated before conclusions can be reached and the right decisions taken. The report has comforting things to say about the level of DDT in human fatty tissue but about little else. Six appendices provide a host of factual information but otherwise serve only to illustrate how difficult it is to make specific recommendations on insufficient data.

The committee's first report in 1964 placed special emphasis on the need for more research into the effects of pesticides in fresh water, and almost one third of the present report is concerned with this topic. Fresh and rain water in Britain contains small but not insignificant amounts of the chief persistent organochloride pesticides, DDT, lindane and dieldrin. The estimated amount lies between 3 and 30 parts in 10^{11}. The report concludes that such a level is within the margins of safety, but precariously so. Experiments have indicated that if the concentration of any organochloride residue should rise to more than 1 part in 10^{10}, freshwater fish and other aquatic organisms would be at considerable risk.

The report suggests three methods of approaching the problem. First, a coordinated system of surveillance should be established in collaboration with the River Authorities and the Natural Environment Research Council to plot the pattern of pesticide distribution in rivers, lakes and reservoirs. Second, the ways in which pesticides may affect organisms against which they are not directed must be examined in much greater depth. The final recommendation deals with the destruction of pesticides. It is difficult to destroy pesticides once they

have become diluted in rivers and lakes, and the report suggests that biological methods of neutralizing harmful chemicals should be sought and applied before factory wastes are voided into rivers and streams.

This last point emphasizes a new and disturbing feature of chemical pollution. The report mentions that increasingly larger amounts of the polychloro-biphenyl compounds (PCBs) are being detected, especially in bird carcasses. These are not pesticides, but are widely used in industrial processes. Neither their distribution in the environment nor their effect on living organisms is yet known in detail, but they are distantly related chemically to the organochloride pesticides, and like these compounds they are persistent, and become concentrated to a high degree in animal tissues.

The report describes some methods of pest control which might be substituted for pesticides, including the use of natural predators and sterilization, but notes that, in general, the progress made in developing these techniques has been disappointing. Pesticides remain the most economical and effective form of pest control, but further research is clearly required if the pesticides are to be controlled as effectively as the pests.

SATELLITES

Back to Nature

from our Soviet Correspondent

THE recent launch of Kosmos 368 comes as an interesting variation in a series of satellites whose payloads have been either unannounced, or which consist of physical experiments. Kosmos 368 is stated to include "scientific apparatus for testing experimental life support systems for laboratory animals, for the further study of the effect of space flight on living organisms and for the continuation of the investigation of the physical characteristics of space".

It is several years since the Soviet space programme concentrated on the orbiting of test animals. This new emphasis on life support systems, following hard on the heels of clear hints that Moon missions, let alone interplanetary missions, will remain unmanned at least in the foreseeable future (see *Nature*, **228**, 6; 1970), seems to indicate that there is to be a new attack on the problems of weightlessness. Such an attack may be a preliminary to establishing a manned space station as a base for manned exploration of the Moon or planets.

The Kosmos series of satellites, inaugurated on March 16, 1962, has long been recognized to be not only a regular programme of investigation of the upper atmosphere but also a useful label to attach to a diversity of launchings, including unmanned precursors of manned missions, failures of comsats or interplanetary probes and a number of special projects such as testing of electrical generators, automatic docking and the orbiting of dogs.

HIGHER EDUCATION

Bursting at the Seams

by our Education Correspondent

IF children now in Britain's junior schools are to be given the same opportunity of having a higher education as those now in the sixth forms of secondary schools, the number of places available in institutions of higher education must nearly double in the next ten years. By the same token, unless dramatic savings can be made in the cost of higher education, government expenditure in that sector must also nearly double by 1981. These are the chief conclusions to be drawn from reports published by the Planning Unit of the Department of Education and Science and the Scottish Education Department (*Student Numbers in Higher Education in England and Wales*, HMSO, 12*s*; *Student Numbers in Higher Education in Scotland*, HMSO, 2*s* 3*d*).

What the planning unit has done is to estimate the numbers of school leavers who are likely to achieve at least the minimum qualifications for entry into higher education during the 1970s. This estimate is then used to predict the number of places which must be made available to maintain the demand for them at its present level. The predictions are based on trends established during the 1960s and publication of these reports simply puts flesh on the skeleton which the DES has used for the past year as a basis for its discussions on the development of higher education.

The report suggests that by 1981, 835,000 full-time students will have to be accommodated in higher education establishments in Great Britain, compared with about 509,000 in 1971. The universities' share of these students, the report suggests, may be about 54 per cent, or 460,000. The costs of such an expansion are reckoned to increase expenditure on higher education in England and Wales from £590 million to £980 million over the next ten years.

These predictions are, however, simply the results of a planning exercise which seeks to point out the consequences of maintaining the demand for places at its present level. They do not imply any government commitment to higher education expansion and they make no attempt to assess the extent to which existing establishments can increase their capacity. Moreover, no account is taken of any possible changes in the structure of higher education. The chief function of the reports is therefore to provide Mrs Thatcher, Secretary of State for Education and Science, with a framework in which to plan the development of higher education and they also help to underline the urgency with which policies must be formulated.

The vice chancellors have already told the government that the number of places which existing universities can reasonably hope to provide by 1981 is 50,000 less than the DES predictions suggest should be made available (see *Nature*, **226**, 790; 1970). If the shortfall is to be taken up by establishing new universities, a commitment must therefore be made very soon; otherwise the new capacity will be established too late to affect the situation by 1980. The report also suggests that the greatest increase in demand for places is likely to be in the advanced higher education sector—the sector which includes the polytechnics whose role has still to be fully worked out.

It is the projected costs, however, that are likely to cause the most concern. The estimates suggest that expenditure on higher education will grow at 5·4 per cent a year, which means that higher education would take a larger and larger share of government expenditure. Mrs Thatcher will therefore have to convince her cabinet colleagues that the axe must not fall as heavily on higher education as on other areas, or new ways of saving on unit costs must be found. The vice

chancellors and the AUT have already rejected most of the measures canvassed by the Labour government, and Mrs Thatcher must therefore look very closely at alternative suggestions, such as introduction of a two-year degree course. The alternative is to cut back on the number of places and to restrict entry to higher education during the 1970s.

Nobel Prizes

Peace Award for Agriculturist

THE 1970 Nobel Prize for Peace, worth more than £32,000, has been awarded to Dr Norman Borlaug, geneticist and director of the Mexican International Maize and Wheat Improvement Centre (IMWIC). Although the announcement came as something of a surprise, Dr Borlaug's contribution towards easing the world's food problem makes him eminently worthy of the honour. This contribution can be measured by the number of developing countries which in recent years have greatly improved their wheat yields using the seed stocks pioneered by Dr Borlaug and his research team.

Norman Ernest Borlaug was born in Cresco, Iowa, in 1914, and received a PhD in plant pathology from the University of Minnesota in 1942. He joined the Rockefeller Foundation in 1944 and was assigned as geneticist to an agricultural project which the foundation was carrying out in collaboration with the Mexican Government. Envisaged originally as a scheme to help Mexican agriculture, IMWIC has since become well known for the practical aid it gives to stimulate cereal production in countries struggling to break out of the vicious circle of hunger and poverty.

Dr Borlaug is best known for his work on the development of dwarf wheats—high-yielding crop plants into which have been bred dwarfing genes from the Japanese 'Norin' varieties. These hybrid plants are very much shorter than normal wheat, which means that the wheat-stalk can support a heavier head of grain without collapsing.

But this is only half of the picture. Dr Borlaug had the foresight to understand that developing superior types of cereal seed was not enough. It was essential that the seed should be brought to the attention of the governments of needy countries, grown using adequate irrigation and fertilizer, and the crop skilfully harvested and distributed. He tackled the problem in two ways: by constant travelling to advise and persuade governments and industries to provide the necessary funds and facilities, and by training, in Mexico, young agricultural graduates from many of the countries which could put the newly developed seed to best use. The benefit of Dr Borlaug's vision has been felt already in India, where wheat yield has more than doubled in three years.

Two Physicists honoured

THE news that Professor Hannes Alfvén of the University of California, San Diego, has been awarded a joint share in the physics prize will come as a pleasant surprise to astronomers, who have long felt the need for such recognition of astronomy. Although the award is for physics, and not specifically for astronomy, Alfvén's work on plasma physics and magnetohydrodynamics has been used chiefly in astronomy rather than any other field. Born in Sweden in 1908, Alfvén has held many official posts in Swedish government councils and academic societies; he is a Fellow of the Royal Astronomical Society, and was awarded a Gold Medal by them in 1967 for "his fundamental work on cosmical electrodynamics".

Whilst it has been a feature of Alfvén's career that many of his ideas are considered somewhat eccentric, there have usually been elements of great value within his elaborate theories, so that piece by piece he has built up an impressive list of positive contributions to astronomy. His work is fundamental to the concept of "frozen" lines of magnetic force being held inside a plasma, a phenomena of great importance to any understanding of the processes occurring in the magnetosphere, the atmospheres of stars, and interstellar matter.

With the Nobel honours in physics falling in the past three years to nuclear or high energy physicists it comes as no surprise that Professor Louis Neel, a solid state physicist, has been selected to share this year's award. Although Professor Neel is perhaps most widely known for his pioneering work on the theory of antiferromagnetism he became involved in a very broad range of problems throughout many aspects of magnetism.

Born in Lyon in 1904, Professor Neel spent the early part of his scientific life at the University of Strasbourg, where he published two of his celebrated papers explaining the basis of antiferromagnetism.

In 1954 he became director of the Institut polytechnique. Three years later he took charge of the Centre d'Etudes Nucléaires at Grenoble, and has devoted considerable effort within the last decade to building up the large campus at the University.

It was just after the war that he published his theory of ferrimagnetism, a type of magnetism which has assumed growing importance in recent years because of the electrical properties that seem to be associated with it. Neel showed how the magnetic structure of a ferrimagnetic material, was related to, but essentially different from, that in antiferromagnets. During the immediate post-war period he also carried fundamental work on the properties of small magnetic particles, which pointed the way towards the development of modern magnetic materials. His other accomplishments include a study of the domain structure of ferromagnetic materials and work on the theory of coercivity in ferromagnets.

Biochemist for Chemistry Prize

PROFESSOR LUIS F. LELOIR receives the chemistry prize for the light which his research shed on carbohydrate metabolism, in particular the synthesis of glycogen. In the 1950s he demonstrated that the elongation of the α-1→4-glucan chain proceeded by a stepwise transfer of the glucose moiety from uridine diphosphate glucose to the non-reducing end of the polysaccharide. The wider relevance of nucleoside diphosphate sugars soon became apparent and this discovery complemented the enzymological work on phosphorylase for which C. F. and G. T. Cori and B. A. Houssay were awarded the Nobel prize for physiology and medicine in 1947. Although well known internationally, Leloir has worked almost exclusively in his own country, in a villa on the outskirts of Buenos Aires, and in far from ideal conditions. His meagre funds were to a large extent from his own resources and from a benefactor after whom his laboratory, the Fundación Campomar, is named.

NEW WORLD

Scientists and Senators against Arms Race

by our Washington Correspondent

THE scientific community may not have fielded any candidates in next week's elections but it will have at least a small voice in determining the composition of the next Senate and the way it votes on certain pertinent issues. The organs of that voice are two lobbying groups, the Council for a Livable World and the Federation of American Scientists*, both of them governed and largely supported by scientists. Up to now the two groups have been concerned almost exclusively with the arms race but the Federation is preparing to broaden its scope to include matters of more special concern to scientists such as employment and federal funding of science.

The Federation, founded after the last war by physicists concerned to see the control of atomic weapons in civilian hands, lapsed into a long quiescence once this task was done and has only come to life again since the appointment of a full time director in July this year. The Council for a Livable World has a more recent and sturdy history. Formed in 1962 by the physicist Leo Szilard with the purpose of halting the arms race, the Council focuses its efforts on the Senate, holding seminars on controversial military issues and contributing to the campaign chests of Senators or candidates who share its views. A tangible measure of the Council's strength is that this year it has 12,000 supporters and hopes to have contributed some $400,000 by election day to twelve Senate candidates.

The full time director of the Council is Thomas A. Halsted who from an office on Capitol Hill arranges the seminars, advises the Council on how it should act on particular issues, and coordinates activities with other arms control groups. Halsted is not a scientist by training but worked for two years in the US Arms Control and Disarmament Agency before becoming director of the Council in 1967. Policy decisions are taken by the Council's board of directors of which the chairman is William von E. Doering, professor of chemistry at Harvard. The twelve member board shares three of its members in common with the council of the Federation of American Scientists—Bernard T. Feld, professor of physics at MIT, who is also the president of the Council for a Livable World, Matthew S. Meselson, professor of biology at Harvard University, and George W. Rathjens, professor of political science at MIT.

The Council's campaign funds are small in relation to need and have to be spent judiciously for effect. The Council's money would be a drop in the bucket in states like New York or California but in places like Montana small sums can go far. Races where both candidates are either favourable or unfavourable to the Council's ideas are not worth investing in. A candidate's party is not a consideration but in fact the Council has supported only five Republicans and thirty-five Democrats in the four elections in which it has been involved. Thirty of the Council's forty candidates have won their races, of which perhaps the most gratifying was that of Senator McGovern who in 1962 received a contribution of $20,000 and came home by a margin of 597 votes. This year the Council is supporting candidates such as Senator Albert Gore of Tennessee, chairman of the Senate Disarmament Subcommittee and an important opponent of the anti-ballistic missile system (ABM), who is facing what is expected to be a close fight. Another ABM critic, Senator Frank Moss, is being supported for re-election in Utah, and a new candidate, Howard M. Metzenbaum, already victorious over astronaut John Glenn in a primary, has the Council's backing in the Ohio race against representative Robert Taft.

The Federation of American Scientists has also been active in the elections. This week it entered into public battle with the American Security Council, a political group targeted against liberal candidates and claiming that the United States has allowed the development of a "megatonnage gap" with the Soviet Union. But the Federation's existence as a political force lies in the future. At the moment it has only 1,500 members whose dues scarcely suffice to support the present level of activities. After many moribund years the Federation was aroused, among other things, by the noise of the ABM debate in the Senate, which showed that scientists' voices could be heard even if they did not prevail. Jeremy J. Stone, who became the Federation's full time director in July, is a mathematician with a long standing interest in the arms race and experience at the Hudson Institute and the Harvard Center of International Affairs.

In anticipation of a broader membership, the Federation has extended its interests from just arms control to matters of more particular concern to scientists. The present brochure describes the FAS as standing for six policies: arms limitation and disarmament, the reconversion of defence industry to peaceful purposes, improvements in the quality of life by the enlightened application of science, informing Congress on matters of concern to the FAS, rights of scientists in respect of travel and security regulations, and a "vigorous scientific community". Under the last heading the Federation expresses its concern with the administration and funding of science and imbalances in the supply and demand in scientific specialties.

The basic policies of the Federation are decided by the 24 member council but considerable latitude is allowed to members; individual chapters, for example, may make public pronouncements as long as they are not in conflict with general policy, and in practice the

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director, Dr Stone, has wide discretion on day-to-day issues.

The success of the membership campaign is crucial to the Federation's future, which its recent few months of activity have at least established as promising. The Federation's lobbying efforts during the ABM debate did not take the opponents of the system to victory, or even as close to it as last year's tied vote, but at least were effective enough to draw criticism from several pro-ABM senators. The Federation was defended by Senator Cranston who praised its "invaluable" expertise.

By the nature of their business lobbying groups such as the Federation and the Council for a Livable World cannot claim any given proportion of the anti-ABM vote as being due to their efforts. But seen in retrospect there has been a significant shift in the Administration's position on ABM, to which the intensive lobbying efforts of the scientific community has certainly contributed in some measure. Halsted has recently described the "pivotal role" played by scientists in the lobbying effort: "They gave senators and congressmen confidence in their ability to counter Administration arguments; they contributed invaluable information and advice to grass-roots citizens and organizations, which they often started. . . . And the controversy in turn has had an important effect on the scientific community itself, providing the stimulus to the formation of lasting activist political organizations and alliances through which concerned scientists could become more effectively involved in discussion of public policy". The Council has been the channel through which much of the scientific evidence in the ABM debate was delivered and to that extent can take credit for what the evidence has achieved. It has at any rate received the enviable though maybe ironic accolade from Senator Charles Mathias that on issues such as arms control and ABM, "the Council for a Livable World is one of the most effective organizations in Washington . . . with the possible exception of the Pentagon".

In the coming months Halsted sees the Council's activities being directed towards getting productive results from the Strategic Arms Limitations Talks, of which the third round starts on November 2 in Helsinki. Another issue will be the Geneva protocol on chemical and biological weapons which President Nixon has asked Congress to ratify though with certain reservations. The Council at least has the advantage of having an unchanging goal in sight, the limitation of the arms race. It remains to be seen how far the Federation of American Scientists can preserve unanimity in extending its activities to the more controversial private affairs of the scientific community. What with the downturn in Federal funding of academic science and in military spending, the unemployment that this has brought in its train, and a vaguely felt but widespread disenchantment with science and technology, scientists as a group are probably more ready to be politicized than ever before. For different reasons neither the National Academy of Sciences nor professional bodies such as the American Association for the Advancement of Science can openly step down into the political arena. The Federation of American Scientists, if it plays its card right, could find itself representing a solid constituency. With admirable braggadocio it is already billing itself as the "largest lobbying arm of science".

DRUGS

Cyclamates Farce not appreciated

by our Washington Correspondent

A BRUTAL inquiry into the management of the cyclamates farce has left the Food and Drug Administration with little that could be called a reputation. Reporting on the hearings it held in June, the House Subcommittee on Intergovernmental Operations has accused the FDA of failing to protect the public against possible health hazards associated with cyclamates despite a clear legal obligation to do so, of aggravating the consequences of its lethargy by allowing the use of cyclamates in food to reach massive proportions, of contravening its own regulations by allowing foods containing cyclamates to be marketed as drugs, and of sundry other offences and misdemeanours. Many of these charges have been denied by Dr Charles Edwards, the present Commissioner of the FDA.

The salient tergiversations of the cyclamates melodrama have been as follows. On Saturday, October 18 last year, acting on the basis of evidence received by the FDA five days previously, Mr Robert Finch, the then Secretary of the Department of Health, Education and Welfare (HEW), announced that cyclamates were to be removed from the list of substances generally recognized as safe (the GRAS list). The announcement sparked off a train of equally precipitate bans in other countries, notably Britain, where hitherto respectable scientific advisory committees hastened to follow Mr Finch's footsteps without troubling to see where he was going. They, like many others, failed to appreciate the significance of what Mr Finch also said at the time, "I expect that in the future these products will be labelled as drugs, to be consumed on the advice of a physician".

A month later, on November 18, it transpired that Mr Finch had indeed correctly predicted the conclusions to be arrived at by a group of scientists known as the Medical Advisory Group. The group, appointed by HEW after Mr Finch's announcement, duly reported that cyclamated products should continue to be made available for the benefit of diabetics on a non-prescription, drug-labelled basis. Following this finding, the FDA drew up the appropriate regulations which allowed cyclamated products to be sold as before, except that they now carried a label saying they were for use only by diabetics or obese people under medical supervision.

Cyclamates enjoyed their new lease of life as drugs only until August 4 this year, when the HEW Medical Advisory Group on Cyclamates met once more and rescinded its previous decision, declaring that cyclamates should now not even be allowed as drugs. The reason given for this volte-face was that new evidence on the carcinogenic effects of cyclamates in rats brought the permissible safe level for human consumption so low that very few calories would be saved thereby, hence cyclamates were ineffective as drugs. The "new evidence" became available in the form of a FDA memorandum dated November 26, 1969, one week after the group's original decision to allow the use of cyclamates as drugs.

The FDA's conduct in the cyclamates case clearly warranted some investigation and the enquiry became the subject of hearings in June before the House Sub-

committee on Intergovernmental Operations chaired by representative L. H. Fountain (see *Nature*, **226**, 1084; 1970). In the report now published, which serves as a resonant epilogue to the drama it describes, the Subcommittee has put together a withering indictment of the FDA's role at each stage of the proceedings. The chief failings, as Mr Fountain's subcommittee sees them, can be summarized as follows:

- A report prepared by the National Research Council (the operating arm of the National Academy of Sciences) in 1955 stated that the available scientific data did not assure the unrestricted use of cyclamates by children or pregnant women. Nevertheless, when the Food Additives Amendment was passed in 1958, the FDA included cyclamates on the GRAS list, thus foregoing the opportunity afforded by the amendment of putting the onus on the manufacturers to establish the compound's safety.
- The courts have held that when there is genuine difference of opinion among qualified experts about the safety of a substance, it does not deserve a place on the GRAS list. Yet despite a recommendation by the National Research Council in 1962 that cyclamates should be restricted to dietary foods and a further report in 1968 warning against the totally unrestricted use of cyclamates, the FDA did not remove the substances from the GRAS list.
- "Lethargy appears to have played a role in FDA's inaction in this matter" and the lack of appropriate restrictions "also set the stage for unfavourable economic consequences for those who built up large inventories of cyclamate-containing products". (This conclusion may affect the fate of legislation to indemnify food manufacturers which is now pending before the House Judiciary Committee.)
- The decision to permit cyclamates to be marketed as drugs after the ban in October was taken by Mr Finch, the Secretary of HEW, not by FDA, despite the fact that responsibility for enforcing the relevant act (the Federal Food, Drug and Cosmetic Act) had been delegated to the Commissioner of the FDA.
- The HEW's Medical Advisory Group that recommended cyclamates should be available as drugs did so on the basis of scientific evidence no different from that on which the FDA had recommended they be removed from the GRAS list. In any case the real issue at this stage was whether the law permitted Mr Finch's already announced intention to allow the marketing of cyclamates as non-prescription drugs; this was a question that the Medical Advisory Group was not qualified to determine.
- In order to implement Mr Finch's decision that cyclamates should be marketable as drugs, the FDA resorted to a process that was illegal in several respects. Despite their long use in foods, cyclamates were declared to be new drugs, and marketable subject only to the filling of a new drugs application containing the required labelling. But although new drugs cannot legally be marketed without adequate evidence of their safety, the form of the regulations issued by the FDA provided that cyclamates could be approved for sale without any such supporting evidence. Since no such evidence is in fact available, the proposed use of the regulations "was obviously intended to circumvent the law". This question aside, the FDA's regulations were improper in that although they classified cyclamates as prescription drugs they also required a declaration that made the substances prescription drugs by statutory definition.
- All these actions were taken in the face of serious dissent among the FDA's medical staff who, in a position paper of January 30, clearly expressed their doubts as to the legality of the regulations: "To approve cyclamate-containing foods as safe and effective drugs within the concept of our enforcement of the New Drugs provision of the Act", the paper said, "is untenable. . . . Under the principles we strongly adhere to in permitting drugs to be marketed, these products should not be allowed on the market. To approve a New Drug Application for these products is not supportable medically or legally".
- The new evidence cited by the HEW Medical Advisory Group after its August 4 meeting as grounds for rescinding its earlier decision was all known and available to the group at the time the earlier decision was taken.
- The FDA put the wrong questions to the National Research Council panels that reported on cyclamates in 1962 and 1968. To determine if cyclamates were "generally recognized as safe" the FDA should not have asked whether the panel itself so regarded cyclamates, but whether there was a substantial difference of opinion among experts as to their safety. In fact such a difference did exist at the time the two reports were made.

The Fountain Subcommittee concludes its report with the dry observation that decisions about the safety of foods and drugs should be made in compliance with scientific and legal requirements "and without regard to economic or other extraneous considerations". The Secretary of HEW is admonished in future to let the FDA get on with its job without interference in individual cases, and both the FDA and the HEW are advised not to pass the buck on matters they can decide themselves to outside scientific advisory bodies. The present heads of those two organizations must be profoundly thankful they were not in office at the time the principal actions in the cyclamates drama were being unfolded.

In a statement issued at the same time as the report of the Fountain Subcommittee Dr Charles Edwards defended his predecessor's actions each step of which, he said, was taken with the advice of counsel: "at no time did we take a position inconsistent with the Food, Drugs and Cosmetics Act as we construe it". An even blander statement from the department of HEW actually dares to praise the Fountain Subcommittee for its constructive review of the department's actions, adding that the subcommittee's interpretation of the facts and the law are in this instance erroneous.

JOURNALS

Ap.J. loses its Star

by our Astronomy Correspondent

THE resignation of Dr S. Chandrasekhar as editor of the *Astrophysical Journal*, which has come to be regarded as the foremost of the journals that astronomers read, has set in motion changes that will mark a new era in the journal's seventy-five year history. Dr Chandrasekhar, who is professor of theoretical astrophysics at the University of Chicago, has been editor since

1952, and makes no secret of his belief that eighteen years has been too long. By all accounts his job has been arduous, and until recently Dr Chandrasekhar personally dealt with much of the technicalities of producing the 400-page monthly journal and the thinner letters section that appears separately, as well as the scientific editing. Like editors everywhere, his selection of papers for the journal is sometimes criticized. The competition for space in the *Astrophysical Journal* is strong, and last week Dr Chandrasekhar was moved to say that he has come to hate astronomers just as much as astronomers hate him. Between 25 and 30 per cent of the manuscripts submitted for the main journal have to be rejected, and the rejection rate for the more prestigious letters section which contains short items of urgent interest is 50 per cent.

Dr Chandrasekhar was sixty this year, and by resigning from the *Astrophysical Journal* now he hopes to find more time for his scientific work before he retires from the University of Chicago. His resignation comes into effect on April 1 next year. From then on new manuscripts submitted for the main part of the journal will go to Dr Helmut A. Abt, of the Kitt Peak Observatory who will be acting editor, and for the letters section to Dr D. E. Osterbrock, of the University of Wisconsin. But with the six or seven month publication delay in the main journal, Dr Chandrasekhar expects to continue his involvement with the journal until the November issue next year.

The changes that Dr Chandrasekhar's resignation will bring about arise because the University of Chicago Press which publishes the *Astrophysical Journal* wants to divest itself of its interest by the end of next year now that there will be no University of Chicago astronomers involved in running the scientific side of the journal. It is thought that the press is unhappy about continuing to publish the journal because their control over it would be lessened while they would continue to be financially responsible. It is expected that the American Astronomical Society will take over the journal, and a committee to study the changeover will probably be formed at the December council meeting. The committee will report back in the spring and the deal will have to be approved by the membership next summer.

In the past the profit for the University of Chicago Press has been the interest on a trust fund maintained at 25 per cent of the operating cost of the journal, held in reserve as a cushion against an operating deficit.

At present the role of the American Astronomical Society in the running of the *Astrophysical Journal* is as a consultant, and the council of the society appoints the five member editorial board. What usually happens is that Dr Chandrasekhar submits a list of the people he would like to fill a vacancy, and the council has made the final selection. It is not yet known who will look after the mechanics of publishing the journal, but the likeliest possibility seems to be the American Institute of Physics which already publishes the *Astronomical Journal* for the society. It would then seem appropriate for the *Astrophysical Journal* to merge with the *Astronomical Journal*, which has come to have very much the look of a poor relation, and has remained chiefly a medium for papers on celestial mechanics whereas the *Astrophysical Journal* reflects all branches of astronomy. According to a spokesman for the society, however, it is more likely that the two journals will become three by the division of the *Astrophysical Journal*. Although the letters section will continue monthly as before, the main part of the journal will appear twice a month from January. Instead of the present 400 pages per month there will be two issues of 250 pages. To begin with the mix of papers will be the same in both issues, but eventually there could be a subdivision by subject matter, involving the two issues of *Astrophysical Journal* and the *Astronomical Journal*. It appears therefore that there are the beginnings of a rationalization of the astronomy journals on the west of the Atlantic to match the merging of the European journals into *Astronomy and Astrophysics*. Although it is not always good that the number of publications open to astronomers should be reduced, the journal that stood aloof from the European merger, the British *Monthly Notices of the Royal Astronomical Society*, has recently seemed to be very much out on a limb.

PLANETS

Roll Up for the Grand Tour

NASA has begun to solicit help from the scientific community for the definition phase for the so-called grand tour missions to the outer planets. Although there is no funding yet for the two launches which are being considered—the first in 1977 to Jupiter, Saturn and Pluto, and the second in 1979 to Jupiter, Uranus and Neptune—work will have to start soon on the detailed design of the spacecraft if they are to catch the fortuitous line-up of the outer planets which makes the mission possible. An invitation for participation dated October 15 has been issued to scientists in the United States and elsewhere. The definition phase of a mission involves the selection of the basic properties of the spacecraft, its power, data handling capabilities and so on, and the best orbit to provide the most valuable return of scientific information. In the words of the NASA invitation "It will be the responsibility of the scientists selected to develop mission plans, define typical payloads, and participate in trade-off studies to maximize the value of each mission within its particular constraints".

NASA plans to hold a briefing for interested scientists in Washington on November 17, after which proposals for participation giving details of the proposed investigation and instrumentation are to be submitted by January 18. NASA hopes to have finished the selection process by March 15, and will form an imaging science team, and a radio science and celestial mechanics team. Although it is hard to recall any involvement from abroad in previous NASA definition studies, NASA is stressing that the competition is open to foreign scientists, who should forward their proposals through the National Space Agency. Letters of intent from US scientists have to be received by NASA by November 9 and from foreign scientists by November 16, and these letters do not have to be endorsed by the National Space Agency.

NEWS AND VIEWS

Deluge Unabated

THIS issue of *Nature* includes another batch of five articles about the DNA polymerase activities found in RNA tumour viruses; this completes the identification of the enzyme activities involved in producing double stranded DNA from the single stranded RNA genome. And suspicions that the DNA duplex product is integrated into the host cell genome must harden with the finding in the virion of other types of enzyme activity.

For example, Temin and his colleagues, who must marvel at the goldrush they and Baltimore started (*Nature*, **226**, 1209, 1211; 1970), now report (page 424) that Rous sarcoma virions contain at least two and probably three enzyme activities in addition to RNA dependent DNA polymerase. One is a DNA dependent DNA polymerase which Spiegelman's group has also detected in several RNA tumour viruses (*Nature*, **227**, 1029; 1970). Říman and Beaudreau report (page 427) that the DNA dependent DNA polymerase in avian myeloblastosis virions preferentially copies templates rich in guanosine and cytosine. They also confirm that these particles contain DNA, although its source is still obscure.

The third enzyme activity which Mizutani, Boettiger and Temin have detected is an endonuclease, the existence of which in Rous sarcoma virions may, they believe, explain why the double stranded DNA molecules transcribed off the endogenous viral RNA templates by the two polymerases are so much smaller than the template RNA. The idea is that any larger DNA which is made is promptly cleaved by this nuclease. Furthermore, because this DNA endonuclease activity is reduced when an ATP generating system is added to their assay brew, Mizutani *et al.* suggest that the Rous sarcoma virion also contains a ligase activity for joining DNA molecules. It is, of course, impossible to resist speculating about the roles of these four enzyme activities during the infection of cells by Rous sarcoma virus. Perhaps the two polymerases effect the synthesis of a double stranded DNA containing all the information required to specify progeny single stranded RNA genomes. The endonuclease and the ligase, the activities of which are presumably restricted in natural infections perhaps by topographical factors, might then provide the machinery required to integrate the viral DNA into the genome of the host cell. In other words the RNA tumour viruses may carry into the cell all the enzymes needed for their unique replication cycle.

Further characterization of the two polymerase activities continues apace. Levinson's group (page 433) has shown, perhaps not surprisingly, that actinomycin D inhibits DNA dependent DNA polymerase but not RNA dependent DNA polymerase. And Spiegelman's indefatigable zealots (page 430) have compared the efficiency of a wide range of synthetic nucleic acids as templates for the polymerases in six RNA tumour viruses. In brief they find that single and double stranded RNAs and double stranded DNAs act as templates for the synthesis of DNA, but synthetic RNA–DNA hybrids are by far the most effective templates. Poly dC·poly rG, for example, stimulates by very nearly two orders of magnitude more polymerization of deoxyriboside triphosphates than any natural template. As Spiegelman and his colleagues note, such effective templates will be extremely useful in monitoring the activities of the polymerases in viruses and infected cells and following the activities during purification. An intriguing possibility is that these templates could be used to try to detect the polymerase activities in other cases, such as white blood cells in patients with leukaemia. If this were to give positive results, the implications for clinical cancer research would be as important as they are obvious.

But of course any precise characterization of the polymerase activities, and for that matter the characterization of the endonuclease and putative ligase activities, depends on the development of ways of separating the active enzymes from the virions. Todaro and his colleagues report on page 435 a step in that direction. After treating murine leukaemia virions with detergent they find that the RNA dependent polymerase activity sediments with the nucleoid fraction and in a fraction with a light buoyant density. Under the electron microscope the nucleoid fraction appears to be free of envelope material and intact virions while the light fraction is seen to consist of fragments of membrane and nucleoids. These observations, together with the finding that rat anti-murine sarcoma virus serum inhibits the polymerase activity in the nucleoid fraction but not that of intact virions, indicated that the RNA dependent activity resides in the nucleoid. The polymerase enzyme(s) may well give rise to one of the group-specific antigenicities of the RNA tumour viruses which are also known to reside in the nucleoid and as Todaro and his colleagues note, by using monospecific antisera it should be possible to identify the polymerases with a particular group-specific antigen. Synthetic nucleic acid templates and group specific antisera directed against these enzyme activities look as if they will be powerful tools in the hunt for these activities, and by implication tumour viruses in human cancer cells.

What Happens inside Pulsars?

IT is now generally accepted that pulsars are associated with rotating neutron stars, but there is no really satisfactory detailed explanation of the mechanisms whereby the rotational energy of the star is transformed into the kind of radiation observed. NP 0532 in the Crab Nebula is the youngest pulsar known; it is still emitting visible and X-ray pulses as well as radio pulses and has been the object with the greatest interest to observational astronomers—and often a frustrating source of negative information which pulls the rug from under almost any detailed model put forward by the theoreticians. There was an outstanding illustration of this behaviour in September last year, when there was a sudden increase of this pulsar frequency. Having confounded much of the astronomical community by this change, NP 0532 perversely refused to suffer other similar events, even though the "starquake" model put forward to account for them required that they should occur at least once a month.

New insight into the mechanisms operating in this pulsar (and presumably in others too) is provided by the work reported on page 445 of this issue of *Nature*. Observations of the optical pulses from NP 0532 were made at the Smithsonian Astrophysical Observatory for the period September 1969 to April 1970, which includes the occasion of the marked jump in frequency. The measurements are sufficiently accurate to point to the dominant mechanism in the transfer of rotational energy into radiative energy. This accuracy is obtained by studying the pulsar over several consecutive nights. Extrapolation from the end of one night's observations to the beginning of the next is effected by a least squares fit to the pulsar's previous behaviour. The sudden jump in frequency on September 22, 1969, seems to have caused a cumulative deviation from the predicted behaviour on subsequent nights, and because this deviation is non-linear at least one other parameter than the frequency of the pulsar must have changed. There is a great deal of evidence for disturbed activity after the jump, but the overall behaviour is close to that expected of a system in which magnetic dipole radiation is the dominant mechanism.

Even better agreement with the predictions of this model is obtained when the behaviour of the pulsar just before the jump is interpreted rather differently than has been accepted previously. The original report on the frequency change from the Arecibo radio observatory described the erratic behaviour of the pulsar just before the jump as "quasi-sinusoidal", and this has been taken in some quarters as evidence for a planetary companion to NP 0532. Unfortunately, like so many experimental data, there is no unique curve fitting the observations, and in this case the changes in the data can be well fitted by two straight lines rather than one sine curve. This fitting now seems much more plausible, for, in conjunction with the magnetic dipole radiation law and assuming also a small change in the moment of the dipole (only about 1 part in 2 million), there is good agreement between the theory and subsequent observations.

Small errors forming a background noise are present throughout the data; although even the detection of this very low level noise would have seemed a triumph a few years ago, it is sufficient to prevent any possibility of using the variations in pulsar frequency caused by the Earth's movement in the Sun's gravitational field to measure accurately the gravitational redshift. In spite of this extra blow to the hopes of some theoreticians (which emphasizes the apparently capricious nature of pulsars), there may be some comfort in the suggestion that there really are starquakes on NP 0532, and that it is the frequent occurrence of rather small quakes which produces the noise.

GALAXY FORMATION

Early Protospirals

from our Observatories Correspondent

JAN OORT, who retired this year as director of the Leiden Observatory, has made important contributions to the study of the structure of our Galaxy. Recently one of his principal concerns has been the origin of the high velocity clouds of neutral hydrogen which are observed to be falling into the galactic plane at high and intermediate galactic latitudes. The clouds, which have velocities of up to about 200 km s^{-1}, have been detected by 21 cm observations made mostly at the Dwingeloo radio observatory in Holland. In the past Oort and his collaborators have discussed two possible origins for the clouds. One explanation is that they result from giant explosions in nearby spiral arms which have ejected neutral hydrogen into the galactic halo. According to this view it is largely by chance that the material is observed to be falling into the galactic plane in the vicinity of the Sun. In any case Oort has rejected this explanation (*Astronomy and Astrophysics*, **7**, 381; 1970), and instead has concluded that intergalactic gas is streaming into the Galaxy. A rough estimate of the total flow is 2×10^{17} hydrogen atoms per cm^2 of the galactic plane per million years. This corresponds to an increase of the Galaxy's mass of 0·9 per cent in 10^9 years.

Oort attempts to relate the observation of infalling hydrogen to a theory of the formation of galaxies. He first considers the origin of the angular momentum of spiral galaxies. This subject has had a controversial history. Many theoreticians suppose that galaxies formed as a result of gravitational instability soon after the decoupling of matter and radiation in the Universe. At that time the scale of the Universe was 1/1,000 of its present value. Spiral galaxies could not have contained their present angular momentum because they were too small. Starting with Hoyle in 1951, various people have explored the possibility that angular momentum was imparted to protogalaxies by extraneous couples—for example, a nearby cluster of galaxies.

Oort reports new calculations which show that this mechanism fails by two orders of magnitude to explain the present angular momentum of spirals. Consequently he suggests that protospirals were not formed by gravitational instability in a very early phase of the Universe, but that they date from a much later period when their formation was caused by large scale mass motions which at the same time provided their angular momentum. The idea that large scale "turbulence" has played an important part in the formation of galaxies was suggested in the early 1950s by von Weizäcker and Gamow.

For a protospiral to contain enough momentum it must have formed when the scale of the Universe was about 1/30 the present value. A protocloud formed at that time would initially expand with the Universe, but at a reduced rate. Consequently the actual collapse into a galaxy could occur at an epoch as late as 10^8 years after the initial "big bang". Oort points out that this is an attractive feature of this theory because counts of the extragalactic radio sources (the log N–log S relation) indicate that most of the powerful radio sources in the Universe were born at this comparatively recent epoch.

Oort speculates that the present infall of hydrogen into the Galaxy results from material initially at the outer boundaries of the cells from which protogalaxies were formed. This intergalactic gas must have a high temperature—of order 10^5 to 10^6 deg—and Oort suggests that it might be detectable through its thermal X-ray emission.

SUPERNOVAE

Clues about Pulsars

by our Cosmology Correspondent

THE frequency of supernovae has become of particular interest recently, because of its bearing on the number of pulsars expected in our Galaxy. Without this inducement to encourage detailed investigations, estimates ranging from one supernova per galaxy in 350 years to one every 15 years have been put forward by various authors. Now, a search has been carried out to find the frequency of supernovae in spiral galaxies, and this work also relates the frequency to the mass and luminosity of the galaxies in which they occur (G. A. Tammann, *Astron. Astrophys.*, **8**, 458; 1970).

Tammann has studied only Sb and Sc spirals, but all such galaxies with known luminosity classes and which are within the distance of the Virgo cluster have been investigated, having a total of forty-five supernovae. Other types of galaxy and more remote spirals have not been studied, for they have produced too few supernovae for reliable statistical analysis, but the number of supernovae seen in all galaxies out to the chosen limit during 1959–1969 gives an average of one such event per galaxy in 140 years. This must represent some upper limit, for there must have been some unobserved events. The Sb and Sc galaxies themselves behave differently, both from each other and within their own types. There is a strong dependence on luminosity class, and the frequency in Sb galaxies is down by roughly a factor of 3 on that in Sc galaxies; overall, the spirals have a higher frequency of supernovae than that found when all galaxies are included in the sample, the highest frequency found by Tammann being one in 16 years for ScI galaxies. The ratio of type II to type I supernovae seems the same for all galaxies (roughly 2:1).

Only nine of the thirteen known galaxies in which more than one supernova has occurred fall within the limits of this study, but all of these lie towards the higher luminosity classes in a way which is both significant and surprising. It would be natural to assume that brighter galaxies have more supernovae just because they contain more stars, but it seems that the correlation of supernova frequency with mass is non-linear, the frequency of events rising rather more rapidly than the mass.

Finally, and of greatest interest for investigators of pulsars, Tammann applies his results to M 31, M 33, and our Galaxy, which is generally accepted to be a type Sb or Sc. Using values intermediate between those appropriate for these two types, and taking the Galaxy's mass as 17×10^{10} $M_\odot$ gives a rate of one supernova every 26 years, with a possible error of ± 10 years (this figure is modified to one in 65 years for an Sb spiral of the same mass and one in 17 years for Sc). Overall, in spite of the incompleteness of records covering the past thousand years, this figure agrees with observation, and perhaps suggests that a new supernova is imminent. For M 31, using the latest mass estimate of 20×10^{10} $M_\odot$ the predicted rate is one event in 55 years (the only observed supernova in M 31 occurred in 1885) and for M 33 the rate should be one event in 73 years, which is at least not inconsistent with the lack of any observed supernova in that galaxy. Accepted theory suggests that the rate of star formation is greater in Sc than in Sb galaxies, which could explain the different supernova rates between the types. The interesting consequences of these differing rates on the heavy element abundances in different classes of galaxy are discussed by Tammann, and perhaps these results should be used as a basis for further observations along this line of investigation.

EARTH'S MANTLE

Rifts and Ridges

from our Geomagnetism Correspondent

CONTINENTAL rift valleys have often been regarded as the equivalents on land of the median valleys of mid-oceanic ridges. The East African rift, in particular, is usually drawn as an extension of the world wide undersea rift system, a procedure which seems to be compatible with the geometry of the system. But the evidence for this assumption is rather shaky. By implication, the East African rift should be a region of rising convection currents in the mantle, which would imply a higher than average flow of heat. Measurements of heat flow in the African rift are few; but available data suggest that the flow is not abnormal. Twenty measurements from Lake Malawi, for example, gave an average of 1·0 μcalorie cm^{-2} s^{-1}, which is well below the world average and comparable with that for the South African shield. To be sure, five other measurements were in the range 1·62 to 2·88 μcalories cm^{-2} s^{-1}, but these were easily explained as the result of a local heat source.

To the heat flow evidence must now be added geochemical evidence, for Murray (*Earth Planet. Sci. Lett.*, **9**, 34; 1970) points out that the products of volcanism in the African rift valleys differ both in composition and volume from those in the median

valleys of mid-oceanic ridges. The African rifts are sites of undersaturated, predominantly mafic volcanics erupted in comparatively small volume from single vents. Murray interprets these products as the end result of partial melting in the mantle at depths of more than 100 km. When melted, to reach the surface the liquids would have to pass through a considerable distance in which temperatures were colder than the solidus of the surrounding peridotite. In these conditions, the liquids would be fractionated at high pressures, become nepheline-normative and decrease in volume.

In regions of high heat flow, on the other hand, partial melting in the mantle would occur at depths of less than 60 km, and the liquids would thus avoid passage through peridotite at sub-solidus temperatures. Greater volumes of such magmas would reach the surface than in low heat flow areas, olivine would be the principal crystal phase to separate and, if no previous fractionation had occurred, the magmas at the surface would be tholeiitic. Accordingly, this model predicts voluminous oceanic tholeiites at mid-oceanic ridges; and these are observed. Murray's models are based on recent high pressure experimental results on the origin of basaltic magmas.

From the heat flow data and the nature and volume of the surface volcanics, Murray concludes that the upper mantle conditions below the East African rifts and mid-oceanic ridges are fundamentally different. It follows from this that because mid-oceanic ridges are regions of upwelling mantle material, the African rifts probably are not. This does not apply to the northern end of the rift valleys in Ethiopia, of course. There, heat flow is high, the volcanics are more voluminous and dredging has indicated the presence of typical oceanic tholeiites beneath the Gulf of Aden and the Red Sea. The northern end of the rifts is well established as a region of young sea floor spreading. Murray's view, however, is that the southern end cannot even be regarded as an area of "embryonic" spreading.

FISH

South African Clinids observe Rules

from our Marine Vertebrate Correspondent

THE clinids constitute the dominant group of fishes that live permanently on the shores of South Africa from south-west Africa to Natal; north of both of these provinces they are replaced by other families of shore fishes. Within this area the clinids predominate, and the composition and abundance of the thirty-three species found there vary with the changing physical conditions in the same way as those of other intertidal animals.

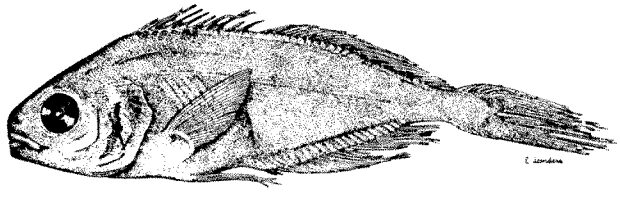
The late T. A. Stephenson and his colleagues, in their now classical distribution studies of the intertidal fauna and flora of South Africa, pointed out that the distribution of these fishes, as known in 1948, agreed with the general trends on that coast. The precise ranges of most species, however, were not then well known. Recent field work has produced a better understanding of their distribution, and Mary-Louise Penrith has summarized the data in a study of the distribution of the fishes of the family Clinidae in southern Africa (*Ann. S. African Mus.*, **55** (3), 135; 1970).

In general, fishes are unhelpful animals in littoral distribution studies, either on account of their mobility, or because they are very adaptable to various habitats, or because many of them possess a pelagic larval or post-larval stage. The South African clinids, however, are exceptional in that they are viviparous, sedentary animals, and speciation having proceeded lavishly might be expected to be restricted in their habitat selection. They are therefore extremely appropriate fishes for distribution studies.

Penrith has shown that their distribution corresponds very well with the faunal regions proposed by Stephenson. The west coast populations extend from Lüderitzbucht (South-West Africa) to Cape Point; a south coast group of species extends from there to East London, where the east coast group begins, extending northwards to Laurenço Marques. Some minor assemblages partly overlap these zones or are found in two adjacent zones but never more than two. Penrith concludes that because the clinids conform to the South African intertidal provinces established by other workers and these have been shown to be temperature dependent, then temperature must be a factor in their distribution also. This probably operates in two ways, the direct effect of temperature on the fish being of paramount importance, but its effect on other organisms which go towards constituting a suitable habitat is of considerable significance. An example of the latter is cited in *Clinus cottoides* which feeds on the legs of acorn barnacles. It is abundant on the south coast where dense balanoid barnacle zones are characteristic, but absent on the west coast where the balanoid zone is absent because of the different temperature.

FISH

New Nomeid



A new member of the family Nomeidae, discovered in the eastern Pacific, has been named *Psenes sio*, in honour of the Scripps Institution of Oceanography. The specimen shown here, which is immature, is 75·5 mm long and has been described by R. L. Haedrich (*Breviora*, No. 351; 1970).

PEPTIDES

New Paths to Structures

from our Molecular Biology Correspondent

PROTON magnetic resonance work on proteins, especially since the advent of the 220 MHz instrument, has often appeared to the observer as somewhat of a scramble for the spectacular, but not too arduous, *coup*. At the same time, however, some sober application has gone into the more modest area of peptide structure. From comparison of observed and calculated couplings of the protons on the α-carbon and peptide nitrogen atoms, and from other features of the spectrum, attempts have been made to deter-

mine the conformations of peptides explicitly, and in small, especially cyclic, structures, such as those of some antibiotics, considerable success has been achieved. Each compound, however, has presented its own problems, and the degree of conviction that structural conclusions have carried has depended on luck, as well as ingenuity. Some of the more glib operators in the field have also evolved what might be termed a conversational, rather than a rigorous approach to the divination of structures, and the bemusement of the outsider.

A rational general strategy, which should have wide appeal, has now been outlined by the Rockefeller group (Gibbons, Nemethy, Stern and Craig, *Proc. US Nat. Acad. Sci.*, **67**, 239; 1970). They have considered the conformation-dependence of backbone proton coupling constants in the light of the known—or at least calculated—restrictions on the peptide geometry. The various theoretical, as well indeed as the vulgarly empirical, model building routines that have been developed in different laboratories have by and large homed in nicely on the energetically favourable values for the two dihedral angles that define the conformation of a peptide chain. Each of these combinations of the angles must be associated with a particular value of the coupling constant. The latter can be derived from data on simple amide derivatives, in which substituents on the carbon determine the rotational angles, by driving the N—H bond into an optimally staggered position. The coupling constants, which in practical terms represent the splitting of the observed resonances, differ considerably. It is also possible to make use of a semi-empirical relation between the relevant dihedral angle (φ) and the coupling constant, developed by a Russian group of workers. From the values of the dihedral angles at the minima in the energy contour map, corresponding to right and left handed α-helices, pleated sheets, and so forth, coupling constants for these forms are deduced. For example the values for the right and left handed α-helix are about 2 and 8 Hz, and a typical coil value is 7 Hz. So far as can be ascertained with the sparse data at present available, predictions are consistent with experiment. The strategy then is to determine the possible dihedral angles, which according to the analytical relation are compatible with the observed coupling constants, and then eliminate those which do not correspond to favourable regions in the conformational map. Much work on model compounds is still required, and it will long be necessary to make use of evidence from other sources.

A number of important hand to mouth methods in fact remain available to assist the deduction of structures. A good example of their use, in relation to a cyclohexapeptide, ferrichrome, which is implicated in iron transport in microorganisms, comes from Llinás, Klein and Neilands (*J. Mol. Biol.*, **52**, 399; 1970). Because of the paramagnetic line-broadening induced by the iron atom which is chelated by this peptide, the aluminium analogue has been used. Introduction of the metal produces striking changes in the nuclear magnetic resonance spectrum, involving especially amide N—H proton frequencies. The solvent-dependence and temperature-dependence of the several frequencies are used to determine which groups are relatively free, and which restricted by internal hydrogen bonds, or other conformational factors, including coordination with the metal. The rough structure which is deduced for ferrichrome in solution resembles the crystal structure of a rather similar species, and involves a kind of antiparallel β-fold, which, it now seems, may be common in small cyclic peptides. Approximate calculated dihedral angles by the semi-empirical method are compatible with those observed in the X-ray model of the analogue.

DNA TUMOUR VIRUSES

Most Fastidious Viruses

from our Cell Biology Correspondent

WHEN polyoma virus or simian virus 40 transforms a cell at least some of the viral genes and their products persist in the neoplastic cell. In the case of cells transformed by SV40 the entire genome persists, whereas in cells transformed by polyoma virus it is not clear whether all or only part of the viral genome becomes stably associated with the cell. But in either event the nature of the viral genes which persist, and are apparently transcribed in transformed cells, is one of the central questions of tumour virology.

Unfortunately, although the small DNA tumour viruses are very simple structures and probably contain only about half a dozen genes, they reproduce, in extremely complex hosts, eukaryotic cells. As a result there is no way yet to select amber and ochre conditional lethal mutants which could be used to define the functions specified by the viral genome. Of course suites of temperature sensitive mutants of polyoma virus can and have been isolated but, as with all temperature sensitive mutants, they are leaky. These obstacles led Benjamin first to predict and now to isolate a third class of conditional lethal mutants of polyoma virus–host range mutants (*Proc. US Nat. Acad. Sci.*, **67**, 394; 1970).

Benjamin's ingenious idea was that it should be possible to isolate mutants of polyoma virus which fail to replicate and kill normal permissive host cells, but which can grow in such cells previously transformed by polyoma virus. This selection, of course, depends on the isolation of a murine 3T3 cell which has been transformed by polyoma virus. Normally these cells support the lytic growth of the virus and are killed, but either because the occasional cell is infected with a spontaneous mutant virus incapable of killing the cell, or because the occasional cell is itself a mutant which cannot support the lytic cycle of the virus, it is possible to isolate from an infected population rare 3T3 cells which are transformed by polyoma virus.

When Benjamin had obtained such a cell, 3T3-Py-3, he could infect it with mutagenized stocks of virus and select those viruses which grow only on 3T3-Py-3 cells. So far he has isolated four such mutants which yield less than two infectious progeny per infected 3T3 cell, but between 200 and 600 infectious particles per infected 3T3-Py-3 cell. The restricted host range of the mutants is not the result of some trivial failure in the uncoating of the virus particles or some requirement for a dividing host cell because infectious DNA isolated from the mutants has the same properties as intact mutant particles. Moreover, the mutant does not grow in stationary or dividing 3T3 cells.

The four mutants do not transform either rat embryo fibroblasts or the BHK line of hamster embryo fibroblasts, although both these types of cell are transformed by wild type virus. This result is not

altogether unexpected, because presumably Benjamin's selection procedure selects for viruses which are defective in one or more functions which are expressed in transformed cells and are also required for the lytic replication of the virus. When mutants grow in 3T3-Py-3 cells they are presumably complemented by a viral gene product or the product of a cellular gene which is depressed by transformation. Because all the mutants fail to transform non-permissive cells there is a chance that the complementing function in 3T3-Py-3 cells, which the mutants lack, plays an integral role in maintaining transformation. In other words the host range mutants may well carry mutations in a transforming gene(s).

MEMBRANES

Miniproteins or Myth?

from our Biochemical Genetics Correspondent

AN analysis of the red blood cell membrane has become an almost statutory requirement of anyone professing the name membrane biologist. The ease with which material can be isolated and purified makes the erythrocyte the favoured test system for new techniques and the touchstone for any new theory of membrane structure.

About half of the red blood cell membrane is protein, the rest being lipid and carbohydrate (44 and 7 per cent, respectively, by weight). The protein fraction displays considerable heterogeneity: Rosenberg and Guidotti, for example (*J. Biol. Chem.*, **243**, 1985; 1968), have separated and characterized several components, some—but not all—of which Berg found to be located on the outside surface of the cell (*Biochim. Biophys. Acta*, **183**, 65; 1969). More recent reports (Phillips and Morrison, *Biochem. Biophys. Res. Commun.*, **40**, 284; 1970; Lenard, *Biochemistry* **9**, 1129; 1970; Rosenberg and Guidotti, *J. Biol. Chem.*, **244**, 5118; 1969) are consistent with a system in which there are two huge proteins of approximate molecular weight 200,000, one of intermediate size (100,000) and about six smaller components (40,000–80,000)—all present in large amounts. And there are also many minor components. Most of the identified fractions—with the exception of that with a molecular weight of 100,000—do not seem to reside on the outer surface of the cell.

In the many laboratories where work has led to this picture of the red cell membrane a cry of astonishment is likely to go up at the report from Dreyer's group at Caltech (Laico *et al.*, *Proc. US Nat. Acad. Sci.*, **67**, 120; 1970) which implies that they all missed the fundamental polypeptide subunits of biological membranes, and suggests that at least 50 per cent of the protein in red cell ghosts is present in this form. The first surprise is that the molecular weight of these subunits is only 5,000—that is, each peptide can only contain at most fifty amino-acids. Some of them are believed to be glycoproteins (for which no evidence, however, is presented). Clearly they must be very small indeed, and Dreyer and his colleagues call them "miniproteins". The second surprise is that they have no N-terminal amino-acids: Dreyer *et al.* suggest that they are cyclic peptides or have blocked N-terminals. The third surprise is the aggregation phenomenon. They surmise (but do not show) that the miniprotein can aggregate to give complexes which cannot be dissociated by any known method: many of the several proteins observed by other groups are, by implication, likely to be artefacts. The only real characterization of these miniproteins Dreyer and his colleagues have carried out is fingerprinting. Miniproteins from red blood cell membranes and mitochondrial membranes give very similar fingerprints.

Unfortunately, these fingerprints were made using subtilisin to digest the miniprotein: this gives no idea of the complexity of miniprotein because of the complete lack of specificity of subtilisin. The fingerprints, then, are not very helpful. Could these miniproteins be degradation products of larger proteins—the cleavage products of a latent membrane peptidase? Dreyer *et al.* must remove this possibility before the miniprotein can be regarded as a fundamental subunit.

ENVIRONMENT

Monitoring for Malformations

from a Correspondent

WHY should anybody monitor for congenital malformations? To detect the influence on the embryo of environmental indiscretions, said Dr E. A. Murphy (Johns Hopkins University) at a symposium on the subject held in Albany, New York, on October 19 and 20. The shorter the interval between insult and recognition of injury, the easier it will be to establish causality. For this reason Drs J. R. Miller (University of British Columbia) and T. H. Shepard (University of Washington) urged monitoring of spontaneous abortions for specific malformations. The history of environmental exposures would thus be obtainable while the mother's memory was still fresh, and an excess of teratogenic effects would be potentially recognizable six to seven months earlier than if observations were limited to the outcome of pregnancies that had gone to term. Unfortunately such surveillance is costly, biased in various ways, and limited in extent.

Epidemics of environmentally induced malformations have sometimes been long delayed in gaining world wide attention; for example cerebral palsy (congenital Minamata disease) among Japanese infants whose mothers, while pregnant, ate fish contaminated with organic mercury. Dr R. W. Miller (National Cancer Institute) suggested the establishment of an international unit to deal with the global implications of such episodes, whether they occur in man or beast.

Because specific major malformations are rare, surveillance for them must be wider than can readily be achieved at present. As an alternative measure, potentially more sensitive, Drs D. W. Smith (University of Washington) and E. B. Hook (Albany Medical College) recommended further study of the relevance of minor malformations (for example abnormal palmar creases) to environmental influences. In the discussion it was suggested that a test of this relationship could be made through the study of Japanese survivors exposed *in utero* to atomic radiation, which is a known teratogen.

Vital and health records contain many unused data that would greatly aid human teratology, especially if a system for automatic linking of records were developed on a large scale. Assignment of a unique number to each child at birth for use on medical and other records would greatly ease computer processing, while preserving the

patient's privacy. Automation is also required for the mass screening of human chromosomes, obtainable at birth from blood of the umbilical cord. The development of scanning devices, which five years ago were expected to be operational in five years, has progressed rapidly according to Dr A. D. Bloom (University of Michigan), but will probably not be operational for another ten years.

Electrophoretic mass screening of blood proteins for new mutations (rare variants) would be formidable on both theoretical and practical grounds, in the view of Dr L. R. Weitkamp (University of Rochester). Screening of blood proteins by immunological reactions may offer rather more hope. The overall message of the conference, however, was that effective systematic mass screening for congenital anomalies does not seem to be imminent.

SLIDES

Away with the Fuzz

LECTURERS will be spared the embarrassment of showing fuzzy, unreadable slides if Mr Haydn Bywater, of the Weed Research Organization, Oxford, has his way. He and his colleagues have standardized a procedure for producing readable slides without the aid of a technical illustrator and elaborate equipment.

Mr Bywater writes: From a black original on white paper a finished slide is produced with white lines against a coloured background. The most critical stage is the drawing of the table or graph, which should have the minimum of lettering. Lower case lettering is easier to read.

The size of the lettering is also vital. A useful standard relates size of lettering to size of screen. Thus the height of the lower case risers (that is the total height of the letter h) should be at least 1/50th of the width of the projected screen. For example, if the original drawing is 20 cm wide, the smallest lettering which can be used is $20/50 = 4$ mm tall. Therefore if a 4 mm pen stencil is used, the graph should not be larger than $4 \times 50 = 200$ mm wide. Pen stencils are easy to use, although "instant" transfer letter systems give a better result.

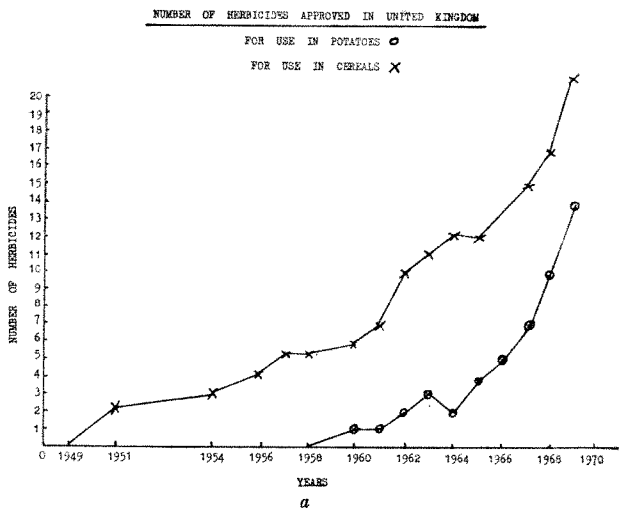
For solid lines black chart tapes give an even, sharp result and are easier to use than a ruling pen. A reasonable thickness of line is 1/200th of the width of the drawing, so that for a graph 20 cm wide the coordinates and other lines should not be narrower than 1 mm. The illustration shows a graph before and after redrawing for use as a slide.

The drawing is next photographed on high contrast, Kodak type 3 Kodalith film, which is available in 35 mm form as well as cut films. It can be handled in a red safelight, Wratten No. 1 or 1a. With 35 mm film the drawing must be framed correctly; a single lens reflex camera is most useful for this because the alignment can be seen through the viewfinder. Exposure should be timed accurately—with two No. 2 (500 W) Photoflood lamps 5 feet from the drawing, exposure time is 3–6 seconds at $f/22$.

The film is processed in Kodak fineline developer, with continuous agitation, for 2·5–3 minutes at 20° C, and fixed and washed as usual. Spotting, if necessary, is done on the rear (shiny) side of the film. The negatives are printed in contact with Technifax Diazochrome material KBL (blue) (Admel International, Weybridge, Surrey). Because this material is only sensitive to ultraviolet light, a darkroom is not needed. A contact printing frame can be used to expose the foil, in contact with the negatives, to direct sunlight for about 2·5 minutes. An ultraviolet printing box can also be used. Overexposure produces a weak colour background, and underexposure causes the letters to be tinted instead of transparent.

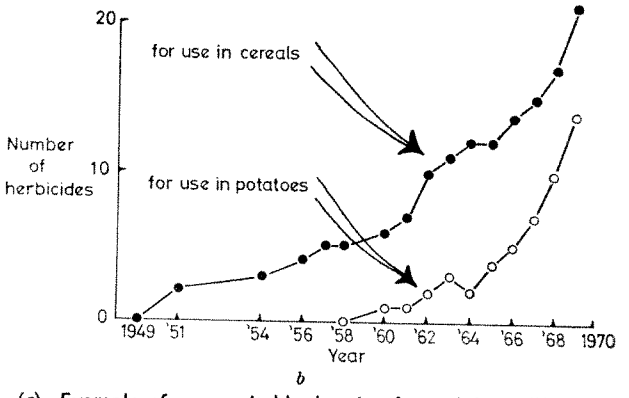
Processing is done in ammonia vapour. A dye line processing machine can be used, or the foils can be suspended in a closed container with a little 0·880 ammonia liquid; a fume chamber should be used. At normal room temperature the colour develops in 2 or 3 minutes; if the room is cold the container can be placed in warm water to speed up the development.

During printing the face of the diazo foil must be in contact with the dull side of the negative. The foil sheets are notched at the top right hand corner when facing the operator, as with photographic film. But when the full sheets are cut for printing it is impossible to tell which side is which unless care is taken to keep all pieces the same way up after cutting. When the colour has ceased to darken, the foil is cut to size and is ready for binding in a slide frame. Other colours are available, but the blue type seems to be the most acceptable to audiences. These slides have the added advantage that both the stick pointer and the illuminated pointer can be used effectively, for they are both visible against the coloured background.



a

Number of herbicides approved in U.K.



b

(*a*) Example of an unsuitable drawing for a slide; (*b*) the graph adapted and simplified.

Research in Muscular Dystrophy

by
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Treatment of the diseases known collectively as muscular dystrophy has not proved very successful. So far, the best results have been achieved by preventive medicine, although pathological and biochemical research is beginning to yield useful information.

THE muscular dystrophies are a group of inherited degenerative diseases causing progressive weakness and wasting of muscles. The individual muscle fibre becomes necrotic, undergoes phagocytosis, and later regenerates. The most active pathological changes may be seen before there is any detectable muscle weakness[1]. Eventually the repetition of this process leads to the extensive loss of muscle fibres, with replacement by fat and fibrosis.

There are several different types of muscular dystrophy[2,3]. In man, the three most commonly encountered, in decreasing order of severity, are first the sex-linked, recessively inherited, Duchenne (pseudohypertrophic) muscular dystrophy affecting young boys, which usually causes difficulty in walking, in rising from the floor and in climbing stairs at about the age of 3 years; most patients are confined to a wheelchair by the age of 10 and die before the age of 20. Second, the autosomal recessively inherited limb-girdle muscular dystrophy is more benign but is usually severely disabling in middle life, while the dominantly inherited facioscapulohumeral type, which is the most benign of all, but which, like the limb-girdle variety, usually begins in early adult life, is often compatible with survival to a normal age, in spite of increasing disability. There is also the relatively common dystrophia myotonica in which muscular degeneration is associated with apparent delayed muscle relaxation (myotonia).

Many other conditions may give a clinical picture similar to muscular dystrophy, including polymyositis, a probable autoimmune inflammatory disorder of muscle[4,5], spinal muscular atrophy, in which there is a primary degenerative process involving the motor neurone[6,7] and various toxic and metabolic myopathies[8,9]. Treatment, prognosis and genetic counselling all depend on accurate diagnosis, and investigations are designed to identify these and many other disorders which affect voluntary muscle.

The search for an effective drug therapy for muscular dystrophy has extended widely. Enthusiastic reports of the effect of vitamins, amino-acid mixtures, nucleotides, anabolic steroids and isoniazid have all proved to be unfounded[10]. Management rests on the established practice of maintaining mobility, building up relatively unaffected muscles, preventing contractures and treating the ever-threatening respiratory infections (and, in the Duchenne type, cardiac failure due to associated cardiomyopathy). Surgical lengthening of contracted muscles and tendons is of limited value, but possibly future advances in immunosuppressive therapy and organ transplantation will allow whole muscle transplantation; it is difficult to see that this could ever be of more than limited value in view of the large numbers of muscles which are usually involved.

The greatest success in recent years in the management of muscular dystrophy has been in preventive medicine, with genetic counselling and the detection of female carriers of the gene responsible for the Duchenne type muscular dystrophy. Women who already have an affected boy and have another affected male relative in the female line of inheritance are definite carriers of the gene. They can be advised that each pregnancy has a one in four chance of producing a further affected child and a one in four chance of producing another female carrier. It is now becoming possible to determine the sex of the foetus *in utero* early in pregnancy, thereby allowing a therapeutic abortion should the foetus be male[11]. Women with a brother or uncle affected with the disease are possible carriers. The hemizygous carriers usually have little or no muscle weakness detectable clinically, but it has proved possible to detect more than 80 per cent of them by a combination of the estimation of serum creatine kinase, quantitative electromyography and muscle biopsy[10,12]. Efforts are being made to improve the rate of carrier detection by various approaches including computer analysis of the electromyographic interference pattern[13], by estimations of total body potassium and rubidium turnover[14–16], by study of the isoenzyme pattern of lactate dehydrogenase in sera and muscle[17], by a search for specific muscle antibodies[18–20], and by the investigation of electrocardiographic abnormalities[21]. The object of this research is to reduce to zero the number of carriers which go undetected, and thus to reassure female relatives of patients that they have no more chance of having an affected child than any other member of the population. Thus genetic counselling may reduce the number of boys affected with muscular dystrophy, but only to the level of the spontaneous mutation rate.

Pathological and biochemical research into muscular dystrophy is in progress, although it must be admitted that pointers to the underlying nature of the disease process are only just beginning to appear. One of the problems of investigating human muscle disease is the difficulty of obtaining sufficient material by muscle biopsy

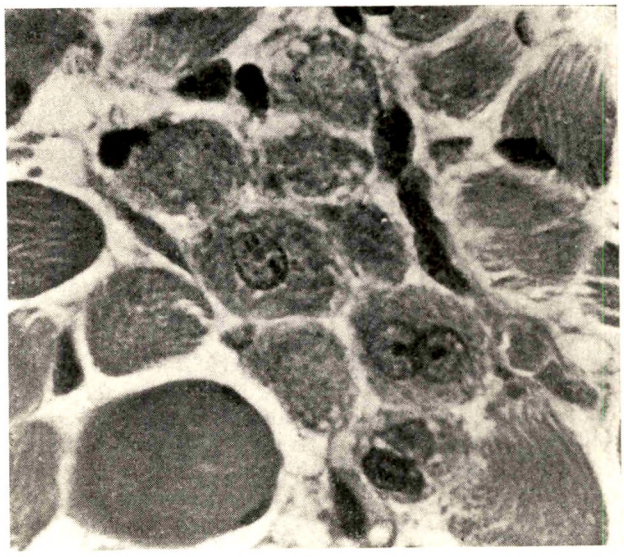


Fig. 1. A transverse section of a quadriceps biopsy obtained from an early (preclinical) case of muscular dystrophy of the Duchenne type. The group of muscle fibres in the centre of the section show sarcoplasmic basophilia with stippling of the sarcoplasm due to the sparse myofibrils; their nuclei are large and vesicular with prominent nucleoli. These histological characteristics are indicative of regenerative activity. (Stained with haematoxylin and eosin, ×640.)

for multi-disciplinary investigations. The discovery of several genetically determined muscle diseases in animals, including mouse, chicken, hamster and lamb[22], has overcome this problem to some extent. Unfortunately these disorders differ in many ways from the human disease; for example, mouse dystrophy is more like human dystrophia myotonica than Duchenne muscular dystrophy. In the latter condition, although it seems that the initial lesion is a segmental necrosis of muscle fibres, and that regeneration is active (Fig. 1), there is still doubt as to whether such regeneration is ever effective. The study of dystrophic muscle in tissue culture has aided the elucidation of this problem. Recent studies have not supported the earlier difficulty experienced in growing multinucleated muscle fibres in tissue culture from human dystrophic muscle[23]. In cultures of dystrophic mouse muscle, however, the proportion of morphologically abnormal myoblasts is greater than in control cultures, and these myoblasts do not seem to fuse into multi-nucleated muscle fibres as rapidly as normal[24–26]. Furthermore, measurements made on single cells in tissue culture, which indicate that the amount of both nucleolar and cytoplasmic RNA may be decreased both in myoblasts and fibroblasts from these cultures[26–28], may provide an explanation of these abnormalities.

Biochemistry and Electrophysiology

The search for a primary biochemical defect in the muscle of patients with muscular dystrophy is hampered by the very pathological changes that the disease produces[29]. Muscle biopsies are diluted and contaminated with biological systems derived from connective tissue, fat and macrophages. A major advance can be expected when tissue culture techniques are developed to allow the relatively pure culture of large quantities of dystrophic muscle for biochemical investigation[30]. The most striking biochemical abnormality in patients with Duchenne muscular dystrophy is the very high level of serum creatine kinase. This, however, seems to be simply an index of damage to the muscle cell, and is not due to a specific defect in creatine phosphate metabolism. In spite of the great biochemical advances recently made in understanding many forms of muscle disease, such as the glycogenoses[31,32], the biochemical basis of muscular dystrophy is still in doubt. Recent work on the activity of cathepsin in normal and dystrophic human muscle has shown that in dystrophic muscle there is a relatively greater increase in the splitting of added protein than in autolytic activity[33]. The calcium affinity of sarcotubular vesicles obtained from the muscle of patients with early Duchenne type dystrophy is less than normal[34], but these and the many other biochemical studies have not so far revealed any fundamental pathogenetic abnormality.

The histochemical characterization of muscle, and the recognition of its two principal types of fibre, has provided considerable impetus to the investigation of muscle disease[35–37]. Histochemical studies, however, suggest that both types of fibre are involved equally early in human muscular dystrophy[37,38], although occasional fibres may contain a high activity of all the enzyme systems studied[39]; in the dystrophic mouse[40], electrophysiological investigations have suggested that type II muscle fibres are affected more severely than type I.

Electrophysiological investigations of muscular dystrophy involving microelectrode studies of single muscle fibres have revealed evidence of a decreased resting membrane potential in dystrophic muscle, and of fibres or segments of fibres which are functionally denervated[41–43]. It is possible that "cracking the code" of muscular dystrophy may come from an entirely new approach, such as a consideration of the role of the motor neurone in the disease. Thus recent observations[44] have suggested that the total number of motor units in the extensor digitorum brevis muscle of the foot is greatly reduced in patients with muscular dystrophy. More attention to the "trophic" role of the motor neurone in this and other muscle diseases is clearly required. Plainly, however, much more work on the nature of the primary morphological and biochemical lesion which causes the muscle to waste must be done before the cause of the condition can be elucidated, and before hopes can be revived that an effective treatment is in sight.

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Frequencies for Radio Astronomy

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At present the scope of research in radio astronomy is limited by the allocation of frequencies, some of which have to be shared with other radio services. When the International Telecommunications Union reconsiders all frequency allocations next year, astronomers are hoping for an improvement.

THE allocation of bands of frequencies to the many radio services is a matter for agreement between nations. A series of conferences, arranged by the International Telecommunications Union (ITU), have dealt with various parts of the spectrum and with various services, so that there is now an agreed allocation for all frequency bands from 10 kHz to 40 GHz. In this agreement, the needs of scientific research must be presented, along with those of all radio services, by national administrations and not directly by the scientists. Coordination of the individual national proposals must therefore be achieved through the combined action of three international scientific unions, IAU (astronomy), COSPAR (space) and URSI (radio), which jointly delegate members to an Inter-Union Commission for the Allocation of Frequencies for Radio Astronomy and Space Science (IUCAF). This Commission discusses the scientific case for protecting various frequency bands for space research and radio astronomy, and informs scientists and administrations throughout the world of the desirable and practical steps that can be taken to improve and extend the allocations.

There will be a new World Administrative Conference of the ITU in June 1971, covering frequency allocations for all space activities, notably for satellite communications. IUCAF has made several suggestions for revising the allocations, especially for radio astronomy; these have been passed on to national administrations, and there is reason to hope that most of the IUCAF suggestions will become firm proposals with a good chance of acceptance at the conference.

Space research seems to be fairly well catered for in the existing regulations, apart from a difficulty in obtaining the use of some low frequency bands for ionospheric research. IUCAF is seeking an improvement in the protection of the "guard bands" around standard frequency transmissions for this purpose.

The more complex problem of radio astronomy is set out in the table, which lists existing allocations together with the latest IUCAF proposals. Two kinds of allocation are required: a series of bands to cover the whole spectrum at roughly octave intervals, for continuum radiation, and some individual bands to cover the most important radio spectral lines.

Some bands already allocated are shared with other services, such as "fixed and mobile", that is communications such as taxi radio. This is only possible when radio observatories and the other users are geographically widely separated; even so, sharing is often a serious limitation on the sensitivity of the radio astronomical work. There are also differences between the three "regions" of the world, defined by ITU. In any table the word "shared" covers a range of meanings. The ITU regulations include "secondary allocations", "shared allocations" and various forms of footnotes; all these are here designated as "shared".

The table represents the present state of discussions. There will be many more discussions during the coming year, and many weeks of work at the conference itself. IUCAF has asked to have an observer at the conference, so that the scientific case for the various proposals is available at all times. Fortunately many of the people concerned with formulating the next international agreement realize that the electromagnetic spectrum is a natural resource that can be despoiled as effectively as any other, and it is to be hoped that considerable improvement will be obtained in the status of frequency allocations for scientific research.

Table 1. EXISTING ALLOCATIONS OF FREQUENCIES TO RADIO ASTRONOMY AND PROPOSED CHANGES

Frequency band	Present status	Proposed changes
Approx. 10 MHz (bandwidth 20 kHz)	None	This band is needed for low-frequency observations, especially of extragalactic sources
Approx. 20 MHz (bandwidth 20 kHz)	None	As at 10 MHz. Proposals for a band at 22 MHz are expected
37·75–38·25 MHz	Shared	Improved protection is needed, although sharing may be necessary
73–74·6 MHz	Region 2 only (N. and S. America)	No extension to other regions seems possible
150·5–153 MHz	Region 1 only (Europe). Shared	No improvement seems possible
404–410 MHz	Shared	Small regional differences should be removed, giving an international allocation
606–614 MHz	Allocated to television, but so far unused	IUCAF asks for this band to be allocated to radio astronomy
1,400–1,427 MHz (the hydrogen line)	Exclusive allocation to radio astronomy	An extension to lower frequencies—down to 1,390 MHz—is needed for observation of galaxies with large redshifts
1,664·4–1,668·4 MHz (the OH line)	Shared	Protection is sought from airborne transmitters in this band, and an extension to 1,660–1,670 MHz seems possible
1,612 and 1,720 MHz (other OH lines)	None	Some local protection is sought for individual observatories observing these lines
2,690–2,700 MHz	Exclusive allocation to radio astronomy	An extension down to 2,670 MHz, on a shared basis, is requested for wide-band observations of extragalactic sources
4,990–5,000 MHz	Exclusive in Region 2 (N. and S. America), shared elsewhere	Improvement of protection, and an extension down to 4,950 MHz, both seem possible and are requested by IUCAF
10·68–10·70 GHz	Exclusive allocation to radio astronomy (with some exceptions)	An extension down to 10·60 GHz is requested
15·35–15·40 GHz	Exclusive allocation to radio astronomy (with some exceptions)	No change is proposed
19·3–19·4 GHz	Exclusive to radio astronomy, with some exceptions	This band is needed for satellite communications, and will be relinquished by radio astronomers
23·6–23·5 GHz	Proposed for exclusive allocation to radio astronomy	Replacement for the 19·3–19·4 GHz band; also covers some NH_3 and H_2O lines
31·3–31·5 GHz	Exclusive to radio astronomy with some exceptions	No change is proposed
40–240 GHz	A series of bands, for example, 88–92 GHz	It is proposed to preserve wide bands in this region initially for reception only

There are also requests for some protection to be given to observations of the three following spectral lines: formaldehyde, 4,825–4,835 MHz; formaldehyde, 14·485–14·515 GHz; water vapour, 22·21–22·26 GHz.

X-rays from Domestic Colour Television Receivers in Britain

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With British domestic colour television receivers, the genetic dose from X-rays is negligible, and the X-ray emission from individual sets is normally well within the internationally accepted limit.

RECOMMENDATIONS for limiting the emission of X-rays from domestic television receivers have existed for many years[1–3]. Throughout this period, measurements of the radiation from black-and-white sets have indicated compliance with the recommendations, and the dose to viewers was deemed negligible[4].

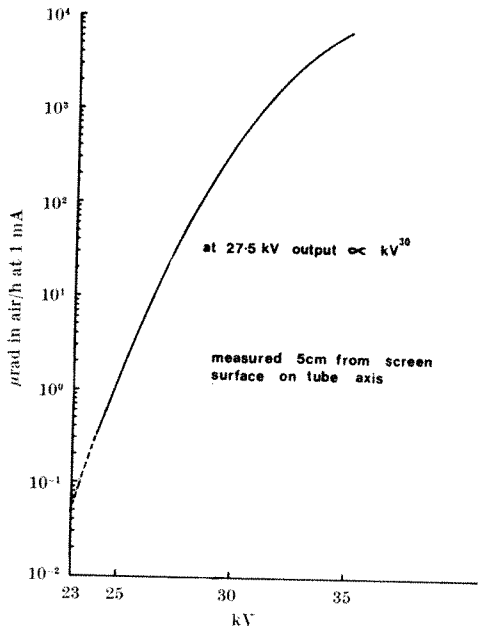


Fig. 1. Variation with tube voltage of the X-ray dose rate at 5 cm from the screen surface on the tube axis.

Colour sets operate at higher voltages than black-and-white sets, so that the possibility arises of increased emission of more penetrating X-rays. In a preliminary investigation in 1965, the Radiological Protection Service examined prototype receivers and found that manufacturers were aware of the new difficulties. Towards the end of 1969, when colour transmission on all three national channels was imminent and the production of receivers had become significant, a full-scale survey was undertaken; its objects were to estimate the radiation dose that the population is likely to receive and to determine the maximum emission from individual receivers. The survey was confined to the products of British companies, two picture tube makers and seven set makers, which supply virtually all the equipment on the British market.

The picture tube is the only source of X-rays in sets produced at present, and the maximum dose rate occurs at the front screen. Both tube companies make three sizes, but the six tubes can be reduced to two groups, thin screen and thick screen, the tubes within each group having practically identical X-ray outputs. Thin screen tubes are used by a majority of receiver manufacturers and have a greater X-ray output than thick screen tubes; but the assumption that all tubes are thin screen leads only to a slight overestimate of the dose received by the population and greatly simplifies treatment of the results. The thin screen tube with average X-ray output can be called the representative tube.

Techniques of Measurement

We made X-ray measurements chiefly with a plastic scintillation detector encased in 0·38 mm aluminium; for a limited amount of dosimetry we used a high-resolution X-ray spectrometer with a germanium (lithium) detector; the principal use of the spectrometer, however, was in matching the picture tube spectra to the spectra of the various X-ray beams used for calibration and depth dose measurements. Blank rasters were used throughout the survey to avoid fluctuating picture content.

On the basis of a study by Braestrup and Mooney[5], the International Commission on Radiological Protection (ICRP) recommends[3] that the X-ray exposure rate at 5 cm from the surface of any television set should not exceed 0·5 milliroentgens per hour, which is equivalent to a dose rate limit of 435 microrads in air per hour (μrad in air/h). Both tube companies make identical claims for X-ray emission from their products, namely that the ICRP limit is not exceeded at the maximum tube rating, which can be taken as 27·5 kV, 1 mA. Their claims were tested by haphazardly selecting one hundred thin screen tubes at each factory and measuring the dose rates 5 cm from the screen centre. The X-ray outputs were found to be an order of magnitude below the ICRP limit. The output distributions were normal in both cases and narrow spreads indicated very close control over tube production. In fact, the X-ray output 5 cm from the screen surface of the representative tube at 27·5 kV, 1 mA was 39 μrad in air/h with 30 per cent standard deviation. As is indicated in Fig. 1, X-ray output from the representative tube depends sharply on tube voltage. The output is, of course, directly proportional to tube current.

Estimating Dose Rates

Front screens are reasonably uniform in thickness, and when dose rates at the screen are measured, they too are reasonably uniform. The spatial distribution of dose rate at large distances from the tube face is, however, non-uniform, because of oblique attenuation of some X-rays in the screen. The dose rate is maximal on the tube axis and remains relatively near the axial value inside a 60° cone with apex at the screen; beyond 30° to the axis the dose rate decreases rapidly. The fall-off of the axial dose rate with distance from the screen is gradual at first, but from 70 cm outward tends to follow the inverse square law. The dose rate at 2·5 m is about a fiftieth of the dose rate at 5 cm, 2·5 m being the average viewing distance. The British Broadcasting Corporation conducted a survey of viewing habits on our behalf (B. P. Emmett, personal communication); the results indicated that the great majority of persons sat within the 60° cone. The assumption that all viewers are exposed to the axial dose rate does not therefore lead to a serious overestimate of viewer dose. The results of the survey of viewing habits are given in Table 1.

Table 1. SURVEY OF VIEWING HABITS

Persons surveyed	3,000
Households surveyed	950
Viewing distance	2·5 m
Hours viewing per year	1,000

Simulating Tissue Doses

It was not practicable to measure the dose at various depths in tissue-like material with a picture tube because of the low X-ray outputs; heavily filtered beams from a constant potential X-ray set were used instead. The spectra from both sources were matched according to equivalent or photopeak keV but, as expected, the picture tube spectra were narrower. Water was used to simulate tissue, and thermoluminescent dosimeters were used for dose measurement. Doses at depths of 1 cm and 7 cm were taken as indicative of the doses to the male and female gonads and were related to the dose at the position of the water surface; the results are given in Fig. 2. Picture tube voltage and current during normal viewing are typically 23·3 kV and 0·5 mA. Allowing for this fact, and assuming that there is a colour receiver in every household, and that male and female are equal in number and child expectancy, the annual genetically significant dose was estimated to be 0·4 microrads. The annual genetically significant dose from natural sources in Britain is about 100 millirads, with 20 per cent difference between areas of sedimentary and igneous rocks[4]. Thus the additional dose received by the public from television X-rays will be only 0·0004 per cent of that from natural sources and can therefore be regarded as negligible.

How Safe is the Viewer?

The maximum X-ray emission from individual receivers was determined at the set makers using regulation-emission charts as described by Ciuciura[6]. In test conditions of service maladjustment, or circuit failure tending to maximize X-ray emission, or both conditions combined, some manufacturers' receivers could be made to exceed the ICRP limit slightly. Picture quality was inferior even for marginally excessive dose rates. As test conditions were made more extreme, picture quality deteriorated further and sets operated merely momentarily before breakdown. The limiting output was judged to be about 5 millirads in air per hour at 5 cm from the screen, that is, approximately ten times the ICRP limit. The probability of obtaining excessive emission from a television receiver, except deliberately in severe test conditions, is, however, slight. Indeed, an evaluation of the procedures for setting up receivers during manufacture indicated that the dose rate 5 cm from the surface of any receiver leaving a factory is unlikely to exceed 15 μrad in air/h.

Some older British colour sets, no longer in production, used thermionic valves in the high voltage circuits: these were a shunt regulator tricde and a half-wave rectifier, both capable of emitting X-rays. All the valves were made by one company and have design features that minimize X-ray emission; nevertheless, in adverse operating conditions, the dose rate 5 cm from unshielded valves could reach about 100 millirads in air per hour. In all these receivers, however, the valves were placed in steel compartments of minimum thickness 0·5 mm, with the result that, in normal operating conditions, the dose rates at the surface of compartments are of the order of 1 μrad in air/h; even in abnormal conditions the dose rate outside any receiver resulting from the high voltage valves is unlikely to exceed the ICRP limit. It is essential, however, that the shielding afforded by the compartment should not be impaired.

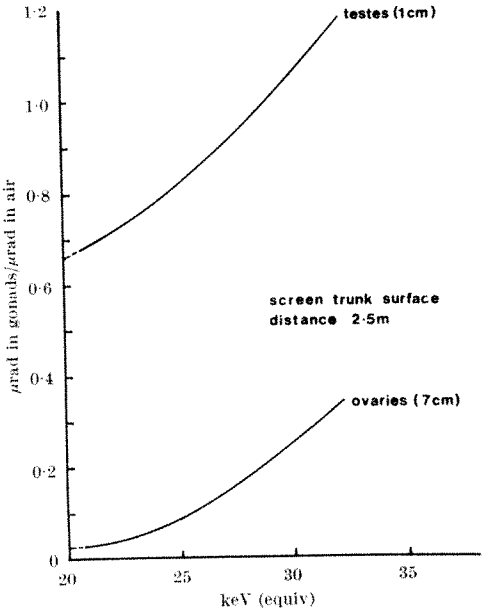


Fig. 2. Gonad dose per unit dose in air at position of trunk surface. The correspondence between keV (equiv) and picture tube kV is approximately 20–20, 25–26, 30–32, 32–35.

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Drug Pushers in the United Kingdom

by

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A survey of twenty-two young narcotic addicts suggests that socially damaging forms of drug selling, or "pushing", must be discriminated from those which are socially irrelevant. This could have an important bearing on the way that current legislation is applied in Britain.

THE recent spread of drug dependence in Britain has focused attention on the selling, or "pushing", of drugs as a possible cause of this "epidemic", but there have been few studies of the drug "pusher". In a detailed study of the use of heroin in a provincial town, Kosviner *et al.*[1] found no evidence of anyone "pushing" heroin: "supplies for those not on prescription were generally obtained by different users going each week to 'score' (buy) from registered addicts in London".

For obvious reasons opportunities for studying drug pushers and their habits are severely limited. The original aim of this investigation was to compare the psychosocial characteristics of a group of heroin pushers with those of a group of heroin users who did not sell drugs. Heroin pushers were to be operationally defined as individuals making a profit of £15 or more a week from the sale of heroin.

The investigation soon ran into difficulties. Heroin pushers, as defined, were hard to discover. Heroin users were easy enough to find among our patients, but when their histories were probed they were found to contain, almost without exception, episodes of drug selling. They therefore could not be used as a satisfactory control series, even though most did not meet the operational definition of heroin pushers.

At this stage we decided to modify the study to cover the selling of addictive drugs of any kind and in any amount, and to base it on interviews with narcotic-using patients who were willing to tell us of their experiences. The operational definition of heroin pushing, and the search for a control series, were abandoned.

The Enquiry

It was difficult to arrange interviews with so mobile and unpredictable a group and the material was drawn from the following sources. D. P. interviewed ten out of fifteen narcotic addicts in a provincial town who were under his care. D. M. interviewed the other five. D. M. also interviewed five narcotic addicts or users under the care of colleagues in the London area and two ex-patients in prison.

A semi-structured form of interview was used, and absolute confidentiality was guaranteed to the subjects. Enquiries about drug-selling experiences were made with great tact and care, emphasizing past rather than current activities. It was unlikely that patients would admit selling drugs which were prescribed currently by either of us, or that even within the confidential setting of the enquiry they would give information incriminating themselves or others. Five of them, however, said that they were still engaged in selling drugs.

The Eysenck personality inventory (EPI)[2] was administered to twenty addicts and Foulds's hostility and direction of hostility questionnaire (FHDHQ)[3] was also completed in thirteen cases. No psychometric data were available in two cases. Most of the interviews were conducted in 1969.

When analysing the general characteristics of our subjects, we found that the ages of the men ranged from 19–34 (mean 22·9, median 22). In seventeen cases there was no evidence of gross parental separation or deprivation before the age of 14. Two of the subjects had been adopted in early childhood, and the parents of three others separated before the children were fourteen. Ten of them described a very marked disparity in relationships with their respective parents, nine having a much better relationship with their mothers than with their fathers, one saying that the converse was true.

The social class of father showed that this was by no means an economically deprived group of people. The British Registrar General divides the population into five classes, and of our sample the fathers of seven out of twenty were in social classes 1 or 2 (professional, semi-professional or self-employed) and nine of the remainder were in class 3 (skilled tradesmen or clerks). Two subjects were not classifiable. Seven of the subjects were or had been married, and of the fifteen single men, four had one or more children. Those currently (or immediately before entering hospital or prison) in full time employment consisted of one motor mechanic, one fitter, one painter and decorator, one car cleaner and one student. The remaining seventeen were unemployed and, of these, thirteen were drawing social security benefits. This proportion of unemployed is characteristic of addicts seen at the clinic where most attended, but may be higher than the national figure.

As far as personal drug dependence was concerned, fifteen of the subjects were, or had recently been, prescribed heroin for maintenance treatment at a National Health Service clinic. One was similarly prescribed morphine and two methadone. Three claimed to be dependent on heroin from illicit sources and one was classified as a polyaddict.

Two-thirds of the group had ceased full-time education at fifteen (the minimum school leaving age in England). One-third had some form of further full time education after fifteen. They were predominantly highly mobile, itinerant people, who changed their addresses every few weeks or months. They were also mostly separated from their family roots.

Experiences of Drug Pushing

The reported episodes of drug selling took place during 1962–70, with a peak in 1967. Subjects were asked for how long in the past they had been in the habit of selling drugs. The answers varied from 4 weeks to 8 years. In round figures, half had sold for 6 months and more, and half for less than 6 months. Two did not answer this

question. These were retrospective statements; all but five said that they had stopped pushing. Table 1 shows the drugs that were sold. It was not easy to obtain information about the source of these drugs, but over-prescribing to the patient by private doctors, before the new drug regulations came into effect in the United Kingdom in April 1968, accounted for most of the heroin.

Table 1. DRUGS SOLD BY THE SUBJECTS

No. of subjects	Drugs
7	Heroin
1	Heroin and cocaine
1	Heroin and cannabis
1	Chinese heroin
1	Morphine and methadone
5	Cannabis
4	Amphetamines, including 'Drinamyl'
2	Cannabis and LSD

In twenty cases an estimate of the average weekly profit was given. It ranged from £0·5 to £100 with a median of £12. In one other case the profit took the form of personal hemp supplies instead of cash. One subject refused to reveal his profit (Table 2). Amphetamines seem to have been the most profitable commodity in this small series. The figures speak for themselves up to a point. To judge them fairly, however, we should also show a table of the amounts spent by the same patients in buying drugs for themselves. In most cases of heroin selling, profits were ephemeral, being almost immediately spent in buying drugs to the same value for personal use.

Table 2. AVERAGE WEEKLY PROFIT

Drug	Mean (£)	Median (£)	Range (£)
Heroin	30	9	2–100
Cannabis	14	11	6–20
Amphetamines	31	45	12–75
LSD	50	—	—

Twenty subjects admitted having a criminal record when juvenile crimes were taken into account. Seventeen of them had been in trouble before they started selling drugs. Their motives for starting and ceasing to push drugs are summarized in Table 3.

Table 3. MOTIVES FOR STARTING AND STOPPING TO PUSH DRUGS

	No. of subjects
Starting	
Profit, with which to buy drugs for personal use or pay private doctors' fees	12
Profit for keep and other reasons	8
To oblige other users (and profit)	3
Self aggrandizement	4
Idealistic motives	3
Stopping	
Free treatment available in new clinics	8
Police action or pressure	5
Fear of police action	3
Distaste for transactions	3
Beaten up by rival pusher	1
Insufficient reward	1
Not applicable (still selling)	5

Psychometric Findings

The FHDHQ is designed to measure hostility or aggression, and the score expresses the total hostility reported by the subject. The direction of hostility score also indicates whether aggression is directed principally inward or outward at others.

The EPI is better known and provides a measure of neurotic tendency and of extroversion as opposed to introversion (Table 4).

The reliability of questionnaire findings in addicts is open to question for various reasons. The pushers interviewed did not, on these tests, differ from a larger group of male narcotic, needle-using addicts interviewed in Brighton. Both groups had significantly higher total hostility and neuroticism scores than normals. The scores resembled those in mixed neurotics except in that the direction of hostility was less intropunitive than in the neurotic group.

As far as heroin was concerned, our subjects said that much of their drug selling experience was a consequence of the pre-1968 overprescribing by private doctors. It seemed inevitable and natural to the otherwise impecunious addict that he should sell part of his supply of drugs in order to pay doctors' fees and keep himself. "We all had to find our script or fee money, and one would help the other out. Anybody who attended a private doctor and didn't push was a millionaire."

Table 4. RESULTS OF THE EYSENK PERSONALITY INVENTORY

		FHDHQ		EPI form A	
		Total hostility	Direction of hostility (+ = intropunitive)	Neuroticism	Extroversion
Pushers (n = 20) (present study)	Mean	18·8	+2·2	14·1	11·3
	(s.d.)	(7·5)	(7·0)	(4·7)	(3·2)
Male injecting addicts* (n = 60)	Mean	21·9	+0·1	13·3	11·3
	(s.d.)	(8·0)	(6·8)	(5·9)	(3·5)
Mixed neurotics[4]	Mean	18·4	+6·2	14·4	10·0
	(s.d.)	(7·5)	(6·7)	(5·5)	(4·3)
Normal subjects[4]	Mean	13·0	+0·5	9·1	12·1
	(s.d.)	(6·2)	(4·6)	(4·8)	(4·4)

* Unpublished work of J. A. Greenup and D. P.

Moreover, it seems to be in the make-up of the young narcotic addict to buy, sell, borrow or (according to variations in his fortune) lend small quantities of drugs to and from other users. Such transactions were seen by most of the individuals in this series as matters of mutual convenience with which society ought not to feel concerned. "It was more or less spreading it out. You might sell it one day and buy it back the next." Bartering of drugs was also extensive, particularly of injectable heroin, morphine, methadone and methylamphetamine.

The securing of maintenance supplies of drugs from National Health Service treatment centres was a common reason for abandoning drug selling. Apart from the precarious, hand to mouth life-style inseparable from private doctor prescribing, some addicts have, however, learned that the austerity of life on social security benefits can be alleviated by systematic drug selling, especially of cannabis.

Simple profit in cash or kind was the dominant motive for selling given by these subjects. The commonest attitude expressed was essentially amoral. One hemp-seller remarked: "It was an easy way out—less reprehensible than breaking and entering or personal violence, an unfortunate necessity because I'm unable to work." Most of those who had sold drugs claimed to limit sales to established users. A minority of five, however, had on occasion sold heroin or other drugs to non-users, with little concern for the consequences.

A positive wish to "turn on" non-users of drugs was expressed by only one subject, who claimed at one time to have sold amphetamines to school children. He regarded drug taking as a form of "communion with life". One sold drugs because it was "the big thing to do", another for excitement ("the glory of it"); only one for a sense of power. Another man recalled how he had for a time sought to "show the light" to other heroin addicts by getting them to take LSD and so achieve a cure of their heroin habit. In similarly mystical terms another spoke of his wish to aid "seekers of the third eye" with "the truth", through selling them good quality cannabis: he did not, however, disguise his first motive, which was profit at 100 per cent per deal.

The range of selling activities varied enormously even within this small series. Few of the subjects revealed any link with large-scale importers or other operators. The exceptions included a man who said that he had sold Chinese heroin, obtained from an agent in Soho, regularly to a clientele of four to five people, at a profit of £10–£12 weekly. The few who had dealt in sizable quantities of cannabis had evidently been in touch with suppliers trading on a very considerable scale. In the main, however, the transactions described were limited on any one occasion to quantities of drugs appropriate to one individual's immediate personal use.

Table 5. CHARACTERISTICS OF SUBJECTS ACCORDING TO TIME SPENT SELLING AND PROFIT MADE

	No.	Mean age	"Higher" education	Main drug sold: Heroin	Cannabis	LSD	Amphetamine	Profit £15 or more	"Abnormal" motives
Duration 6 months or more	9	24·0	4	3	4	2		6	4
Duration less than 6 months	11	21·6	4	5	4	2		4	2
Weekly profit £15 or more	10	22·5	3	4	4	2		(10)	4
Profit less than £15	12	25·1	5	6	6	1		—	1

The series was split into a subgroup who had sold drugs for 6 months and over, and the remainder who had sold drugs for less than 6 months. It was also split into those who claimed a weekly profit of £15 or more and those who said that their profits were less. Table 5 shows a comparison between these subgroups in respect of certain variables. By "abnormal" motives are meant motives other than simple profit or obliging (temporarily or reciprocally) other drug users. The apparent differences do not reach statistical significance. There is an interesting tendency, however, for those who were more heavily involved in selling drugs to report "abnormal" motives more often than the other subjects.

Fear of police action seemed to be a potent factor in deterring these subjects from selling drugs, and none conceived the present scale of penalties as other than extremely severe.

All of the drug selling activities in this investigation were highly illegal. In medico-social terms, however, they amount to a very wide spectrum. We are inclined to endorse the addicts' own views that much of the petty trading that goes on among themselves is socially no more harmful than the inherent patterns of their lives. The importance of trading in cannabis is debatable, and dependent on the view taken of the harmfulness of this substance and of the sort of arguments which followed the Wootton report[5]. We would speculate that, within this particular series, the most dangerous activities were the selling of amphetamines and LSD.

There is a widespread belief that a clear distinction can be drawn between the (evil) drug pusher, who is not addicted, and the (sick) addict who does not push. This belief is based on a false dichotomy, if our findings are at all representative, in that most of the young non-therapeutic addicts with whom we have been in touch have at some time sold drugs. In current legislation, public anxiety and anger about the spread of drug misuse are being expressed in still heavier penalties for trafficking. To apply such penalties indiscriminately would be to disregard the social realities of the drug scene.

We thank Drs Derek Richter and David Murray Shaw for encouragement and Drs D. Beckett and Hilary Jones for permission to interview their patients. We also thank Maryse Metcalfe and Anthony Johnson for practical suggestions and the drug pushers for giving up their time.

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A DNA-dependent DNA Polymerase and a DNA Endonuclease in Virions of Rous Sarcoma Virus

by

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The virion contains a DNA-dependent DNA polymerase which replicates duplex DNA and an endonuclease which attacks it. A ligase system may also be present.

THE replication of RNA tumour viruses seems to involve a DNA intermediate—the DNA provirus[1,2]. Virions of RNA tumour viruses contain an RNA-dependent DNA polymerase (refs. 3 and 4 and S. Spiegelman, M. Green, R. Gilden and W. Levinson, personal communication). The data reported here suggest that virions of Rous sarcoma virus contain in addition to this RNA-dependent DNA polymerase an endonuclease for DNA and a DNA-dependent DNA polymerase.

Schmidt–Ruppin virus (SRV) was prepared, purified and disrupted as before[4]. At times the virus was incubated for 20 min at 37° C with 20 μg/ml. pronase before the final sucrose density gradient centrifugation. The polymerase assay contained 10 nmoles of dATP, dCTP and dGTP, 7·5 nmoles of ATP (in 0·02 M Tris-HCl buffer (*p*H 8·0) containing 0·4 mM EDTA and 10 mM dithiothreitol), 50 nmoles of $MgCl_2$, 100 nmoles of KCl, 2·5 μg of phosphoenolpyruvate, 10 μg pyruvate kinase, 2·5 μCi of ^{3}H-dTTP (12 Ci/mmole) and 0·025 ml. of enzyme (7–10 μg of protein) in a total volume of 0·125 ml. Incubation was at 40° C. At different times samples were taken for counting as described before[4] or were extracted and analysed on sucrose or CsCl or Cs_2SO_4 gradients using standard methods. T7 (originally supplied by Dr H. Lozeron, University of Wisconsin) was grown and labelled with ^{14}C-thymidine using standard methods. DNA was extracted with phenol, and dialysed against 0·01 M NaCl–0·01 M Tris buffer (*p*H 8·0).

Disrupted virions of SRV were incubated in a standard polymerase assay until there was no further increase in ^{3}H-dTTP incorporation (2–6 h). The entire reaction mixture was then analysed on a 5–20 per cent sucrose gradient at *p*H 8·0. Twenty per cent of the counts sedimented at greater than 200S (Fig. 1*a*). If the reaction mixture was digested with pronase and the product purified on a CsCl equilibrium gradient (when there is an almost complete recovery of counts) (Fig. 1*c*) and then analysed in a neutral sucrose gradient, a broad peak of

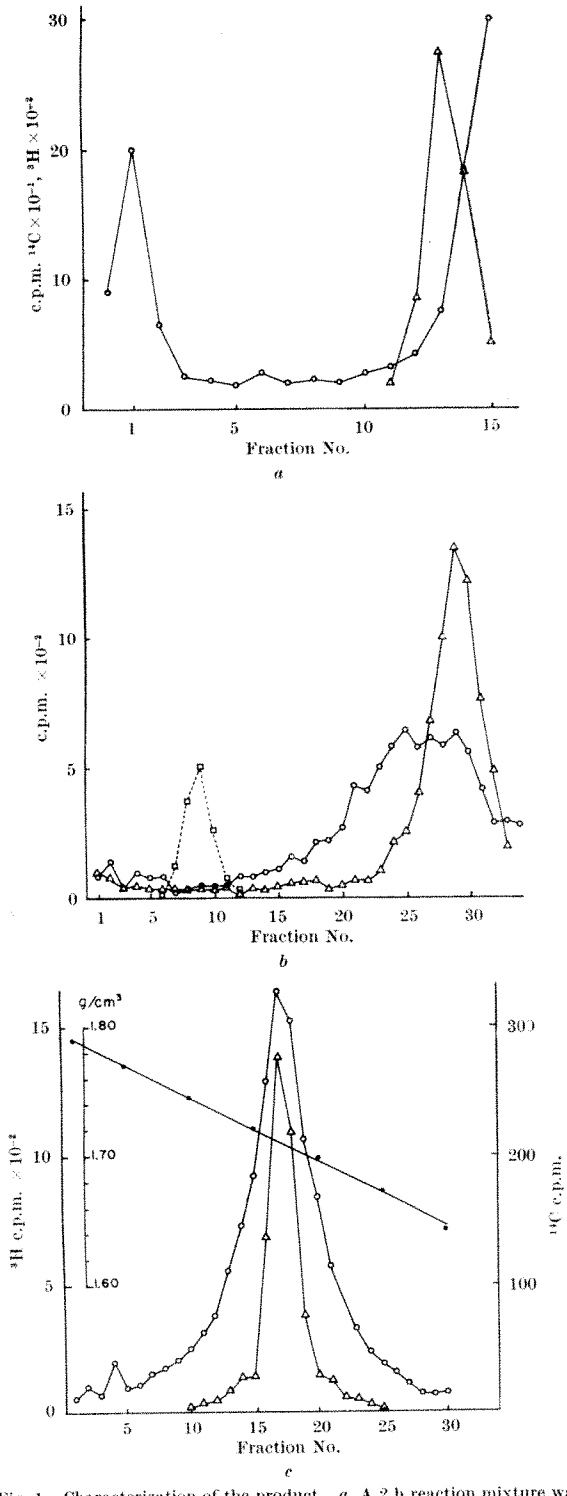


Fig. 1. Characterization of the product. *a*, A 2 h reaction mixture was layered on a 5–20 per cent sucrose gradient (0·1 M NaCl–0·01 M EDTA–0·05 M Tris-HCl buffer, *p*H 8·0) and centrifuged at 30,000 r.p.m. for 1 h in an 'SW 39' rotor. 0·3 ml. of 70 per cent sucrose was used as a cushion and ^{14}C-labelled T7 DNA was present as a marker. Drops were collected from the bottom of the tube and acid insoluble counts were determined. ○, DNA product; △, T7 DNA marker. *b*, A 6 h reaction mixture was incubated for 2 h with 200 μg/ml. pronase and centrifuged to equilibrium on a CsCl gradient. Peak fractions were pooled and dialysed against 0·015 M NaCl–0·0015 M sodium citrate. The dialysate was layered on a 5–20 per cent neutral sucrose gradient (as in *a*). ○—○, or a 5–20 per cent alkaline sucrose gradient (0·3 M NaOH–0·3 M KCl–0·01 M EDTA, *p*H 12·5), △—△, and centrifuged at 45,000 r.p.m. for 2·5 h in an 'SW 50·1' rotor. T7 DNA was used as a marker, □- - -□. Drops were collected from the bottom of the tube and acid insoluble counts determined. *c*, A 6 h reaction mixture was incubated for 2 h in 200 μg/ml. pronase mixed with ^{14}C-labelled T7 DNA and centrifuged in a CsCl gradient at 30,000 r.p.m. in an 'SW 50·1' rotor for 64 h. Drops were collected from the bottom of the tube and acid insoluble counts were determined. ○, ^{3}H counts (product); △, ^{14}C counts (T7 DNA).

5–16S material was found (Fig. 1*b*). If the phenol extracted or the overnight pronase digestion product was analysed on a neutral CsCl equilibrium density gradient, a peak with a density of 1·71 g cm^{-3} was found (Fig. 1*c*). In a neutral Cs_2SO_4 equilibrium density gradient a single peak with a density of 1·42 was found. A DNA–RNA hybrid would probably have a density of about 1·8 in CsCl and about 1·49 in Cs_2SO_4 (ref. 5).

These results suggested that the product was short double-stranded pieces of DNA. To determine the effect of denaturation on the sedimentation rate, a late reaction mixture was digested with 200 μg pronase for 1 h and analysed on an alkaline sucrose gradient (*p*H 12·5). All of the counts were found at the bottom of the tube. The same was found when the pronase-digested product was extracted with ether or heated at 100° C before analysis on the alkaline gradient. This result seems to be due to binding of single-stranded DNA to some other material, perhaps a protein. If, however, the late reaction mixture was first purified on a CsCl equilibrium gradient and then analysed on an alkaline sucrose gradient at 5–8S, peak was found (Fig. 1*b*).

To characterize further the product as double-stranded DNA, it was exposed to exonuclease I before and after denaturation with heat. It was found (Table 1) that the product was resistant to digestion by exonuclease I until after denaturation and is therefore double stranded DNA[6]. These results suggested that there might be enzyme activities other than the RNA-dependent DNA polymerase in the virion, that is a DNA-dependent DNA polymerase and an endonuclease for DNA.

Table 1. SENSITIVITY OF PRODUCT TO DIGESTION WITH EXONUCLEASE I

	Control (c.p.m.)	Exonuclease I
Native	10,800	12,000
Denatured	9,500	3,900

A standard reaction mixture was treated with 200 μg/ml. of pronase (Calbiochem) at 38° C for 2 h and then extracted with phenol. The aqueous phase was used for assay directly or after denaturation by heating in a boiling water bath for 10 min followed by rapid cooling. The assay was carried out at *p*H 9·5 for 60 min at 38° C with 23 units/ml. of exonuclease (kindly supplied by Dr R. Wells, University of Wisconsin) or buffer, and the amount of acid insoluble counts was determined.

To study the DNA-dependent DNA polymerase without disturbing its relationship to the proteins in the nucleoid, disrupted virions were pretreated with 50 μg of ribonuclease A for 60 min at 0° C to destroy the RNA template, exogenous DNA was added and a typical polymerase assay was done. It was found (Table 2) that there was a ten to fifteen-fold stimulation of incorporation of ^{3}H-dTTP by native calf thymus or *E. coli* DNA. The stimulation was reduced two-fold by denaturation. This template dependence was confirmed (personal communication from John Coffin) by the isolation by equilibrium sucrose density gradient centrifugation of a polymerase activity from ^{3}H-uridine labelled virions. This activity contained no radioactivity and banded at a density greater than that of the ribonucleoprotein nucleoids. This polymerase activity was almost completely dependent on added RNA (from mouse liver) or added DNA.

To find out more about the activity of this DNA-dependent DNA polymerase, ribonuclease-treated dis-

Table 2. EXOGENOUS DNA AS TEMPLATE

DNA added	^{3}H-dTMP incorporated (pmoles)	Ratio
None	2·3	1
Calf thymus		
Native	23	10
Denatured	13·2	5·8
E. coli		
Native	32·2	14
Denatured	20·2	8·8

Disrupted virions were pretreated with 50 μg/ml. of ribonuclease A (Worthington) for 60 min at 0° C. 1·0 μg of calf thymus DNA (Worthington) or *E. coli* DNA (obtained from Dr H. Kubinski, University of Wisconsin) in 0·015 M NaCl either native or denatured by heating in a boiling water bath for 10 min followed by rapid cooling, was added, and a standard polymerase assay was carried out for 1 h. Counting efficiency of 20 per cent was assumed.

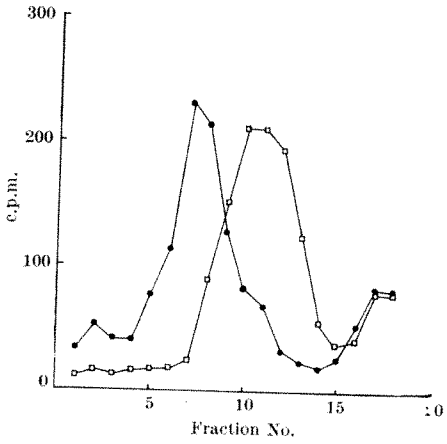


Fig. 2. Endonuclease for DNA. ^{14}C-labelled T7 DNA was incubated for 1 h at 40° C in 0·125 ml. containing pronase treated whole or disrupted virions (about 10 μg protein), treated for 30 min with 0·5 per cent sodium dodecyl sulphate 200 μg/ml. pronase, layered on neutral sucrose gradients (0·1 M NaCl–0·05 M EDTA–0·05 M Tris-HCl, *p*H 8·0) and centrifuged for 2·5 h at 45,000 r.p.m. in an 'SW 50·1' rotor. Drops were collected from the bottom of the tube and total counts were determined. ●, Non-disrupted virions; □, disrupted virions.

rupted pronase-treated virions were incubated with different single and double stranded homopolymers. Poly rA did not act as a template, and poly dT was a poor template (Table 3). Poly dA acted as a poor template in the presence of only dTTP, and also in the presence of only dATP. There is probably some small amount of contamination of poly dT in the sample so that poly dA·dT is the actual template (personal communication from R. Wells). The poly dA was a good template in the presence of both dTTP and dATP. Poly rA·dT was a fair template for poly dT and poly dA synthesis, even in the presence of only one substrate. Poly dA·dT was the best template for synthesis of both poly dT and poly dA. Synthesis was better when both dTTP and dATP were present in the reaction mixture. The activities of exogenous DNA as template were, within a factor of 10, the same as the activity of the endogenous RSV RNA as template.

To determine if the small size of the product was the result of nuclease action, virions were incubated with labelled T7 DNA and the size of the T7 DNA was analysed on sucrose gradients[7]. Incubation with whole virions reduced the *s* value of T7 DNA. To remove this external enzyme activity, virions were incubated with 20 μg/ml. pronase for 20 min at 37° C before sucrose gradient centrifugation. These virions had no endonuclease activity, unless they were disrupted with 'Nonidet' (Table 4). To determine if any exonuclease activity was present in the virions, labelled chicken DNAs were incubated with about 10 μg of disrupted virions and the amount of acid insoluble counts remaining was determined. No exonuclease activity was found.

To determine some of the requirements for the endonuclease activity inside the virions, labelled T7 DNA was incubated with disrupted pronase-treated virions and various other components of the polymerase assay, and the size of the T7 DNA was determined by sucrose gradient centrifugation (Table 4 and Fig. 2). $MgCl_2$ was required for endonuclease activity. In the presence of the ATP generating system endonuclease activity was reduced. In the presence of the dNTP alone no reduction in endonuclease activity was found. These experiments suggest that in addition to the endonuclease there may be a ligase system in the virion.

We have demonstrated that virions of Rous sarcoma virus contain several enzyme activities: an RNA-dependent DNA polymerase, a DNA-dependent DNA polymerase, and an endonuclease for DNA. All are present inside the virion as shown by their resistance to pronase digestion. It is not known whether they represent separate enzymes or separate activities of one enzyme.

Table 4. REQUIREMENTS FOR ENDONUCLEASE

System	Endonuclease
Non-disrupted virions, $MgCl_2$	−
Disrupted virions, $MgCl_2$	+
Disrupted virions	−
Disrupted virions, $MgCl_2$, dNTPs	+
Disrupted virions, $MgCl_2$, PEP, ATP, pyruvate kinase	±
Disrupted virions, $MgCl_2$, dNTPs, PEP, ATP, pyruvate kinase	±

^{14}C T7 DNA was incubated and analysed as described in the legend of Fig. 2 with the various components of a standard polymerase assay added. All virions were pronase-treated. A positive endonuclease (+) was a shift of greater than 10S (see Fig. 2). A ± reaction was a broad peak that shifted less than 10S.

The result of the action of these enzymes in a cell-free system is to make small pieces of double-stranded DNA from a single-stranded RNA template. There seems to be a transitory RNA–DNA hybrid intermediate, but double-stranded DNA is present from 1 min after the start of the reaction (unpublished data). Further, the enzyme seems to prefer an ordered DNA-like structure as template (personal communication from R. Wells). For example, poly dA·dT is a better template than poly dT, poly dA or poly rA. Poly rA·dT is a better template than poly rA (for poly dT synthesis) and a better template than poly dT (for poly dA synthesis). Native *E. coli* or calf thymus DNA are better templates than denatured *E. coli* or calf thymus DNA. With the homopolymers, poly dA, poly rA·dT, and poly dA·dT, there was more incorporation when both dTTP and dATP were present in the reaction mixture than when only one was present. These results suggest that this DNA-dependent enzyme system prefers double stranded templates, and, perhaps, copies both template strands at the same time. In short incubation times such as we have used, other DNA polymerases do not seem to have the same marked specificity for double-stranded templates[6].

In the cell-free enzyme system we have used, the endonuclease in the virions appears to cleave the double-stranded DNA as it is synthesized, and to cleave exogenous DNA.

In a completely native undisturbed system as in cells infected by virus, the actions of these enzymes might be restricted by topological factors, such as the location of the nucleoid and the presence of the viral RNA template, and so be more specific than indicated by these experimental results. The endonuclease might then act as a component of the machinery to integrate the viral double-stranded DNA into the host cell chromosome[8].

These results support the protovirus hypothesis[9] that RNA tumour viruses come from natural elements replicating DNA to DNA and DNA to RNA to DNA and controlling cell multiplication. They also raise the possibility that the difference between RNA leukaemia viruses and RNA sarcoma viruses may lie in the presence or absence and in the specificities of these enzymes. These results

Table 3. HOMOPOLYMERS AS TEMPLATE

Template	None		Poly dA·dT		Poly rA·dT		Poly dA		Poly dT	
Substrate	dTMP	dAMP	dTMP	dAMP	dTMP	dAMP	dTMP	dAMP	dTMP	dAMP
α-^{32}P-dATP	—	0·03	—	1·8	—	0·25	—	0·6	—	0·075
^{3}H-dTTP	0·045	—	2·2	—	1·65	—	0·35	—	0·06	—
α-^{32}P-dATP and ^{3}H-TTP	0·065	0·03	5·95	5·4	1·85	0·4	4·9	4·3	0·45	0·47

Values represent pmoles incorporated. Disrupted pronase-treated virions were incubated with 50 μg/ml. ribonuclease A for 60 min at 0° C. 0·4 μg of the indicated templates (kindly prepared by Dr R. Wells) and 0·2 nmoles of α-^{32}P-dATP (550 c.p.m./pmole) and/or 0·2 nmoles of ^{3}H-dTTP ($2{\cdot}65 \times 10^{4}$ c.p.m./pmole) were added and a standard polymerase assay was carried out for 1 h. Samples were counted for ^{3}H and ^{32}P, and the results calculated as pmoles of substrate incorporated, assuming a counting efficiency of 20 per cent for ^{3}H. With 10 μg of poly rA as template, no incorporation was found.

also raise the question of the role of these virus enzymes in cell DNA synthesis in infected cells and in malignant conversion by RNA tumour viruses.

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Viral DNA-dependent DNA Polymerase and the Properties of Thymidine Labelled Material in Virions of an Oncogenic RNA Virus

by

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RNA tumour viruses contain a DNA-dependent DNA polymerase which preferentially uses either single or double stranded DNA rich in guanosine and cytosine as template. The virion also contains DNA of similar composition as well as A+T rich DNA.

MUCH interest has been shown recently in the RNA-dependent DNA polymerase present in various oncogenic RNA viruses[1-3]. Here we describe a DNA polymerase activity and a thymidine labelled material which we found in the virions of avian myeloblastosis (AMV). Using various DNAs as templates we have shown that AMV possesses a DNA-dependent DNA polymerase which acts preferentially on a DNA template rich in G+C to form a DNA product that has the same amount of G+C as its primer. We also demonstrate that a similar kind of DNA having a high G+C content occurs as well as that enriched in A+T in virions of AMV. Early attempts to detect DNA in oncogenic RNA viruses were not successful[4,5].

The virus particles suspended in buffer alone had no enzyme activity. A virus enzyme preparation from purified AMV (see legend to Table 1), used in this study, exhibited an endogenous incorporation of 0·4–0·6 pmoles of dTMP (or dAMP) per 24 μg of protein into polymer as described by Temin[1]. No incorporation of uridine monophosphate into ribopolymers was detectable using an assay system previously described[6] and no further stimulation was observed when RNA or DNA was added to the assay system containing ribopolynucleotides.

Table 1 shows that the enzyme activity, as measured by the incorporation of deoxyribonucleoside triphosphate into acid insoluble material, was stimulated by DNA templates obtained from bird, mammal and bacterial cells. The denatured DNAs were significantly more efficient as templates than the native preparations. The most active of the three DNAs tested was DNA from *Streptomyces chrysomalus* with a G+C content of 70 mole per cent[8]. This template was chosen on the basis of the properties of the thymidine labelled material found in the AMV. With denatured *Streptomyces chrysomalus* DNA the enzyme activity was fifteen times higher than with denatured DNAs from other sources and seventy times higher than the endogenous activity. Table 2 shows that the enzyme present in the AMV virions has the properties expected of a DNA-dependent DNA polymerase.

Omission of one of the three unlabelled deoxynucleoside triphosphates from the complete system in the presence or absence of the template DNA led to a significant decrease in the activity of the enzyme. These results indicate that a heteropolymer is being synthesized. In accordance with the properties of the G+C rich template DNA used (*Streptomyces chrysomalus* DNA), the greatest decreases in activity were measured after omission

Table 1. EFFECT OF DIFFERENT DNA PREPARATIONS ON ^{3}H-dAMP INCORPORATION INTO ACID INSOLUBLE MATERIAL

DNA preparation	Amount (μg)	G+C (mole per cent)	^{3}H-dAMP incorporation (pmoles)
Avian myeloblast, native	32	43	0·32
Avian myeloblast, heated	32	43	0·97
Avian myeloblast, alkaline treated	32	43	0·81
Calf thymus, native	40	39	0·46
Calf thymus, heated	40	39	1·00
Streptomyces chrysomalus, native	40	70	3·02
Streptomyces chrysomalus, heated	40	70	15·10
None	—	—	0·22

Enzyme preparation: AMV was isolated from leukaemic chicken plasma containing $6{\cdot}5 \times 10^{11}$ particles/ml. Plasma was purified by centrifuging twice at 2,500*g*, and the viruses were concentrated by sedimentation through a 15 per cent glycerol layer at 49,000*g* for 60 min in an 'MSE-40' centrifuge. The sedimented viruses were resuspended in 8 per cent glycerol supplemented with NaCl, Tris-HCl (*p*H 7·5) and $MgCl_2$ to a final concentration of 0·1, 0·05, and 0·0025 M respectively. They were further purified by banding at the interphase of 100 and 15 per cent glycerol layers at 63,500*g* for 90 min in an angle rotor 'SW 25·1, L2 65K' Spinco centrifuge. Such a virus preparation checked in a sucrose isopycnic density gradient centrifugation revealed a homogeneous absorbance peak as well as a peak of ATPase activity[7] at a density of 1·165 g/cm^3. The purified virus preparation containing 25 mg of protein, determined according to Warburg, was suspended in 2 × buffer TMG (1 × buffer TMG contains: 0·025 M Tris-HCl (*p*H 8·6), 0·01 M MgCl, reduced glutathione 0·005 M, 0·005 M EDTA, 0·15 M KCl and 10 per cent glycerol). To the virus suspension was added an equal volume of solution containing 'Nonidet' (Shell *P*-40) and dithiothreitol to give final concentrations of 0·25 and 0·065 per cent, respectively[1]. The protein concentration of the enzyme preparation was 12 mg/ml. The enzyme was not further purified and could be stored in an ice bath without loss in activity for 1 week. The enzyme was diluted 1:10 with buffer TMG just before the assay. Enzyme assay: the complete assay mixture (0·2 ml.) contained the following: 8 μmole Tris-HCl (*p*H 8·6), 1·6 μmoles $MgCl_2$, 12 μmoles NaCl, 0·74 μmoles reduced glutathione, 0·2 μmoles of dTTP, dCTP and dGTP (A grade, Calbiochem), $0{\cdot}12 \times 10^{-3}$ μmoles ^{3}H-dATP (Schwarz BioResearch), (2,920 c.p.m./pmole) and 20 μg of virus protein. The reaction mixture was incubated for 45 min at 37° C and the reaction was terminated by addition of sodium pyrophosphate, carrier yeast RNA and trichloroacetic acid. The acid insoluble radioactivity remaining after filtration through membrane filters was determined by scintillation spectrophotometry (Packard, model 3375), using BBOT-Packard as scintillation fluid. The radioactivity of an unincubated sample was subtracted from each value. DNAs used as internal absorbance markers for rate and equilibrium density gradient centrifugation were highly purified ribonuclease treated preparations. Calf thymus DNA and *Streptomyces chrysomalus* DNA were kindly provided by Dr J. Šponar, Biophysical Group of this institute. DNAs were denatured by heating at 100° C for 5 min in a solution of 0·01 NaCl–0·001 sodium citrate (*p*H 7). This was followed by rapid cooling of the samples in an ice bath. DNA samples were incubated in 0·1 M NaOH for 30 min at 22° C. After neutralization the samples were precipitated with ethanol at −20° C.

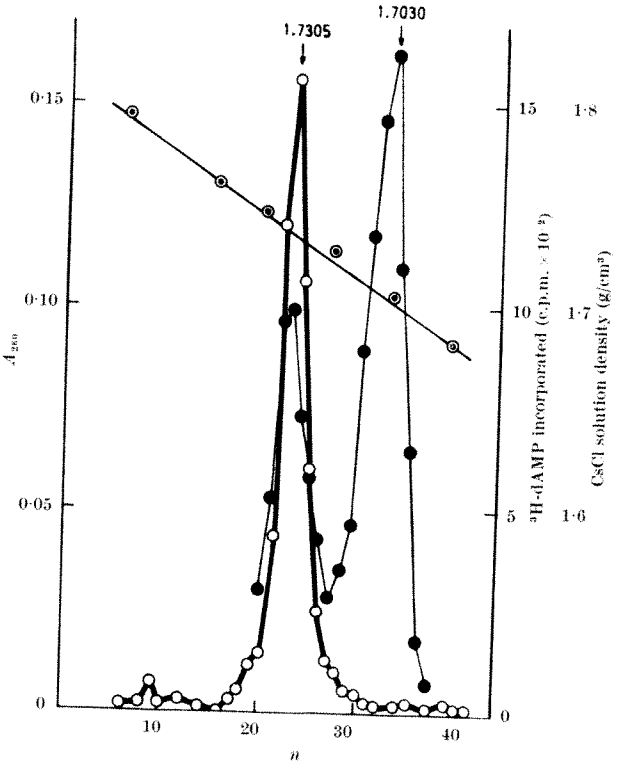


Fig. 1. Buoyant density profiles of template DNA (absorbance profile, ●); product DNA (radioactivity, ○) and marker DNA from avian myeloblasts (80 μg) with an absorbance peak at a density of 1·70. The template was heated DNA (37 μg) from *Streptomyces chrysomalus* which bands at a density of 1·73 g/cm³ CsCl. Reaction conditions were the same as in Table 1. The reaction was run for 45 min at 38° C. 60,000 c.p.m. were incorporated (16·1 pmoles of ^{3}H-dAMP) into acid-insoluble product. The reaction was terminated by adding 2·5 volumes of phenol and freezing the sample in an alcohol-solid CO_2 mixture. The sample was phenol extracted and ether extracted to remove the phenol before putting the sample on a preformed[9] CsCl gradient with DNA from myeloblasts as a marker. The CsCl gradient was centrifuged for 42 h at 37,000 r.p.m. in a Spinco 'SW 39' rotor at 15° C. The radioactivity in the sampled fractions was determined as described in the legend to Table 1; the density of CsCl solutions (○) according to Szybalski[8].

of dGTP; the next greatest decrease was after omitting dCTP. By contrast, the endogenous activity of the enzyme was more sensitive to the omission of dATP, and was less influenced by the omission of dGTP and dCTP from the assay mixture.

Table 2. DEPENDENCE OF ENZYME ON TRIPHOSPHATE AND DNA TEMPLATE

Reaction mixture	Template	^{3}H-dTMP incorporated (pmoles)
Complete	DNA	9·66
−dATP	DNA	3·21
−dGTP	DNA	1·52
−dGTP	DNA	2·10
Complete	0	0·50
−dATP	0	0·03
−dGTP	0	0·10
−dCTP	0	0·08

The complete assay mixture (0·2 ml.) contained 8 μmoles Tris-HCl (*p*H 8·6), 1·6 μmoles $MgCl_2$, 12 μmoles NaCl, 0·74 μmoles reduced glutathione, 0·2 μmoles of dATP and dCTP, and [*methyl*-^{3}H]dTTP (Schwarz BioResearch), $0{\cdot}16 \times 10^{-3}$ μmoles (3,950 c.p.m./pmole). *Streptomyces chrysomalus* DNA was added where indicated, at a level of 24 μg per assay. Isotope incorporated into acid-insoluble material was measured after 45 min incubation at 37° C as described in Table 1.

Material extracted in phenol from a reaction mixture incubated with the enzyme and *Streptomyces chrysomalus* DNA as template was analysed by isopycnic CsCl density gradient centrifugation[9]. An unlabelled myeloblastic DNA was used as the second absorbancy marker. After centrifugation, the radioactivity was situated in the same density region of the absorbance profile as that of the template DNA which, when native, has a density of 1·734 g/cm³ (Fig. 1). This finding suggested the formation of a copy corresponding to the template.

The presence of DNA was shown conclusively by the incorporation of ^{3}H-labelled thymidine into virions produced by myeloblasts cultivated[10] in conditions that eliminated mycoplasma infection (see legend to Fig. 2). The ^{3}H-thymidine label in purified undamaged virus particles was not accessible to deoxyribonuclease and the labelling kinetics were approximately linear during a 12 h labelling period (unpublished results of J. R.).

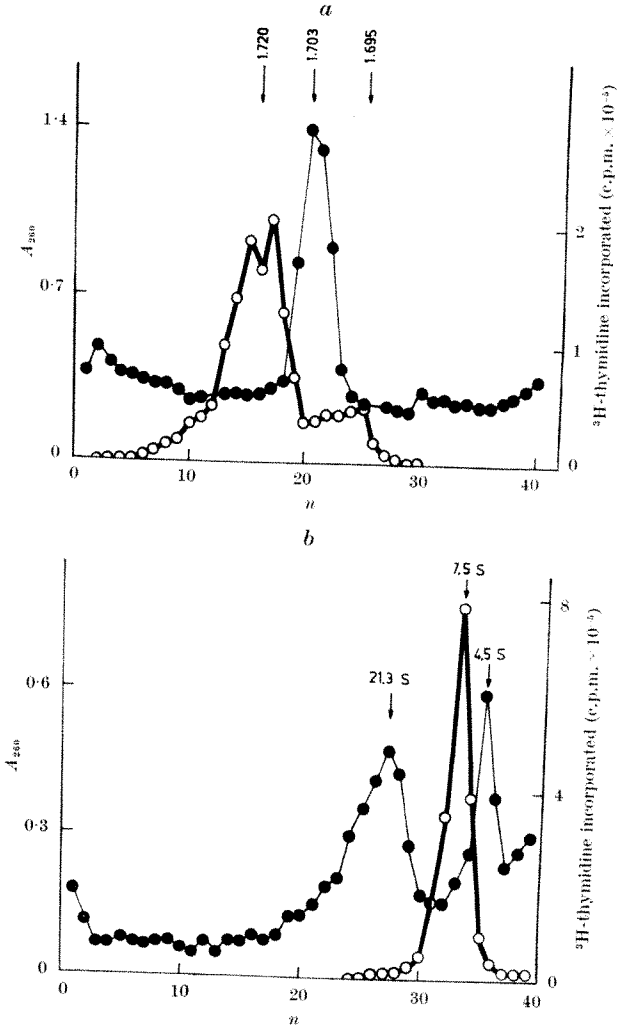


Fig. 2. *a*, Buoyant density profiles of ^{3}H-thymidine labelled material (radioactivity, ○) extracted from AMV virions and absorbance marker (chicken spleen DNA, ●, with a peak at a density of 1·70) after isopycnic CsCl density gradient centrifugation (see legend to Fig. 1). *b*, Radioactivity profile (○) of the same material on a linear (20–5 per cent) sucrose density gradient after 7 h at 25,000 r.p.m. and 4° C in Spinco rotor 'SW 25·1', with absorbance profiles (●) of chicken spleen DNA (21·3S) and *E. coli* B tRNA (4·5S). Labelling of the virus and isolation of viral ^{3}H-thymidine labelled material: 20 ml. suspension of avian myeloblasts ($7{\cdot}6 \times 10^7$ cells/ml.) were cultivated[10] for 7 days in the presence of the usual antibiotics and aureomycin (12 μg/ml.). Viruses from such cultures were void of any mycoplasma when examined by electron microscopy. The cells were labelled for 7 h with 6-^{3}H-thymidine (UVVR, Praha), 250 μCi/ml. (specific activity 25 Ci/mmole) and ^{14}C-uridine (Amersham), 4 μCi/ml. (specific activity 278 mCi/mmole). Viruses produced by leukaemic cells were concentrated and purified according to Erikson[17]. The final virus pellet was extracted with a solution containing 1 per cent sodium dodecyl sulphate (SDS), 0·01 per cent EDTA, and 0·01 M sodium citrate (*p*H 8·5) for 10 min at 22° C, then deproteinized with phenol. The phenol phase was re-extracted with 1 M NaCl. Both aqueous phases were adjusted with solid NaCl to a final concentration of 1 M and spun down with 250 μg of chicken spleen DNA at 39,000 r.p.m. in Spinco rotor 'SW 39' for 24 h at 4° C. The DNA pellet was dissolved in a 0·015 M NaCl–0·0015 M sodium citrate solution (*p*H 7), and was treated with a mixture of ribonuclease A (Worthington, 100 μg/ml.) and T, (Sankyo, 20 U/ml.). It was then inactivated at 90° C for 5 min. Ribonucleases were removed with phenol and the water phase treated with ether. Such samples were used for rate sucrose density gradient and for isopycnic CsCl gradient centrifugation. In the CsCl gradient banded viral DNA, no ^{14}C radioactivity was detected.

The ^{3}H-thymidine labelled material isolated from purified virus had the properties of DNA when banded in isopycnic CsCl gradients. This material was of two kinds: one banded in G + C rich density region; the other had less G + C, being similar in this respect to myeloblastic or chicken spleen DNA which has a G + C content of 44 mole per cent[11]. The presence of G + C rich DNA in the virions was more obvious in experiments with a longer labelling time (Fig. 2*a*) whereas the other type of viral DNA was detected when pulse labelling was used (Figs. 3 and 4).

Fig. 2*a* shows the relative buoyant density of chicken spleen DNA compared with DNA isolated from virus after a 7 h labelling period. In this case a large fraction of the DNA is rich in guanine and cytosine; there is a minor component that is rich in adenine and thymine. Fig. 2*b* shows that both fractions sediment in a sucrose density gradient at 7·5S as a uniform molecular species.

Fig. 3 shows the buoyant density of myeloblast cell DNA relative to that of DNA isolated from viruses produced by cells which were labelled for 60 min with ^{32}P in a phosphate free medium and then cultivated for 7 h with radioisotope in a complete medium that had been used for cultivation of leukaemic chicken myeloblasts[10]. In this case most of the DNA exhibited a lower G + C content while a little was situated in the density region of DNA rich in G + C. ^{32}P-labelled material was resistant to NaOH and sensitive to deoxyribonuclease. A core of deoxyribonuclease resistant material could be observed in the density region corresponding to a lower G + C content (Fig. 3). A total nucleic acid extraction from the same virus preparation, untreated with ribonucleases and analysed in glycerol density rate gradients, was composed of 65S RNA and the low molecular weight component which represented 70 per cent and 30 per cent respectively of the total radioactivity. A considerable part (about 30 per cent) of the low molecular weight material, which is considered to be a mixture of tRNA molecules and degradation products of 65S RNA[5,12–16], was resistant to alkali and sensitive to deoxyribonuclease. After alkali treatment there was a peak in the region of 7–8S (unpublished results of J. R.).

A similar characteristic was also observed after pulse labelling with thymidine (see legend to Fig. 4). Fig. 4

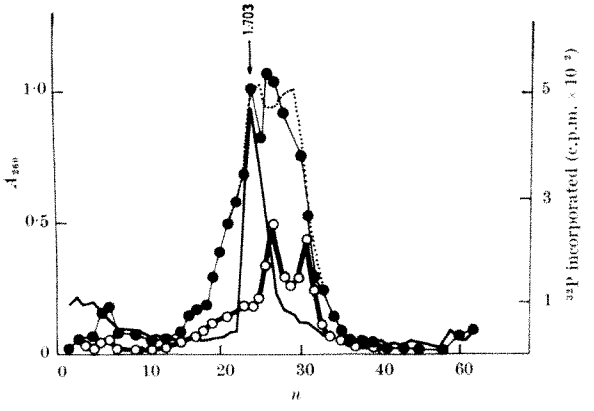


Fig. 3. Buoyant density profiles of ^{32}P-labelled material (●) remaining after ribonuclease treatment of the total viral nucleic acid extract, and its behaviour toward an alkaline and deoxyribonuclease treatment. Such ^{32}P-labelled virus material banded in an isopycnic CsCl density gradient, with myeloblast DNA (——) as an absorbance marker, was subjected in fractions to an alkaline or deoxyribonuclease I treatment. One third of each fraction was treated with 0·1 M NaOH at 22° C, for 30 min, or with deoxyribonuclease I (Worthington, ribonuclease free, 20 μg/ml.), 0·05 M Tris-HCl, 0·05 M (*p*H 8·5), and 0·005 M MgCl$_2$, at 22° C for 60 min. Then the radioactivity was determined in acid insoluble material of the untreated (●), alkali-treated (· · · ·) and deoxyribonuclease treated (○) samples. Virus labelling: in this case a myeloblast cell suspension (20 ml., 8·7 × 10^7 cells/ml.) was labelled for 60 min with 350 μCi/ml. of ^{32}P (carrier-free orthophosphate; Radiochemical Centre, Amersham; specific activity 78 Ci/mg P), in a phosphate-free medium. This medium was then replaced by a complete one[10] and the virus produced during a further 7 h of cultivation was concentrated and purified according to Bader and Steck[15].

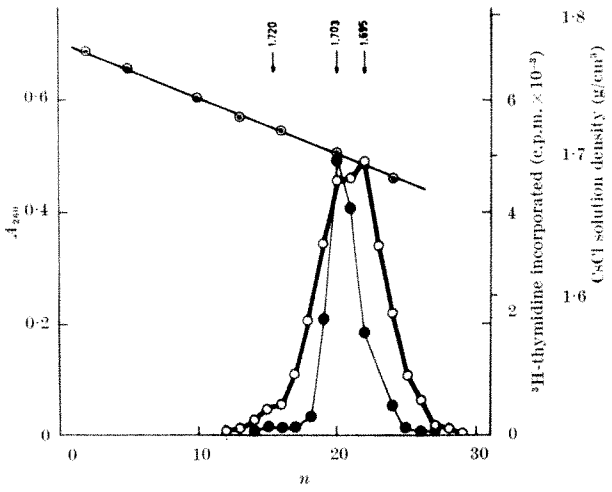


Fig. 4. Buoyant density profiles of 7·5S ^{3}H-thymidine labelled material (○) from AMV virions produced after pulse labelling of avian myeloblasts. Virus labelling: myeloblast cell suspension (10 ml., 7·2 × 10^7 cells/ml.) was labelled with 6-^{3}H-thymidine (UVVR, Praha), 350 μCi/ml. (specific activity 20 Ci/mmole) and with ^{14}C uridine (UVVR, Praha), 2·5 μCi/ml., (specific activity 286 mCi/mmole) in the presence of deoxycytidine (2·5 μg/ml.), deoxyadenosine (0·12 μg/ml.), deoxyguanosine (0·12 μg/ml.) and cytosine (2·5 μg/ml.) for 120 min. The medium was then replaced by an unlabelled medium[10] which was changed four times at 90 min intervals to obtain freshly produced virions unaltered by ageing[18] or not influenced by atypical cell incubation conditions like those represented by a phosphate-free medium. The virus was collected and purified[17] from the joined cell supernatant derived from each unlabelled medium exchange and viral nucleic acid was isolated according to Bader and Steck[15]. The isolated viral RNA was further analysed in a linear 40–10 per cent glycerol density gradient at 37,000 r.p.m., Spinco rotor 'SW 39', at 4° C for 140 min and the peak fraction of the 7·5S thymidine labelled material, selected on the basis of the radioactivity sedimentation profiles, was used for isopycnic CsCl density gradient centrifugation. Myeloblast DNA also served here as an absorbance marker (●). In this, as well as in all other cases, the density of CsCl solution (○) in individual fractions was determined according to Szybalski[8].

shows the buoyant density profiles of thymidine labelled 7·5S material extracted from the virions and selected after a glycerol density gradient rate centrifugation. In this case too, a large fraction of DNA exhibited a lower content of G + C while a small fraction was situated in the density region of DNA rich in G + C.

Our experiments demonstrate the presence of a DNA-dependent DNA polymerase in the virus enzyme preparation used for studying the endogenous activity of an RNA-dependent DNA polymerase in various oncogenic RNA viruses including AMV[3]. The primed reaction uses native and denatured DNA as templated with an outstanding preference for a DNA rich in guanine and cytosine. Such a template was chosen on the basis of similarities in base composition with one of the two kinds of DNAs detected in AMV. It remains to be seen whether such a preference exists for templates similar in their base composition to the second kind of DNA observed in the virions. In view of the high sensitivity of the endogenous virus enzyme to the omission of dATP, an A + T rich DNA might be the proper template for the virus enzyme endogenous reaction. This will be clarified by further definition of the viral enzymes using both types of virus DNA as templates. The function of the viral 7–8S DNA molecules remains to be elucidated. When double stranded, such molecules could carry a genetic message specifying one or two proteins of average size. That they are heterogenous as regards G + C content is evident from their relatively broad buoyant density profiles. This heterogeneity could be explained either by their association with some other component or by chemical changes which affect the buoyant density of a DNA[8]. Other possibilities such as association with very small fragments of RNA cannot be ruled out, even though these DNA types were free of ^{14}C-uridine (see legend to Fig. 2). The labelling kinetics of DNA extracted from virions revealed a synthesis independent of cellular

DNA (unpublished results of J. R.). Thus the origin of these molecules as well as the DNA-dependent DNA polymerase remains an open question.

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Synthetic DNA–RNA Hybrids and RNA–RNA Duplexes as Templates for the Polymerases of the Oncogenic RNA Viruses

by

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Six RNA viruses have now been shown to contain DNA polymerase activities directed by single-stranded RNA, double-stranded RNA and double-stranded DNA. It is further demonstrated that DNA–RNA hybrids, such as synthetic dC·rG, act as even more effective templates.

THE recent reports by Temin[1] and Baltimore[2] of an RNA-directed DNA polymerase in oncogenic RNA viruses have been confirmed and extended[3]. We established that the DNA synthesized is complementary to the viral RNA and that DNA–RNA hybrids appear as early intermediates of the reaction. Both features argue compellingly that viral RNA functions as a template in the synthesis of the DNA.

The RNA–DNA hybrids detected as presumptive intermediates are not likely to be integrated as such into the genome, an event necessary to explain the heritably stable state of the transformation induced by these oncogenic viruses. The required conversion of the RNA–DNA hybrid into a DNA–DNA duplex demands a DNA-directed DNA polymerase. We already had[3] suggestive evidence that DNA strands synthesized late in the reaction were identical rather than complementary to the viral RNA. We set out to find the DNA-directed DNA polymerase implied by these observations and detected[4] the relevant activity in all six oncogenic viruses examined. The DNA-directed polymerase activity thus revealed had a number of interesting properties. There was a clear preference for double-stranded DNA and, except for covalently linked circles, virtually any double-stranded DNA was accepted as a template. In each case, it could be shown by appropriate hybridizations that the DNA synthesized was complementary to the DNA used as the initiating template.

We were still left with the problem of identifying the polymerization reaction which converts the DNA–RNA hybrids into the DNA–DNA duplex. To synthesize natural DNA–RNA hybrids in amounts adequate for enzymatic test is still a formidable task. To avoid the logistic problems involved we decided to use synthetic polynucleotide duplexes. There is ample evidence[5–7] of their usefulness in studies of DNA and RNA synthesis, and the DNA polymerase of *E. coli* has been shown to use the synthetic ribopolymer rA·rU to synthesize the deoxyribopolymer dA·dT (ref. 8). We have found that certain of the synthetic duplexes are not only functional, but are superior to natural templates by almost two orders of magnitude in stimulating polymerization. This finding leads to an extraordinarily useful tool for detecting the enzyme activity. We summarize here the results of our investigations.

Comparison of Templates

The viruses used in the preparation of enzymes were avian myeloblastosis virus (AMV), feline leukaemia virus (FeLV), Moloney sarcoma virus (MSV), Rauscher leukaemia virus (RLV), mouse mammary tumour virus (MTV) and a rat mammary tumour virus (RMTV). Table 1 illustrates the responses of the six RNA oncogenic viral preparations to the resident RNA templates (column 1), exogenous DNA (column 2), and to the hybrid duplex (column 3) formed from polydeoxyadenylate and polyriboguanylate (dC·rG).

The stimulation observed with CEF–DNA is not maximal because for purposes of comparison it was held at the same level (4 μg/ml.), at which the reaction with the dC·rG is optimal. For all six viruses there is clear evidence of a hybrid directed DNA synthesis that is dramatically

superior to the reactions previously studied. The kinetics of DNA synthesis with the three templates (Fig. 1) emphasize the magnitude of the difference in template capabilities. When plotted on the same scale, the synthetic activities observed with the resident viral RNA and CEF–DNA are almost trivial compared with dC·rG.

We examined the template capabilities of as many synthetic polynucleotides as possible. Table 2 shows the response to single homopolymers. It should be noted that in this, as in the tables to be discussed, all measurements involved examinations over 20–30 min periods with samples taken every 5–7 min. The 5 min values are recorded to permit better comparison with those cases in which the kinetics diverge from linearity after 10 min. In all cases shown in this and subsequent tables, both complementary deoxyriboside triphosphates were included in the assay, one being identified by an α-^{32}P label, and the other by a ^{3}H label.

Table 2 demonstrates that of the ribohomopolymers, only rC possesses detectable template activity and here the synthesis lasts only for 5 min. In the case of the deoxyribopolymers, poly dC supports extensive and prolonged synthesis of poly dG, and some later incorporation of dC. Although much smaller, both poly dA and poly dI stimulate the incorporation of the corresponding complementary nucleotides.

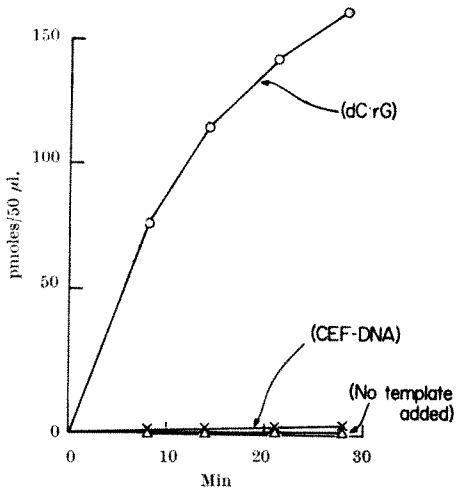


Fig. 1. Kinetics of incorporation of α-^{32}P-dGTP by RLV polymerase. A 0·25 ml. standard reaction mixture was used. The specific radioactivity of α-^{32}P-dGTP was 60 c.p.m./pmole. Methods of preparation of CEF-DNA and dC · rG are described in the legend to Table 1. At the indicated times, 50 µl. samples were withdrawn and the acid precipitable radioactivity was determined as described[3].

Table 1. RESPONSES OF ONCOGENIC VIRAL RNA POLYMERASES TO ADDED DNA AND A SYNTHETIC RNA–DNA HYBRID

Polymerase	pmoles nucleotide incorporated in 10 min/10 µg of viral protein		
	Template		
	None	CEF–DNA	dC·rG
AMV	2·8	5·3	140·0
FeLV	2·1	2·6	30·7
MSV	1·2	1·2	30·0
RLV	1·5	4·3	162·0
MTV	0·1	1·0	35·1
R-MTV	0·1	0·6	21·4

A standard incubation mixture of 0·25 ml. contains 12·5 µmoles Tris-HCl (*p*H 8·3); 3 µmoles $MgCl_2$; 10 µmoles KCl; 2·5 µmoles dithiothreitol; 0·04 µmoles of each of the deoxyribonucleoside triphosphates. To monitor the reaction, α-^{32}P-dGTP was used at a specific activity of 65 c.p.m./pmole. Reactions that were stimulated by added templates were carried out by including the template at a level of 1·0 µg per 0·25 ml. in the standard incubation mixture. The incorporations noted above represent those observed in 50 µl. aliquots corresponding to 10 µg of viral protein. Virus particles suspended in 0·01 M Tris (*p*H 8·3)—0·1 M NaCl—0·002 M EDTA (TNE) at a concentration of 2·3 mg of viral protein per ml. were preincubated for 10 min at 0° C in the presence of 0·2 per cent 'Nonidet P-40' and 0·1 M dithiothreitol. The incubated solution was then added to a standard incubation mixture at a level of 50 µg of viral protein per 0·25 ml. and incubated at 37° C. After incubation for 10 min, 50 µl. aliquots were withdrawn and processed for determining the acid precipitable radioactivity as described elsewhere[3]. The particles designated here as R-MTV were obtained from supernatant fluids of rat mammary tumour[9], R-35 grown *in vitro* at Charles Pfizer Co., Maywood, New Jersey. The source of the other viruses and their methods of purification have been described previously[3]; R-MTV was purified in the same way as the monkey mammary tumour virus. dC·rG was made by mixing equal amounts of poly dC and poly rG (100 µg per ml. in 0·01 M Tris, *p*H 7·4), making the solution 0·2 M with respect to NaCl and allowing the mixture to stand for 15 min at room temperature. The CEF DNA used was made from trypsinized cells from chick embryos according to the procedure of Gillespie and S.S.[10].

Table 2. RESPONSE OF AMV-POLYMERASE TO SINGLE-STRANDED HOMOPOLYMERS

Template	Incorporation (pmoles/5 min/10 µg viral protein)			
rA	dT	(<0·1)	dA	(<0·1)
rC	dG	(3·8)	dC	(<0·1)
rG	dC	(<0·1)	dG	(<0·1)
rI	dC	(<0·1)	dG	(<0·1)
rU	dA	(<0·1)	dT	(<0·1)
dA	dT	(1·2)	dA	(0·2)
dC	dG	(11·0)	dC	(0·8)
dG	dC	(<0·1)	dG	(<0·1)
dI	dC	(1·2)	dG	(<0·1)
dT	dA	(<0·1)	dT	(<0·1)

The composition of the reaction mixtures was as described for Table 1, except that in each experiment only the two deoxyriboside triphosphates were used which corresponded to the identity and the complement of the bases in the template. The template concentration was 1 µg/0·25 ml. of the incubation mixture. The labels used were: ^{3}H-dATP (115 c.p.m./pmole), ^{3}H-dCTP (125 c.p.m./pmole), ^{32}P-dGTP (60 c.p.m./pmole), and ^{32}P-dTTP (110 c.p.m./pmole). 50 µl. of the reaction mixture was withdrawn and acid precipitable radioactivity was determined as described[3] after 5 min of incubation at 37° C.

The abilities of various synthetic double-stranded DNA and RNA homopolymers to serve as templates are recorded in Table 3. Except for rC·rG, all stimulate some incorporation of one of the complementary nucleotide pairs. Of those tested, rI·rC is clearly the best, followed by dC·dG and dC·dI. It is interesting to note that, with the exception of rC·rG, the polyribopolymers are superior to the corresponding deoxypolymers as templates. Table 4 compares the template capabilities of a variety of synthetic DNA–RNA hybrid duplexes. Some of these are the best templates, notably dC·rG and dI·rC.

Table 3. RESPONSE OF AMV-POLYMERASE TO SYNTHETIC DOUBLE-STRANDED DNA AND RNA POLYMERS

Template	Incorporation (pmoles/5 min/10 µg viral protein)			
rA · rU	dT	(15·0)	dA	(0·4)
rC · rG	dG	(<0·1)	dC	(<0·1)
rI · rC	dG	(128·0)	dC	(2·1)
dA · dT	dT	(0·8)	dA	(1·4)
dC · dG	dG	(26·9)	dC	(0·4)
dC · dI	dG	(24·9)	dC	(2·0)

The reaction mixtures and conditions of reactions were as described for Table 1. The duplexes were prepared by using the appropriate polymer components, using the method described for dC·rG (see legend to Table 1). The specific activities of the deoxyriboside triphosphates used were the same as those for Table 2, and the appropriate complementary pair of ^{3}H and ^{32}P-labelled nucleoside triphosphates were present in each reaction.

Table 4. RESPONSE OF AMV-POLYMERASE TO SYNTHETIC HYBRIDS

Template	Incorporation (pmoles/5 min/10 µg viral protein)			
dA · rU	dT	(0·8)	dA	(<0·1)
dD · rG	dG	(141·0)	dC	(1·9)
dC · rI	dG	(44·5)	dC	(1·9)
dG · rC	dC	(<0·1)	dG	(<0·1)
dI · rC	dC	(1·3)	dG	(104·6)
dT · rA	dA	(0·9)	dT	(10·3)

The reaction mixtures and conditions of reactions were the same as described in the legend to Table 1. The hybrids were prepared using the method described for making dC · rG in the legend to Table 1. All other details are as described in the legends to Tables 2 and 3.

There is a striking preference for dGTP and rGTP as substrates. This recalls an analogous situation observed with the Qβ replicase[6] and the DNA-dependent RNA polymerase[7], both of which synthesize poly G if provided with poly C as a template. The preference for dG may

Table 5. TEST OF AMV POLYMERASE TO INCORPORATE RIBONUCLEOTIDES WITH SYNTHETIC POLYNUCLEOTIDE TEMPLATES

Template	Incorporation (pmoles/90 min/10 μg viral protein) Ribonucleotides				Deoxyribonucleotides	
rA · rU	rU	(< 0·1)	rA	(< 0·1)	dT	(73·6)
rI · rC	rC	(< 0·1)	rG	(< 0·2)	dG	(326·0)
dA · dT	rU	(< 0·1)	rA	(< 0·1)	dT	(9·0)
dC · dG	rG	(< 0·1)	rC	(< 0·1)	dG	(109·0)
dC · rG	rG	(< 0·1)	rC	(< 0·1)	dG	(322·0)
dT · rA	rA	(< 0·1)	rU	(< 0·1)	dT	(49·7)

The reaction mixtures and the conditions of experiments were as for Table 1. Here, however, riboside triphosphates were used together with their deoxy analogues. Reactions in the absence of deoxyriboside triphosphates gave the same results. α-^{32}P-labelled deoxyriboside triphosphates and ^{3}H-labelled riboside triphosphates were used to monitor the polyribonucleotide synthesis, and the specific activities for the rATP, rGTP, rCTP, and rUTP were all at 120 c.p.m./pmole. The α-^{32}P-dGTP and α-^{32}P-dTTP used to follow the DNA synthesis were at 60 c.p.m./pmole.

indicate that guanosine is the 5′ terminus and is therefore the first nucleotide inserted. This is in fact the case with the Qβ replicase.

Asymmetric Copying

Tables 3 and 4 show a marked asymmetry in the use of the substrates provided. In no case are the two complementary nucleoside triphosphates incorporated with equal facility. The asymmetry, however, is not absolute, even in the 5 min results recorded in Tables 3 and 4. We found that although there was an initial preference for the incorporation of ^{32}P-dT, ^{3}H-dA is polymerized. A very similar phenomenon is observed (Fig. 2) with ^{32}P-dG and ^{3}H-dC in polymerizations by dI·rC. In some cases, synthesis prolonged for 90 min or more leads to eventual equalization of the incorporation of the two substrates. It should be noted that the use of the preferred substrate (for example, dTTP by dT·rA or dGTP by dI·rC) is not strikingly influenced by the presence of the complementary nucleoside triphosphate in the reaction mixture. As expected, there is symmetrical use and a requirement for the presence of both complementary substrates when an alternating copolymer is used as a template (Fig. 3). Here both complementary nucleotides are incorporated from the very onset at almost equal rates and molarities. Further, unlike the homopolymeric duplexes, leaving out either one of the complementary substrates leads to the complete loss of synthetic activity.

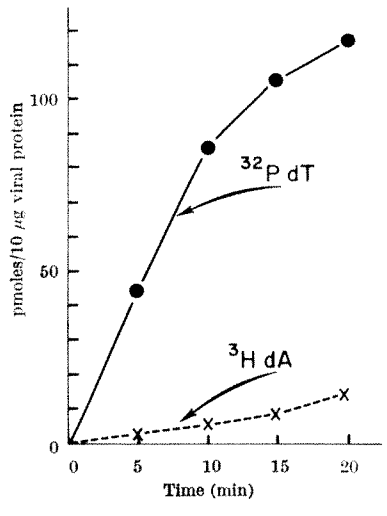


Fig. 2. Kinetics of incorporation of α-^{32}P-TTP and ^{3}H-dATP by AMV polymerase with dT · rA template. A 0·25 ml. standard reaction mixture was used. The specific radioactivities of α-^{32}P-TTP and ^{3}H-dATP were 110 c.p.m./pmole and 115 c.p.m./pmole, respectively. The method of preparation of the hybrid template is described in the legend to Table 1. At the indicated times, 50 μl. samples were withdrawn and the acid precipitable radioactivity was determined as described earlier[3].

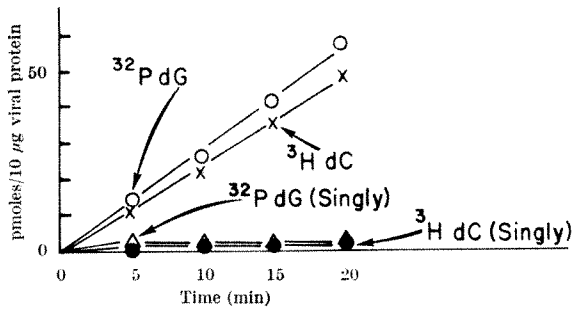


Fig. 3. Kinetics of incorporation of α-^{32}P-dGTP and ^{3}H-dCTP by AMV polymerase with d(I–C) as template. A 0·25 ml. standard reaction mixture was used. The specific radioactivities of α-^{32}P-dGTP and ^{3}H-dCTP were 60 c.p.m./pmole and 110 c.p.m./pmole respectively. At the indicated times, 50 μl. aliquots were withdrawn and the TCA precipitable radioactivity was determined as described earlier[3]. Results are expressed as pmoles of nucleotide incorporated/10 μg viral protein. ○ and × indicate the incorporation obtained when both deoxyribonucleoside triphosphates are present. △ and ● refer to the incorporation obtained when one nucleotide only is present.

Inability to Incorporate Ribonucleotides

It has already been noted[1–3] that the oncogenic polymerases do not synthesize ribopolymers using the viral RNA as a template. In view of the vastly superior template capabilities of some of the synthetic polymers, this question was re-examined and the results are recorded in Table 5. Even after 90 min, no signs of ribonucleotide incorporation could be detected with any of the templates during a period in which excellent polymerization (last column of Table 5) was occurring with the deoxyribonucleotides.

Uses for Synthetic Templates

A detailed examination of the intermediates in the reactions mediated by these synthetic duplexes should be very useful for the study of the chemistry of the polymerization and the factors which determine its asymmetry. Even a casual inspection of Table 1 and Fig. 1 reveals the obvious advantage of using templates such as dC·rG for detecting enzyme in cells or virus particles and following activity during purification procedures. We have now established that oncogenic viruses contain DNA polymerase activities directed by single-stranded RNA, double-stranded RNA, double-stranded DNA, and DNA–RNA hybrids. Further, all oncogenic RNA viruses examined contain all these activities. The number of different kinds of protein molecules required to handle this variety of templates remains to be determined.

We thank Drs F. Bollum, R. D. Wells and D. Brutlag for deoxyribonucleotide polymers; Dr J. W. Beard for a supply of AMV; Drs J. Moloney, F. Rauscher and T. O'Connor of the Special Virus Cancer Program (SVCP) of the National Cancer Institute for their assistance, and personnel of the laboratories associated with the SVCP for cooperation. The work was supported by a contract with the SVCP and a grant from the National Cancer Institute.

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DNA Polymerases of Rous Sarcoma Virus: Delineation of Two Reactions with Actinomycin

by

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The RNA-dependent and DNA-dependent DNA polymerase activities of RNA tumour viruses can be distinguished by their responses to actinomycin D; only the latter is inhibited.

RNA tumour viruses contain an enzyme which can transcribe DNA from an RNA template[1,2]. We have already reported a number of the properties of the DNA polymerase activities associated with avian myeloblastosis virus and Rous sarcoma virus (RSV)[3]. Several of these data suggest that there are at least two enzymatic reactions; synthesis of DNA from an RNA template, with the formation of an RNA : DNA hybrid; and subsequent synthesis of double-stranded DNA. We now present further data which indicate that both enzymatic activities are distributed in a homogeneous fashion throughout the virion population. This confirms recent reports that RNA tumour viruses contain both RNA-dependent[1,2] and DNA-dependent[4] enzymatic activities, and demonstrates that only the latter is inhibited by actinomycin D.

Effect of Actinomycin D

High concentrations of actinomycin D cause a two to three-fold reduction in the rate of DNA synthesis by detergent-treated virus (Fig. 1). Pretreatment of the polymerase reaction mixture with ribonuclease results in a five to ten-fold inhibition of enzymatic activity[1-3,5]. The residual DNA synthesis is unaffected by actinomycin D (Table 1). We suggest that the failure of ribonuclease completely to inhibit DNA synthesis may be due to the capacity of the polymerase to use highly fragmented RNA as template rather than to small amounts of endogenous DNA serving as template[6]. This view assumes that actinomycin D would have no effect on an RNA-dependent reaction, but would inhibit DNA-dependent reactions.

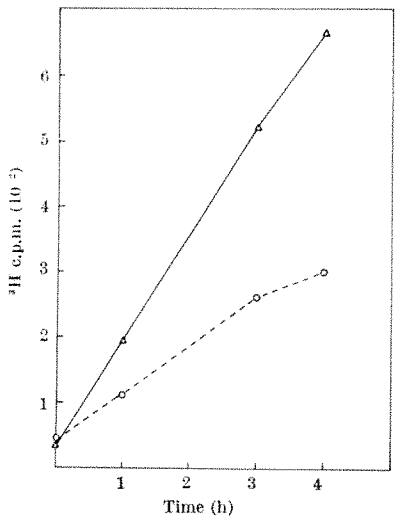


Fig. 1. Inhibition of viral DNA polymerase by actinomycin. Duplicate reaction mixtures were prepared, containing 23 μg of viral protein (purified RSV), 0·05 M Tris–HCl (pH 8·1), 0·01 M $MgCl_2$, 2×10^{-4} M dATP, dCTP and dGTP (Calbiochem), and 0·025 per cent 'Nonidet-P40' (Shell). One sample contained actinomycin D (50 μg/ml.), the other an appropriate volume of the solvent (50 per cent ethanol) used to dissolve the actinomycin. After incubation at 4° C for 15 min, ^{3}H-TTP (11 Ci/mmole, New England Nuclear) was added to 3×10^{-6} M, and incubation continued for 4 h at 37° C. Aliquots for precipitation with acid were taken at the indicated times after addition of ^{3}H-TTP (ref. 3). △—△, Control reaction; ○ - - - ○, actinomycin-treated reaction.

Table 1. INHIBITION OF THE POLYMERASE REACTION BY RIBONUCLEASE AND ACTINOMYCIN

	^{3}H c.p.m. incorporated	Per cent inhibition
(*a*) Control	2,100	—
(*b*) Ribonuclease	700	67
(*c*) Actinomycin	950	55
(*d*) Ribonuclease + actinomycin	720	66

Reaction mixtures similar to those described for Fig. 1 were prepared containing (*a*) an appropriate volume of the solvent (50 per cent ethanol) for actinomycin; (*b*) pancreatic ribonuclease (previously heated at 100° C for 15 min) at 1 mg/ml.; (*c*) actinomycin D, 50 μg/ml., and (*d*) ribonuclease and actinomycin at the foregoing concentrations. After incubation at 4° C for 15 min, ^{3}H-TTP was added and incubation continued at 37° C for 2 h. Samples were then precipitated with acid to determine radioactivity.

As previously reported[3-5,7], the product of the polymerase reaction contains at least two classes of DNA: (1) a complex between nascent DNA and viral RNA which has a heterogeneous, relatively heavy buoyant density in equilibrium density gradients of Cs_2SO_4; some of this material sediments rapidly during rate-zonal centrifugation (about 70S); and (2) DNA with a buoyant density approximately the same as that of the double-stranded DNA of lambda phage, but with a low sedimentation velocity (4–10S); we have shown (unpublished results) that this DNA is double-stranded by analysis on hydroxyapatite. We propose that these two species of nucleic acid represent the products of distinct enzymatic reactions, and that the synthesis of the second class of DNA should be preferentially inhibited by actinomycin D. Comparison of the DNA synthesized by the uninhibited reaction with that synthesized in the presence of actinomycin D has confirmed this hypothesis (Figs. 2 and 3). Synthesis of the rapidly sedimenting product is unaffected by the antibiotic, whereas that of the slowly sedimenting product is substantially reduced (Fig. 2; note the discontinuous scale in (*a*); the drawing is designed to illustrate the similar yields of rapidly sedimenting DNA in the two cases). Similarly, a normal amount of DNA with heterogeneous, relatively high buoyant densities is produced in the presence of actinomycin D (Fig. 3), whereas synthesis of the DNA which bands with phage lambda DNA is completely inhibited. Analysis of enzymatic product on hydroxyapatite demonstrates that the actinomycin-sensitive reaction is responsible for the synthesis of double-stranded DNA (Fig. 4). We conclude that actinomycin D preferentially inhibits the synthesis of one of the two or more classes of enzymatic product. The uninhibited portion of the reaction is responsible for the synthesis of a DNA : RNA hybrid, using viral RNA as template; the inhibited reaction

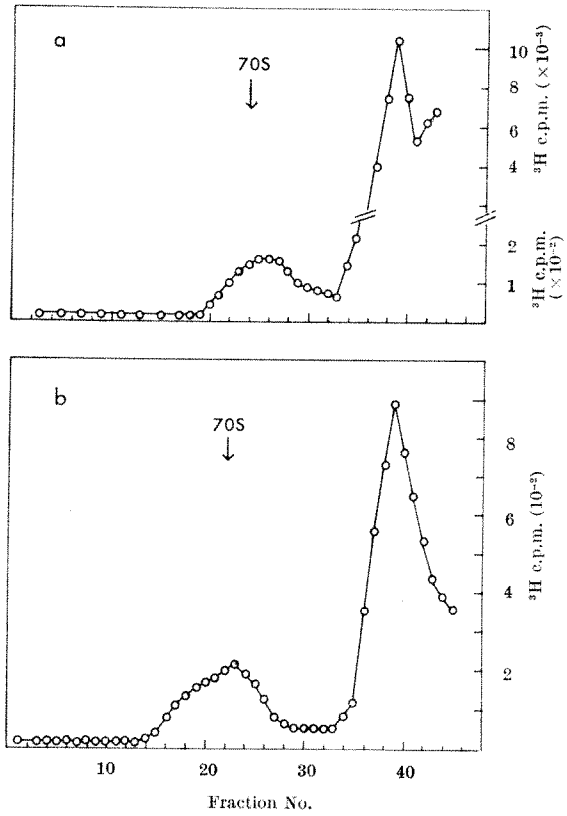


Fig. 2. Zone sedimentation analysis of enzymatic product. Reaction mixtures similar to those described for Fig. 1 were prepared with ten-fold more virus in ten-fold larger volumes. After incubation at 37° C for 4 h, the mixtures were treated with 0·5 per cent sodium dodecyl sulphate and pronase (500 μg/ml.) at 37° C for 45 min, followed by two phenol extractions at room temperature. Portions of the extracted nucleic acids were analysed by centrifugation through density gradients of 15–30 per cent sucrose in 0·1 M NaCl–0·001 M EDTA–0·02 M Tris–HCl (*p*H 7·4) ('SW 65' rotor, 60,000 r.p.m., 70 min, 4° C). The gradients were fractionated and precipitated with acid as described[3]. *a*, Control; *b*, actinomycin-treated reaction.

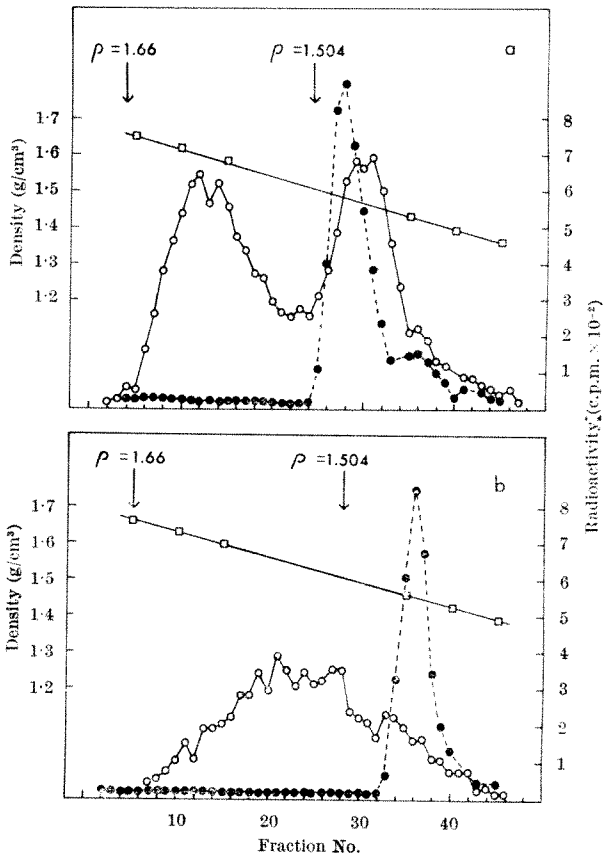


Fig. 3. Equilibrium centrifugation of enzymatic product. Nucleic acids were extracted from reaction mixtures as described for Fig. 2, then dialysed against 1,000 volumes of 0·05 M NaCl–0·002 M EDTA–0·02 M Tris–HCl (*p*H 7·4). Cs_2SO_4 (Gallard-Schlesinger, optical grade) was added to a final density of 1·52 g/cm³, and the solutions centrifuged in an 'SW 50' rotor, 33,000 r.p.m., 60 h, 4° C. Fractionation of the gradients, measurement of densities, and acid precipitation were carried out as described previously[3]. The arrows indicate the approximate expected densities for single-stranded RNA (ϱ=1·66) and hybrid molecules containing equal amounts of RNA and DNA (ϱ=1·504 (ref. 8)). *a*, Control. ³²P lambda phage DNA, denatured with alkali, was included as a density marker. *b*, Actinomycin-treated reaction. Native ³²P lambda phage DNA served as a density marker. ●, ³²P c.p.m.; ○, ³H c.p.m.; □, buoyant density (g/cm³).

synthesizes low molecular weight double-stranded DNA (Fig. 4).

Association of the Actinomycin-sensitive Reaction with Virions

To determine whether the actinomycin-sensitive reaction can be attributed to an integral component of the virion-associated polymerase, we have repeatedly examined the enzymatic activity of virions fractionated by isopycnic centrifugation in density gradients of sucrose (Fig. 5). In every instance, the actinomycin-resistant and actinomycin-sensitive components of the reaction were co-extensive through the virion population. These results suggest that the actinomycin-sensitive reaction is not an artefact from contamination with cellular DNA polymerase.

These findings suggest that synthesis of DNA by viral polymerase is at least a two step process. The putative second step, being sensitive to actinomycin, probably requires a DNA template. We have tested this further by determining the ability of the viral polymerase to respond to exogenous DNA. The initial, RNA-dependent reaction was inhibited by treatment with ribonuclease to reduce the production of intrinsic template for the second reaction. Addition of DNA (sheared, native calf thymus) then stimulated DNA synthesis so that incorporation of precursor exceeded that observed with a control reaction (Fig. 6). The same level of augmented incorporation was obtained by addition of DNA to a reaction which had not been treated with ribonuclease. Results similar to these were recently reported by Spiegelman *et al.*[4]. The effect of exogenous DNA is substantially

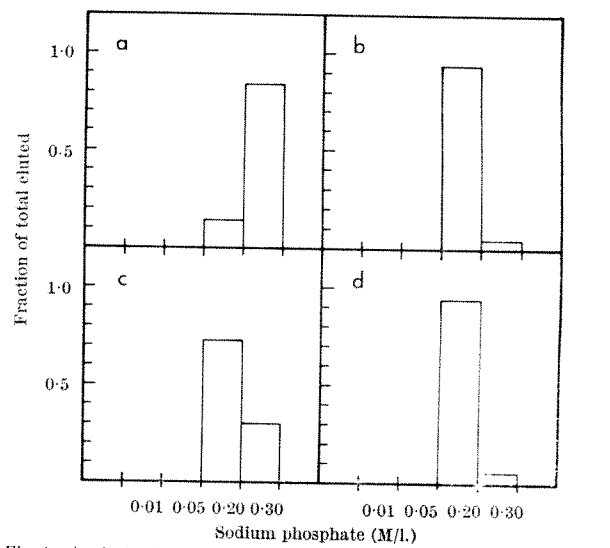


Fig. 4. Analysis of enzymatic product on hydroxyapatite. The products of control and actinomycin-treated reactions similar to those of Fig. 3 were analysed by step-wise elution from hydroxyapatite (our unpublished work). Double-stranded DNA, extracted from chick fibroblasts, and 70S DNA : RNA hybrid, purified by zonal centrifugation, were used as controls. The nucleic acids are treated with ribonuclease in 3 mM EDTA before adsorption to the hydroxyapatite. In these circumstances, hybrid DNA elutes in 0·20 M sodium phosphate; double-stranded DNA in 0·3 M sodium phosphate. *a*, Double-stranded DNA; *b*, purified 70S DNA : RNA hybrid; *c*, control reaction; *d*, actinomycin-treated reaction.

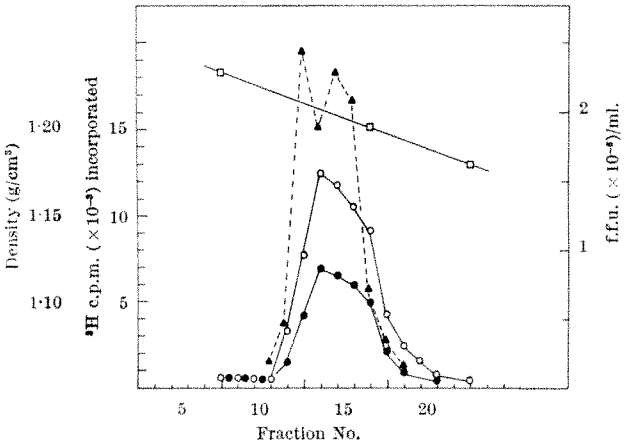


Fig. 5. Association of actinomycin-sensitive reaction with RSV virions. Purified RSV was layered over a preformed gradient of 25–55 per cent sucrose in 0·1 M NaCl–0·001 M EDTA–0·02 M Tris–HCl (*p*H 7·4). Centrifugation was carried out in an 'SW 25·3' rotor, 24,000 r.p.m., 4° C, for 16 h. Samples were taken from the gradient fractions for measurement of enzyme activity, focus-forming units (f.f.u.), and density. Total and actinomycin-resistant DNA polymerase activities were determined with reaction mixtures similar to those described for Fig. 1. ○, ³H c.p.m. incorporated (enzyme activity) in control reaction; ●, ³H c.p.m. incorporated in actinomycin-treated reaction; ▲, focus-forming units; □, density (g/cm³).

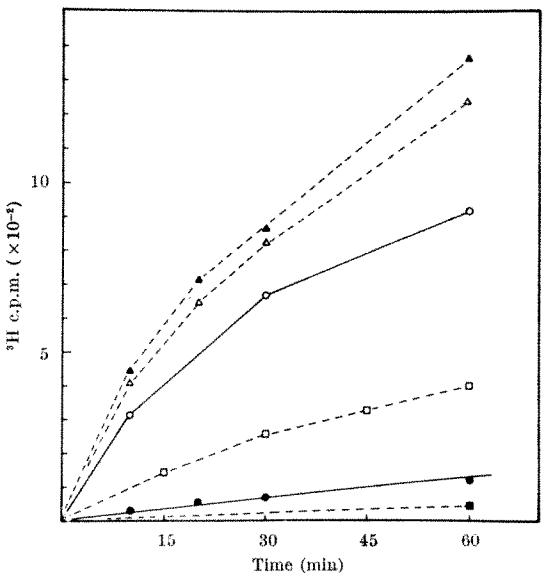


Fig. 6. Response of viral polymerase to heterologous DNA template. Replicate reaction mixtures were prepared and preincubated with actinomycin D and/or ribonuclease as described for Table 1. The reaction was then initiated by addition of ³H-TTP. At the same time, certain samples received 20 μg of native calf thymus DNA, sheared to a molecular weight of approximately 1×10^6–3×10^6. Control reactions contained appropriate amounts of the solvents for actinomycin, ribonuclease and DNA. ○—○, Control (no ribonuclease); ●—●, ribonuclease; △---△, control (no ribonuclease) plus DNA; ▲---▲, ribonuclease plus DNA; □---□, ribonuclease plus actinomycin D plus DNA; ■---■, control plus DNA, but incubated in the absence of detergent.

reduced by addition of actinomycin D to the reaction, and is therefore probably due to a template function of the DNA. No appreciable DNA-dependent activity could be elicited unless the virus preparations were first treated with detergent, an observation which again suggests that the enzymatic activity in question is an integral component of the virions.

Thus the DNA polymerase associated with RNA tumour viruses consists of two (or more) distinct enzymatic activities, one dependent on RNA template, the other on DNA template. The second, DNA-dependent reaction requires at least brief synthesis by the first, RNA-dependent reaction as a source of template[3]. The DNA products of both enzymatic reactions contain nucleotide sequences complementary to the 70S viral RNA[5,7]. The available data suggest a sequence of events in which a short segment (or segments) of the 70S viral RNA is transcribed into DNA, giving rise to an RNA : DNA hybrid with a long tail of single-stranded RNA[3,7]. The reaction then proceeds to the synthesis of double-stranded DNA. It is not yet clear whether this is accomplished directly from the hybrid structure, or whether another, intermediate stage intervenes. It is clear, however, that the final reaction can use double-stranded DNA as a template. This reaction can therefore continue independently of the first reaction, following either the synthesis of a small amount of hybrid or the addition of exogenous DNA.

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Separation of RNA-dependent DNA Polymerase Activity from the Murine Leukaemia Virion

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DNA polymerase activity has been separated from intact virions and is found associated with the nucleoid. Antiserum to the viral proteins inhibits this activity.

An RNA-dependent polymerase activity associated with the avian and murine tumour viruses has been reported[1,2]. In sucrose gradients the purified virions, the DNA product and the enzyme activity cosediment as a ternary complex[3]. The enzyme can be demonstrated in the virions of C-type[4] leukaemia–sarcoma viruses of several species[3,5,6] and in

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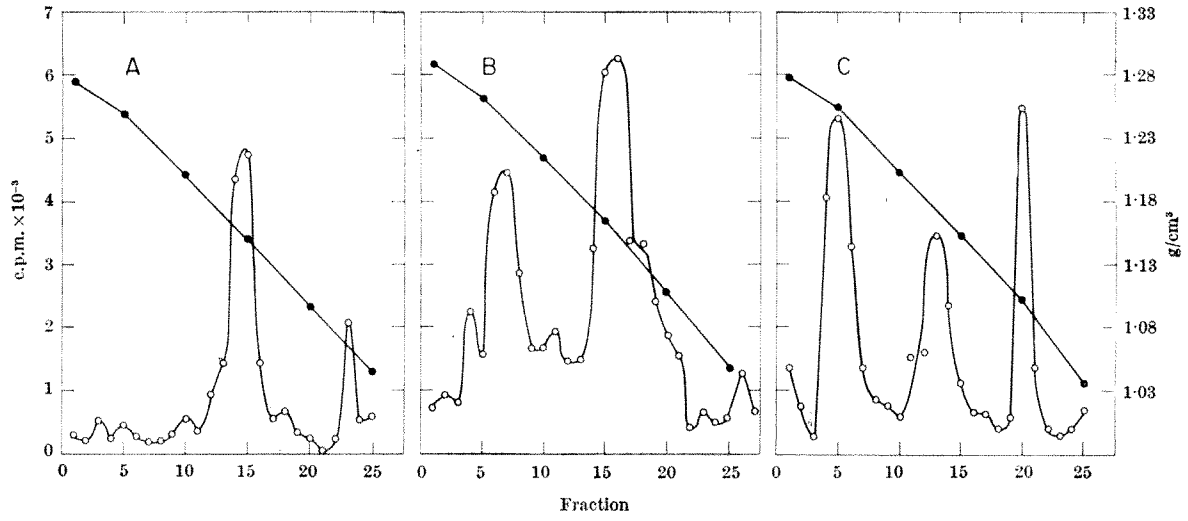


Fig. 1. The treatment of virus in *B* and *C* was as follows. A preparation of Rauscher virus[3] containing 10^7 plaque forming units per 0·10 ml. was incubated at 37° C for 20 min in a 0·20 ml. reaction mixture containing 0·05 M Tris-HCl (*p*H 8·3), 0·05 M NaCl, 0·005 M magnesium acetate, 0·02 per cent 'Triton X-100', 0·02 M DTT and 25×10^{-4} M dATP, dCTP, and dGTP. The 0·20 ml. reaction mixture was then incubated for an additional 90 min at 37° C after being diluted ten-fold in a solution in which the final concentration of each component was: 0·05 M Tris-HCl (*p*H 8·3), 0·07 M NaCl, 0·007 M magnesium acetate, 0·001 M dATP, dCTP and dGTP, 0·02 M DTT, and 0·002 per cent 'Triton X-100'. The mixture was then layered on a 15–60 per cent sucrose gradient containing 0·01 M Tris-HCl (*p*H 8·3), 0·08 M NaCl, 0·008 M magnesium acetate, and 0·006 M β-mercaptoethanol, and centrifuged in a Beckman 'SW-41' rotor at 40,000 r.p.m. at 4° C for 90 min (*B*) or for 15 h (*C*). Fractions of approximately 0·5 ml. were collected from below and assayed for polymerase activity as described by Scolnick *et al.*[3] without added 'Triton X-100'. The virus used in *A* was treated identically except that 'Triton X-100' and DTT were omitted from reaction mixtures before sucrose gradient sedimentation as described above. Each fraction was then assayed for polymerase activity in the presence of 0·012 per cent 'Triton X-100' and 0·02 M DTT.

the B-type mouse mammary tumour virus; the latter may play an aetiological part in carcinomas[7]. By contrast, non-oncogenic viruses, such as influenza and Sendai virus, that contain RNA and develop by budding from the cell membrane do not display this activity[3]. The above findings suggest that this enzyme may play a critical part in cell transformation and cancer development.

We describe here the separation of the enzyme activity from the intact murine leukaemia virion. Both a nucleoid and a light buoyant density fraction contain polymerase activity after detergent treatment and isopycnic banding in sucrose. Partial solubilization of the enzyme represents an initial step in the purification and characterization of this enzyme system.

Experiments with murine leukaemia viruses (Moloney and Rauscher strains) have disclosed a narrow range of optimal detergent concentration for expression of polymerase activity[3]. We assumed that higher detergent concentrations are inhibitory because they disrupt structures essential for enzymatic activity. Conditions were sought which would disrupt virus structure without interfering with active enzyme–substrate complexes. In the presence of 0·02 M dithiothreitol (DTT) and $2{\cdot}5 \times 10^{-4}$ M dGTP, dATP and dCTP, a 20 min treatment of the virion with 0·02 per cent 'Triton X-100' achieved this satisfactorily; virions treated with this concentration of detergent alone were inactive. After treatment the virus preparation was isopycnically banded in sucrose, and the polymerase activity of each gradient fraction was tested.

The results of isopycnic banding of untreated and treated virus are shown in Fig. 1. The activity (Fig. 1*A*) of an untreated virus sample after 15 h of centrifugation at 40,000 r.p.m. was almost entirely associated with intact virus; a low level of activity was found in the light region of the gradient. Fig. 1*B* corresponds to a treated virus sample which was centrifuged for 90 min. Considerable enzyme activity was now present in the region of the gradient (1·24–1·26 g/cm³) where the nucleoid[8] bands. The virus-associated polymerase peak was skewed toward the lighter region of the gradient, indicating the beginning of further separation of enzyme from whole virion. Fig. 1*C* shows a treated virus sample after a 15 h banding. Additional time in the centrifugal field allowed further separation of the activity into subviral components. Here, three distinct peaks of enzymatic activity were demonstrable, one in the nucleoid, one associated with whole virion and one in the lighter region of the gradient.

Electron Microscopy

Fractions showing high levels of polymerase activity were examined by electron microscopy with a Siemens 'Elmiskop 1A' at a base magnification of 30,000 using the negative staining method of Smith *et al.*[9]. Material from the region of the gradient at a buoyant density of 1·16 g/cm³ consisted of a relatively homogeneous population of intact particles (Figs. 2*A* and *B*). There was an occasional disrupted particle in which the intact inner membrane (the capsid of the nucleoprotein core)[10] was visible (Figs. 2*C* and *D*). Material in the high density region can be seen in Figs. 3*A–D* to contain disrupted viral cores. More than 95 per cent of the cores showed neither associated envelope material nor intact capsids. Fig. 3*A* illustrates a rare partially enveloped particle found in the 1·24 g/cm³ fraction. The light fractions in the gradient (1·08–1·10 g/cm³) contained primarily membranes and fragmented nucleoid material (Fig. 3*E*).

Inhibition by Antiserum to Murine Tumour Viruses

Enzymatic activity once freed from the virion should be more readily accessible to interact with added macro-

Table 1. EFFECT OF ANTISERUM ON POLYMERASE ACTIVITY OF NUCLEOID VIRUS AND SOLUBLE FRACTIONS

	³H-TTP incorporation (c.p.m./90 min incubation)		
	Standard assay	+Control serum*	+Anti-MSV serum*
Untreated virus			
Virion fraction	10,800	15,700	14,500
Treated virus			
Nucleoid fraction	14,800	22,500	400
Virion fraction	18,100	23,200	7,100
Soluble fraction	19,700	10,200	300

* Normal rat serum or rat serum prepared against the murine leukaemia–sarcoma viruses[11] was added to the 0·05 ml. reaction mixture at a final concentration of 1·25 mg/ml.

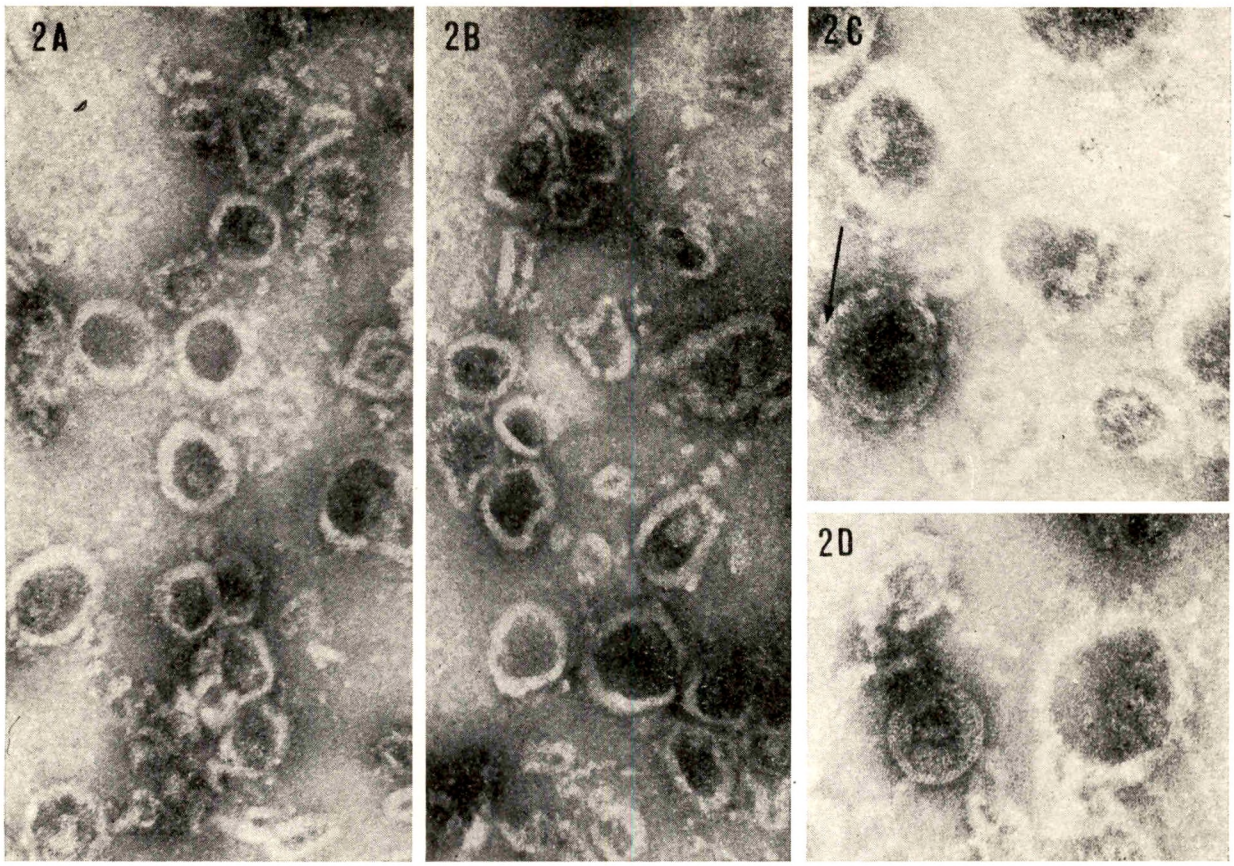


Fig. 2. Pretreated virus from the 1·16 g/cm³ region of gradient shown in Fig. 1*C* (fraction 13). *A* and *B* show enveloped particles (×100,000). The arrow in *C* points to a partly enveloped particle with nucleocapsid visible (×150,000). *D* shows a particle stripped completely of the outer envelope (×150,000).

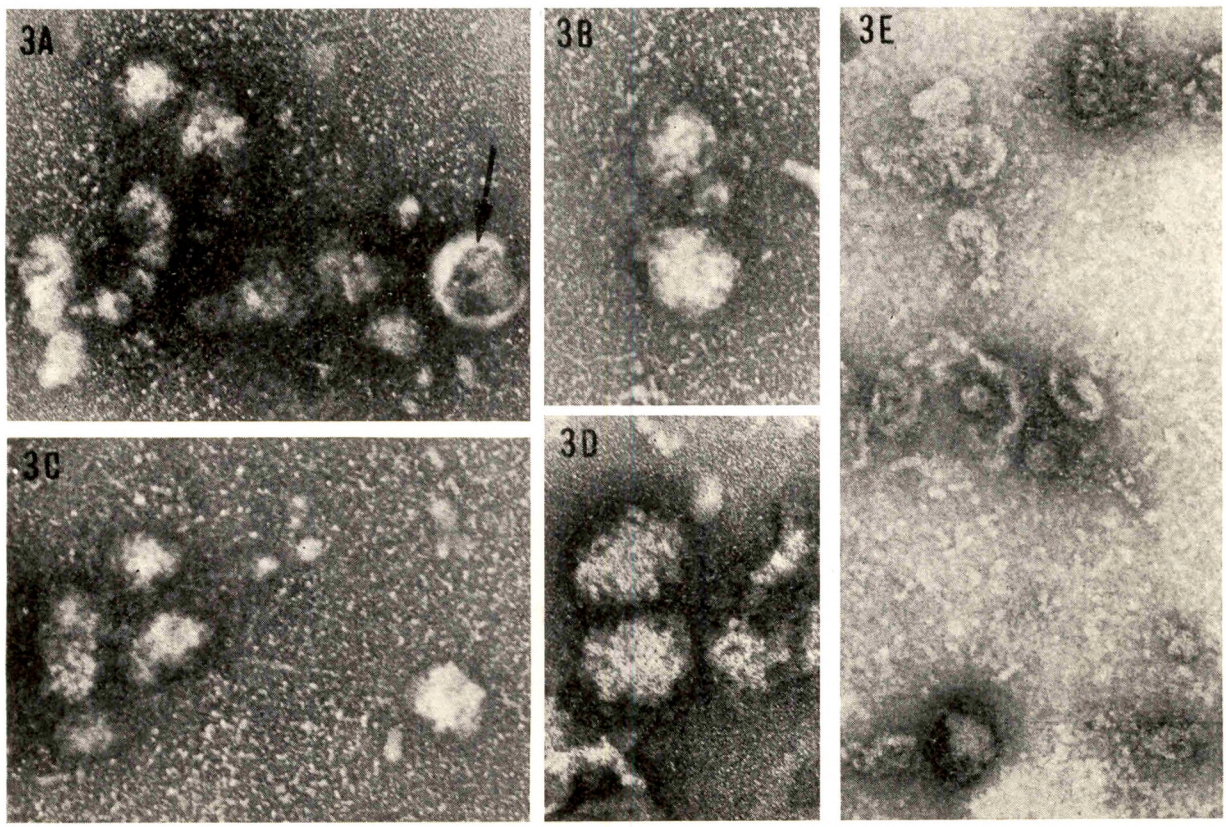


Fig. 3. *A–D* show disrupted nucleoid material at buoyant density of 1·25 g/cm³ from the gradient in Fig. 1*C* (fraction 5). In *A* one partially enveloped particle is visible (×100,000). *E* (fraction 20, Fig. 1*C*) shows predominantly membranous material with occasional nucleoid fragments (×100,000).

Table 2. POLYMERASE ACTIVITY OF VARIOUS GRADIENT FRACTIONS

Sample	μg/ml.*	c.p.m. incorporated/assay/μg
Experiment 1		
Nucleoid (fractions 4–7)	120	1,167
Virion (fractions 11–15)	205	391
Soluble (fractions 20–21)	170	796
Experiment 2		
Nucleoid (fractions 1–7)	210	5,836
Virion (fraction 11)	155	1,381
Soluble (fractions 17–20)	275	1,190

* Protein was determined by the method of Lowry *et al.*[14].

molecules; these interactions cannot occur inside the intact virus. Table 1 shows that the polymerase activity which cosedimented with untreated virions was not inhibited by rat anti-MSV serum. By contrast, enzyme assays on the pretreated virus showed that the same antiserum almost completely inhibited polymerase in the nucleoid and soluble fractions and even partially inhibited enzyme activity associated with the virions. Normal rat serum tested at the same concentration showed no inhibitory effect on the nucleoid or virus peaks. Gamma globulins isolated from various anti-MSV sera inhibited the polymerase reaction while gamma globulins from control sera did not.

Table 2 shows that the nucleoid fraction in two separate experiments showed the highest specific activity (^{3}H-TTP incorporated/μg protein). This represents a three-fold increase in specific activity over that found in the intact virion fraction from the same gradient. These results along with those of electron microscopy of the most active gradient fractions lead us to conclude that DNA polymerase activity is located in the nucleoid.

Polymerase in Viral Fragments

The initial product of the reaction is an RNA–DNA hybrid[6,12]. Recent reports indicate that a later product is double-stranded DNA, suggesting that more than one enzyme activity is present within the virion[6,13]. The fragmentation of the virus as demonstrated here might make possible the experimental separation of the different activities.

Polymerase activity was markedly inhibited in both the nucleoid and more fragmented structures by antiserum directed against viral proteins. Thus disruption of the virion allows penetration of antibody to structures where polymerase activity is inhibited, either by specific or by non-specific binding. Further enzyme inhibition experiments using monospecific antisera directed against the various antigens associated with these viruses may permit identification of the polymerase with a particular group-specific antigen of the nucleoid. Experiments on improved stabilization and purification of the enzyme are in progress.

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Primary Structure of α-Lytic Protease: a Bacterial Homologue of the Pancreatic Serine Proteases

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Comparison of the amino-acid sequence of the bacterial α-lytic protease with the mammalian pancreatic proteases shows homologies in the regions of the N-terminus, the single histidine, an aspartic acid residue, the active serine, and in the positions of the three disulphide bridges. The partial sequence of a trypsin-like enzyme from *Streptomyces griseus* provides further evidence for divergent evolution of this class of enzymes from a common ancestral gene.

PRIMARY structures of several mammalian pancreatic serine proteases, including bovine chymotrypsins A and B[1–4], bovine trypsin[5–7] and porcine elastase[8], reveal extensive structural homologies in their amino-acid sequences and the positions of disulphide bridges. These similarities extend also to the tertiary structures in the case of chymotrypsin A, elastase and trypsin which X-ray diffraction[9,10] and comparative model building[7,9] have shown to have very similar peptide chain conformations. Serine proteases from other organisms, the spiny Pacific dogfish[11], the turtle[12] and the sea anemone, *Metridium senile*[13], possess some enzymatic and sequence similarities with the mammalian serine proteases as, for example, in the presence of the -Asp-Ser-Gly- sequence of the active serine residue.

But proteases from microbial sources seemed until recently to have no homology with the pancreatic serine enzymes. Thus subtilisin BPN′ from *Bacillus amyloliquo-*

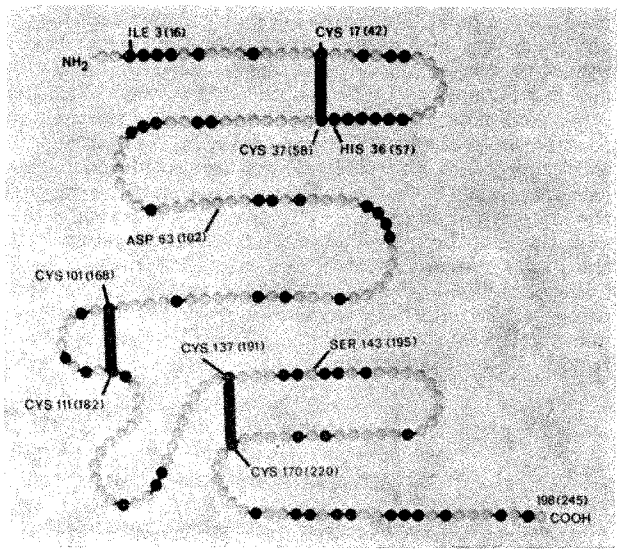
faciens and subtilisin Carlsberg from *B. subtilis*, which are characterized by an active serine sequence Thr-Ser-Met, manifest no homology in amino-acid sequence[14] or in tertiary structure[15] with the mammalian enzymes. They do have a similar arrangement of one aspartic acid, a serine and a histidine residue at the active site, which has presumably developed by convergent evolution to produce enzymes with similar catalytic mechanisms.

The presence of a class of serine microbial proteases, homologous with the mammalian enzymes, has been suggested by the discovery of at least two serine proteases of the soil microorganisms, *Streptomyces griseus*[16–18] and *Myxobacter 495* (refs. 19–21), with many properties in common with the pancreatic serine proteases. The amino-acid sequences about the three disulphide bridges of a trypsin-like enzyme purified from the commercial preparation pronase have a remarkable homology with the corresponding cystine sequences of bovine trypsin[18]. These structures include both the -Asp-Ser-Gly-Gly- active serine sequence as well as the single histidine residue in a histidyl–cystine structure common to all the pancreatic serine proteases whose structures have been elucidated.

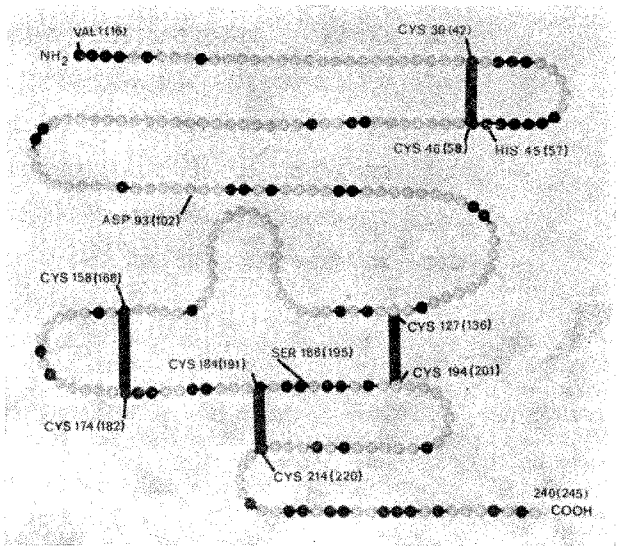
A number of proteases have been isolated from cultures of *Myxobacter 495* and shown to have bacteriolytic activity towards several other soil bacteria[19]. Of these, α-lytic protease has been most fully characterized and has many properties in common with porcine elastase and other pancreatic serine proteases. Like elastase, it hydrolyses casein, elastin and the A and B chains of insulin with a preferred specificity for peptide linkages involving the carbonyl groups of neutral, aliphatic amino-acids[20]. Both α-lytic protease and elastase have a common *p*H-activity profile[22,23], contain the Asp-Ser-Gly-Gly sequence[24,25] and depend for their activity on a single unprotonated imidazole group[23]. This group, which is contributed in α-lytic protease by its single histidine residue, is in an amino-acid sequence homologous with the histidine residue-57 sequence of the pancreatic serine protease family[26].

We report here the complete amino-acid sequence of α-lytic protease, which consists of a single polypeptide chain of 198 residues, with a molecular weight of 19,874. The structures of the three disulphide bridges were determined using the diagonal electrophoretic technique of Brown and Hartley[27], which also provided the sequence of the single histidine residue. Samples of the enzyme were then subjected to treatment with diisopropylfluorophosphate, reduced with mercaptoethanol, and either S-aminoethylated or S-carboxymethylated to form denatured derivatives. After digestion with trypsin or chymotrypsin, the peptides were isolated and subjected to amino-acid and sequential analyses by the dansyl-Edman method. Additional overlap peptides were obtained from peptic and α-lytic digests of the reduced and aminoethylated protein and from cyanogen bromide cleavage of the reduced and carboxymethylated material. Peptides were purified by gel filtration on 'Sephadex', chromatography on 'Dowex-50', and paper electrophoretic techniques.

Table 1 shows the amino-acid sequence of α-lytic protease. Table 2 compares this sequence with those for porcine elastase, bovine chymotrypsins A and B, bovine trypsin and the partial sequence of the trypsin-like enzyme from *Streptomyces griseus*. Here the numbering is based on that of chymotrypsinogen A. In arranging these sequences, appropriate deletions and insertions have been made to maximize homologies, particularly with respect to the positioning of the N-terminal sequence, histidine-57, aspartate-102, serine-195 and the positions of the disulphide bridges. In some cases, the exact assignment of deletions or insertions has been arbitrary; in others, for example residues 192A and 192B, it has been obvious. A schematic representation of the sequences of



a



b

Fig. 1. Bead models of (*a*) α-lytic protease and (*b*) porcine elastase. Numbering is from 1–198 for α-lytic protease (Table 1) and from 1–240 for elastase. Numbers in brackets are for the chymotrypsinogen A sequence as in Table 2. Black beads represent residues which are chemically similar or identical in the two enzymes as defined in Table 2. Disulphide bridges between half-cystines are shown as black bands.

α-lytic protease and elastase is also presented in Fig. 1.

Table 2 and Fig. 1 show immediately that several regions exist where the homology is striking. At the N-terminal end, there is the familiar -Ile-Val-Gly-Gly- sequence present in each of the other proteases with minor variations. Unlike the others, however, where isoleucine or valine is the N-terminal residue formed during the activation of the corresponding zymogen to its active form, α-lytic protease has an extra two residues, Ala-Asn-, at the N-terminal end. Recent X-ray diffraction studies[28] on bovine chymotrypsinogen A coupled with the previously reported tertiary structure of chymotrypsin-A[9] have demonstrated that on the tryptic scission of the arginine-15–isoleucine-16 peptide bond, there is a 180° rotation of the main chain in which the formerly exposed side chains of isoleucine-16 and valine-17 become buried, and the α-amino group of isoleucine-16 approaches the

buried side-chain carboxyl of aspartate-194 to form an internal ion pair. This same ion pair has also been demonstrated in the crystal structure of porcine elastase[29]. We cannot at present determine the relative importance of ion-pair formation, hydrophobic bond formation and the other conformational changes accompanying the zymogen to enzyme transition. But reports that the N-termini of α-lytic protease and elastase can be acetylated without loss of activity[22,30] indicate that, at least in these enzymes, the integrity of an ion pair between the N-terminal residue and the β-carboxyl of aspartic-194 is not essential for the maintenance of the active site conformation. The hydrophobic side chains of residues 16 and 17 which are preserved in all of the enzymes from mammalian and microbiological sources may be of more general importance to this class of enzymes.

90° rotation of its side chain during activation of chymotrypsinogen to provide the histidine-57–aspartic-102 region with limited access to the surrounding aqueous environment. In view of the evidence indicating a similar catalytic mechanism in α-lytic protease, we believe these residues will be shown to be involved in similar structural relationships with the histidine and serine of the active centre.

The next region of the sequence (positions 115 to 167) shows little homology with the pancreatic enzymes, and the assignment of residues and deletions (27 compared with elastase) is rather arbitrary. Clearly this is a region where the tertiary structure of α-lytic protease should differ appreciably from the other enzymes. This is followed by a disulphide loop structure between half-cystines 168 and 182 which in α-lytic protease is smaller (eleven

Table 1. AMINO-ACID SEQUENCE OF α-LYTIC PROTEASE

	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
1	ALA-ASN-ILE-VAL-GLY-GLY-ILE-GLU-TYR-SER-ILE-ASN-ASN-ALA-SER-LEU-CYS-SER-VAL-GLY-
21	PHE-SER-VAL-THR-ARG-GLY-ALA-THR-LYS-GLY-PHE-VAL-THR-ALA-GLY-HIS-CYS-GLY-THR-VAL-
41	ASN-ALA-THR-ALA-ARG-ILE-GLY-GLY-ALA-VAL-VAL-GLY-THR-PHE-ALA-ALA-ARG-VAL-PHE-PRO-
61	GLY-ASN-ASP-ARG-ALA-TRP-VAL-SER-LEU-THR-SER-ALA-GLN-THR-LEU-LEU-PRO-ARG-VAL-ALA-
81	ASN-GLY-SER-SER-PHE-VAL-THR-VAL-ARG-GLY-SER-THR-GLU-ALA-ALA-VAL-GLY-ALA-ALA-VAL-
101	CYS-ARG-SER-GLY-ARG-THR-THR-GLY-TYR-GLN-CYS-GLY-THR-ILE-THR-ALA-LYS-ASN-VAL-THR-
121	ALA-ASN-TYR-ALA-GLU-GLY-ALA-VAL-ARG-GLY-LEU-THR-GLN-GLY-ASN-ALA-CYS-MET-GLY-ARG-
141	GLY-ASP-SER-GLY-GLY-SER-TRP-ILE-THR-SER-ALA-GLY-GLN-ALA-GLN-GLY-VAL-MET-SER-GLY-
161	GLY-ASN-VAL-GLN-SER-ASN-GLY-ASN-ASN-CYS-GLY-ILE-PRO-ALA-SER-GLN-ARG-SER-SER-LEU-
181	PHE-GLU-ARG-LEU-GLN-PRO-ILE-LEU-SER-GLN-TYR-GLY-LEU-SER-LEU-VAL-THR-GLY

Disulphide bridges: 17–37, 101–111, 137–170

A large deletion of fifteen residues (positions 27–38 from the N-terminus) occurs in α-lytic protease as compared with elastase. Similar smaller deletions (three to five residues) also occur in this region with the chymotrypsins and trypsin. This deletion is followed immediately by the "histidine loop" formed by the disulphide bridging between half-cystine residues 42 and 58. The size of this loop (twenty-one residues) is somewhat larger in α-lytic protease than in the pancreatic proteases (seventeen residues) but there is considerable homology, particularly in the region from residues 52 to 58 which includes histidine-57 (part of the active site). Histidine-40 of the pancreatic proteases is replaced by a serine in an α-lytic protease. In the trypsin-like enzyme of *Streptomyces griseus*, this position is occupied by methionine[18]. It therefore seems unlikely that histidine-40 of the pancreatic proteases plays any functional part in the catalytic activity of these enzymes; this conclusion agrees with the position of this residue in the tertiary structures of chymotrypsin and elastase.

Following the "histidine loop" there is a further large deletion (residue 70–88) followed by a region of moderate homology including aspartic acid-102. X-ray diffraction studies have shown that this residue in chymotrypsin is in a "buried" position and seems to be hydrogen bonded to histidine-57 which in turn is hydrogen bonded to serine-195. Blow, Birktoft and Hartley[3] have suggested that the function of this buried aspartic is to increase the tendency to formation of negative charge on the active serine. Although the homology about this aspartic acid residue in α-lytic protease is not as striking as with the pancreatic proteases, it is the only negatively charged residue in the region of the sequence. Further, the hydrophobicity of residue-99 is preserved in α-lytic protease. This residue (isoleucine in chymotrypsin α) undergoes a

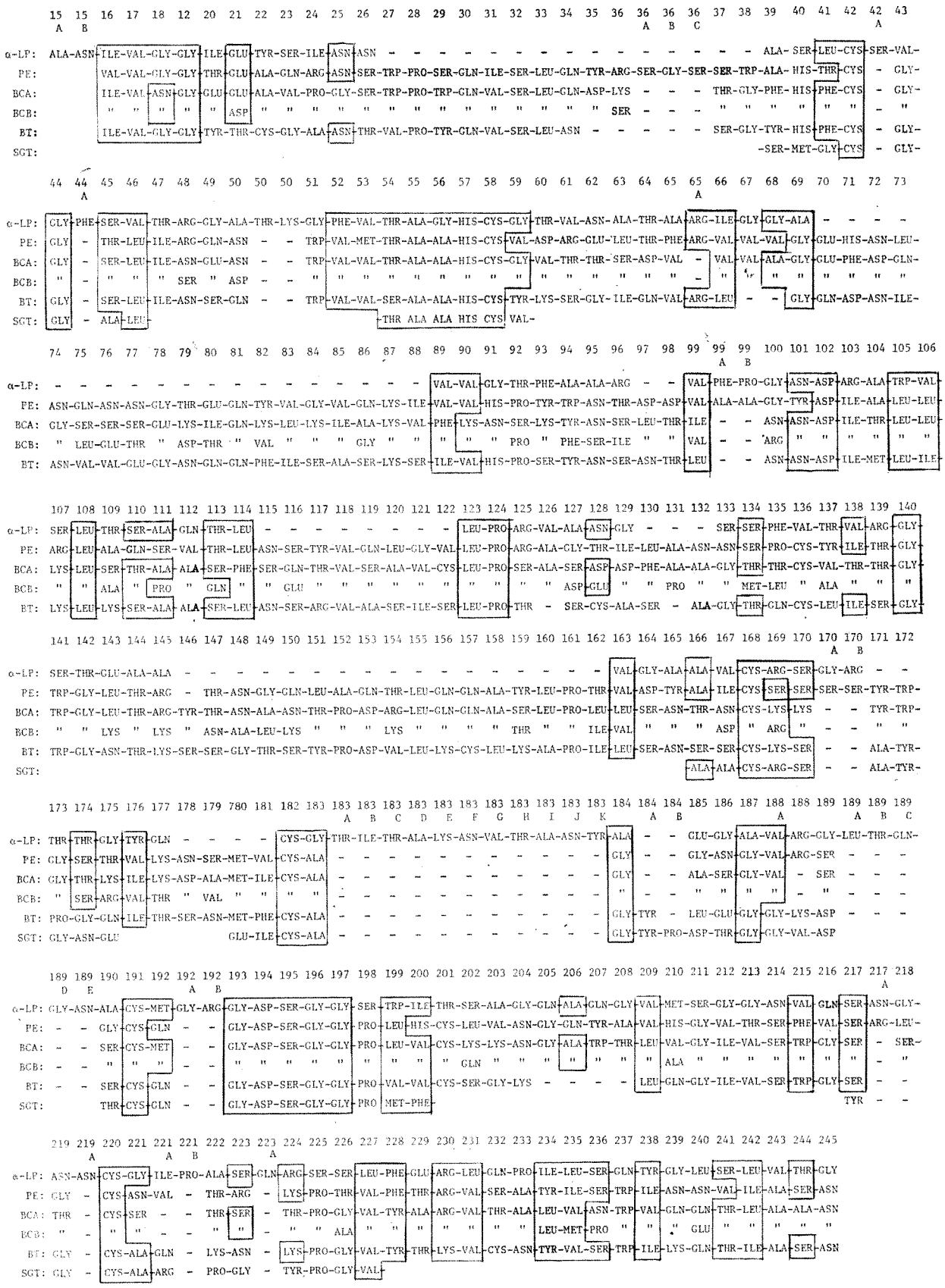
residues) than in the mammalian enzymes (fifteen residues in the chymotrypsins and trypsin and seventeen in elastase). This structure is followed by a sequence in which to maximize the homology of the half-cystine positions it has been necessary to insert a total of sixteen residues compared with the chymotrypsins and elastase. It is tempting to speculate that these insertions are necessary to compensate for the tertiary structural changes produced by the large deletions previously mentioned.

The active serine sequence from half-cystine-191 to residue 197 is identical to most of the chymotrypsins with the notable exception that a dipeptide -Gly-Arg- has been inserted between residues 192 and 193. The tertiary models of both elastase and chymotrypsin at Bristol and Cambridge suggest that this insertion can be accommodated on the exterior of the molecules without serious strain on the conformation of the active site. The remainder of the sequence to the C-terminal end contains approximately the same number of residues as the mammalian enzymes and shows periodic regions of homology, particularly in the region from 227 to 238. There seems to be no problem in accommodating this region of the sequence to the main chain conformation of the other enzymes.

The degree of homology between α-lytic protease and the pancreatic serine proteases described above, and best shown by Fig. 1, undoubtedly reflects a similarity in their tertiary structures, but because of the extensive regions of sequence which show little or no homology and because of the rather large deletions in the bacterial enzyme, we believe that this similarity is limited to only certain regions of the molecule. It will be particularly interesting to determine the structure of this enzyme by X-ray diffraction and we have started such studies[31].

Deductions concerning the evolutionary significance

Table 2. COMPARISON OF AMINO-ACID SEQUENCES OF α-LYTIC PROTEASE (α-LP), BOVINE CHYMOTRYPSINS A AND B (BCA AND BCB), BOVINE TRYPSIN (BT), AND *Streptomyces griseus* TRYPSIN-LIKE ENZYME (SGT)



The numbering is that of chymotrypsinogen A. Insertions in the sequences of other enzymes are numbered 36A, 36B and so on. Deletions are indicated by a dash. Only those residues of chymotrypsinogen-B which differ from chymotrypsinogen-A are indicated. Solid lines enclose residues in which chemically similar residues are present in α-lytic protease and at least two other enzymes. Chemically similar is defined in this paper as: Arg = Lys; Ala = Gly; Asp = Asn = Glu = Gln; Ser = Thr; Val = Trp = Ile = Leu = Tyr = Phe = Met. Disulphide bridges are as follows: αLP, PE, BCA, BCB, BT and SGT: 42–58, 168–182 and 191–220; PE, BCA, BCB and BT: 136–201; BT only: 22–157 and 128–232; BCA and BCB only 1–122.

of the observations described above on the sequence of α-lytic protease must be tempered with caution. It is not impossible that protein structures with some degree of homology could arise in these organisms by a process of convergent evolution. The case for divergent evolution has been considerably strengthened, however, by the isolation and characterization of a trypsin-like enzyme from pronase, a commercial preparation derived from culture filtrates of *Streptomyces griseus*[18]. This enzyme, which has a preferred specificity for peptide bonds of lysine and arginine, like α-lytic protease contains only one histidine residue and three disulphide bonds. Sequence analysis of the half-cystine peptides, however, shows a remarkable homology with bovine pancreatic trypsin. Homology based on identity of residues extends over more than 50 per cent of the sequences included in these disulphide structures. The *Streptomyces* enzyme therefore seems to be intermediate in structural features between those of bovine trypsin on the one hand and α-lytic protease on the other. The evidence is convincing, therefore, that this class of enzymes has evolved at a much earlier point than had previously been appreciated.

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Gene Conversion in *Drosophila*: Non-Reciprocal Events at the Maroon-like Cistron

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A large scale half tetrad analysis in *Drosophila melanogaster* questions the reciprocality of intragenic recombination and demonstrates that all intragenic exchange events at the *ma-l* cistron occur by a conversion mechanism.

Although theories of recombination have usually been concerned with reciprocal exchange[1], experiments have demonstrated[2] that exchange events need not always lead to the production of reciprocal products. Such non-reciprocal events, termed "gene conversions", have been documented at a considerable number of loci in the fungi[3–5]. Investigations in *Neurospora*[2], demonstrating both reciprocal and non-reciprocal events within the same gene, may be contrasted with studies from *Ascobolus*[6] which show that all intragenic events may be non-reciprocal. These observations on the frequency of conversion indicate the importance of understanding this process for a clear insight into the mechanism of recombination in eukaryotic organisms.

In the absence of tetrad analysis, the demonstration of this phenomenon in other systems has been difficult. Suggestions that it may be occurring at the garnet locus of *Drosophila melanogaster* were first reported by Chovnick *et al.*[7], but convincing evidence for the occurrence of conversion in *Drosophila* had not been obtained.

We describe here a large scale half tetrad analysis using attached-*X* chromosomes to question the reciprocality of recombination events between separable site mutants of the maroon-like cistron, and demonstrate that (1) gene conversion does play a role in recombination events in *Drosophila melanogaster*, and (2) all intragenic recombination events at the maroon-like cistron seem to occur by way of a conversion mechanism.

The Maroon-like Cistron

The maroon-like cistron (*ma-l;* 1–64·8) is represented by a group of mutants which affect the eye colour phenotype and which are deficient for at least three enzyme activities: xanthine dehydrogenase (XDH), aldehyde oxidase (AO), and pyridoxal oxidase (PO)[8,9]. XDH converts hypoxanthine and xanthine to uric acid, and is

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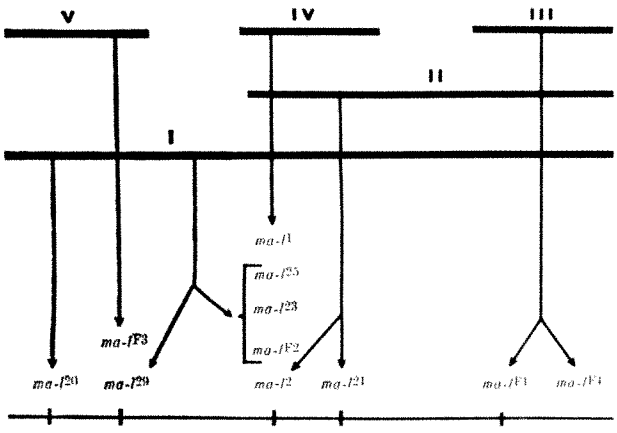


Fig. 1. Complementation and genetic maps of the *ma-l* cistron.

therefore involved in the excretion of purines[8]. Treatment of a developing *ma-l* culture with an aqueous purine solution can kill *ma-l* offspring[10], and this purine lethal effect has formed the basis of a recombination selection system. Recombinational[11] and biochemical[12] investigations indicate that this cistron consists of five complementation groups and a number of separable mutant sites (Fig. 1).

The Half Tetrad Technique

The attached-*X* or half tetrad system in *Drosophila* represents a compromise between tetrad analysis in fungi which enables one to examine each of the four products of a single meiotic event, and random analyses in other meiotic systems which allow the examination of only one of the products of a single meiosis. Attached-*X* females, which bear two *X* chromosomes attached to a single centromere, permit inspection of two of the four products of a single meiosis, for both chromosomes are passed to a single daughter. The *ma-l* half tetrad system provides an opportunity to question gene conversion in higher organisms for three reasons. First, the present system differs significantly from previous recombination studies with *Drosophila* because it does not rely on an examination of outside marker recombinations as indices of intragenic events. Rather, the analysis of *ma-l*$^+$ exceptional female progeny depends on the use of attached-*X* mothers which are heterozygous for two complementing *ma-l* alleles ($ma\text{-}l^{F3}/ma\text{-}l^{F1}$). Females of this complementing constitution possess low XDH activity and display an *ma-l*$^+$ eye phenotype. By allowing an unambiguous intragenic characterization of both strands of an exceptional half tetrad, this technique will permit a distinction between two models predicting the origin of *ma-l*$^+$ chromosomes. Second, because the analysis does not involve the use of outside marker genes, mass matings can be used. Third, the purine selector system can be adapted to kill the normal F1 progeny, which possess low XDH activity, while permitting larvae with higher XDH levels to survive. Thus selection for rare intragenic events leading to the production of *ma-l*$^+$ chromosomes can be performed on large samples of half tetrad bearing progeny from the attached-*X* females.

Origin of *ma-l*$^+$ Chromosomes

Previous recombinational studies[11] with normal (unattached) *X* chromosomes, heterozygous for two *ma-l* alleles, have yielded *ma-l*$^+$ progeny. Subsequent genetic and enzymatic examinations have shown there to be a recombinational mode of origin of the exceptional offspring in those experiments.

If wild type exceptional females arise by classical recombination, two types of *ma-l*$^+$ females can be predicted (Fig. 2*A*). An exchange event between the two alleles involving two strands attached to differing centromeres (*a*) will yield a female bearing an *ma-l*$^+$ chromosome attached to a singly mutant chromosome marked with the proximal $ma\text{-}l^{F1}$ allele. Equally likely, a recombinational event between the alleles involving two strands attached to the same centromere (*b*) will produce a female bearing an *ma-l*$^+$ chromosome attached to the doubly mutant chromosome, $ma\text{-}l^{F1}$ $ma\text{-}l^{F3}$. Conversely, if exceptional offspring result from non-reciprocal exchange, one may similarly predict the occurrence of two types of *ma-l*$^+$ females (Fig. 2*B*). If the distal $ma\text{-}l^{F3}$ allele is converted to wild type (*a*), the resulting female will contain a *ma-l*$^+$ chromosome and a singly mutant chromosome marked by the proximal $ma\text{-}l^{F1}$ allele. If, however, the proximal $ma\text{-}l^{F1}$ allele is converted to wild type (*b*), the exceptional offspring will carry an *ma-l*$^+$ chromosome attached to a singly mutant chromosome marked with the distal $ma\text{-}l^{F3}$ allele.

The salient features of the two models suggest contrasting predictions: with selection for a wild type chromosome, single conversions cannot produce an attached doubly mutant chromosome, $ma\text{-}l^{F1}$ $ma\text{-}l^{F3}$, while single exchanges cannot yield a singly mutant

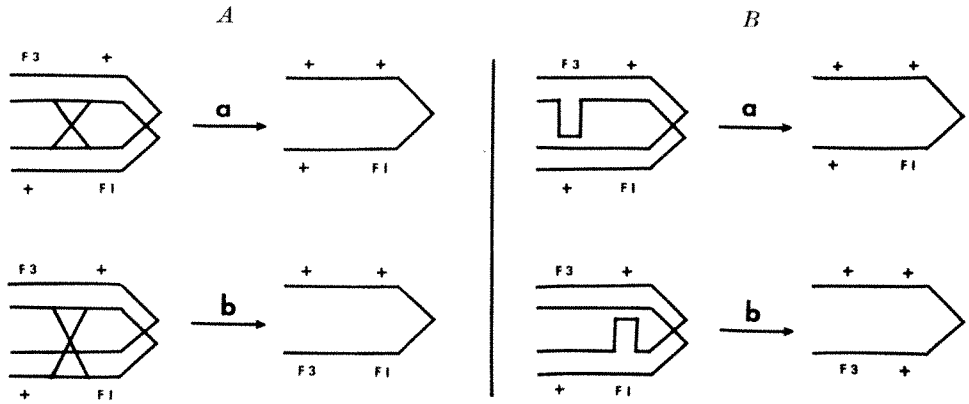


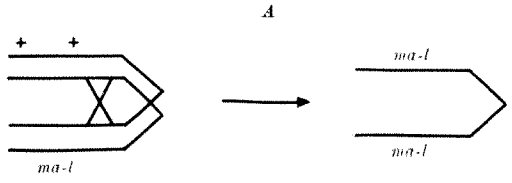
Fig. 2. Mechanisms of origin of *ma-l*$^+$ chromosomes: *A* depicts the origin of *ma-l*$^+$ chromosomes according to the classical recombination mechanism; *B* depicts the origin of *ma-l*$^+$ chromosomes according to a conversion mechanism (diagram does not imply adherence to any particular model). Reversion experiments sampling an estimated 2×10^6 normal *X* chromosomes from $ma\text{-}l^{F1}$ and $ma\text{-}l^{F3}$ homozygous stocks have failed to yield any *ma-l*$^+$ individuals.

chromosome marked by the distal *ma-l*^{F3} allele. Thus, evidence to demonstrate gene conversion may be derived from a consideration of the respective frequencies of the various mutant homologues accompanying an *ma-l*$^{+}$ chromosome.

The Experiment

A single *ma-l*^{F3}/*ma-l*^{F1} attached-*X* female was constructed by standard techniques[12] and fifteen of her daughters were used to initiate separately maintained sublines. A single female was selected from each of these subcultures, expanded two generations, and her virgin grand-daughters were collected to serve as the parental females in a given experiment. Among these collected females, occasional *ma-l* mutant daughters appeared. These represent single exchanges, occurring between the *ma-l* locus and the centromere and involving two chromatids attached to different centromeres. These exchanges render homozygous one or the other of the *ma-l* alleles, and provide a means of insuring that the data will not be biased by using large numbers of parental females containing a recessive lethal on one or the other of their *X* chromosomes (see legend to Fig. 3 for testing details).

The complementing *ma-l*$^{+}$ parental females were mated to non-complementing *ma-l* males. After two days of oviposition, the parents were transferred to fresh culture bottles and the developing cultures were treated with purine. After four successive transfers, the parents were discarded and, on days 12 and 18 of culture, each bottle was examined for the presence of exceptional females. A group of bottles in each experiment was left untreated and the number of emerging progeny provided an estimate of the total sample size.



B

XX/*Y* constitution

	F1	F1 F3	F3
Tester strain			
X/*y*$^{+}$*Y ma-l*^{116}	NC	NC	C
X/*y*$^{+}$*Y ma-l* ^{F3}	C	NC	NC

C, Complementing; NC, non-complementing.

Fig. 3. Analysis of the mutant homologue: *A*, proximal exchange event rendering the mutant chromosome homozygous for testing; *B*, complementation patterns expected from test crosses of the "*ma-l*" mutant half tetrads. For lethal testing, the *ma-l* mutant females appearing among the complementing parental females are mated to a male tester stock bearing a *Y* chromosome marked with the *ma-l*^{116} allele, a member of the *ma-l*^{F1} complementation group. If the female had been rendered homozygous for the *ma-l*^{F1} chromosome, her daughters would exhibit an *ma-l* mutant phenotype. Conversely, if the female had been rendered homozygous for the *ma-l*^{F3} chromosome, the resulting daughters would exhibit a complementing *ma-l*$^{+}$ phenotype. Five mutant appearing daughters from each of the sublines were tested and it was demonstrated that each of the initiator females for each experiment gave rise to both types of attached-*X* chromosomes and therefore possessed no recessive lethals closely linked to the *ma-l* cistron.

An estimated 5×10^6 progeny females were produced, of which thirty were *ma-l*$^{+}$ exceptional offspring. The method of analysis of these rare progeny is depicted in Fig. 3. By virtue of a single exchange between the *ma-l* cistron and the centromere (*A*), the wild type exceptional females will yield daughters homozygous for the mutant strand. The genotypic constitution of these mutant chromosomes may then be determined by matings to two kinds of tester males which bear differently marked *Y* chromosomes. One strain bears a *Y* chromosome marked by *ma-l*^{116}, a member of the *ma-l*^{F1} complementation group[12]. A similar strain, with the *Y* chromosome marked with *ma-l*^{F3}, constituted the second tester stock. *Ma-l* daughters derived from the exceptional *ma-l*$^{+}$ strains are mated to the two test stocks and the genotypic constitution of the *ma-l* chromosome accompanying the *ma-l*$^{+}$ chromosome can be distinguished by the pattern of complementation observed in the daughters (*B*).

Table 1 summarizes the genotypes of the mutant chromosomes of the twenty-nine exceptional females analysed in these experiments. Two points are immediately clear: (1) of the twenty-nine exceptional females, none carried the doubly mutant *X* chromosome predicted by the recombination model, and (2) approximately half of the mutant chromosomes were marked by the distal *ma-l*^{F3} allele, as predicted by the conversion model. The singly mutant homologue marked by the distal *ma-l*^{F3} allele could be produced by a classical recombinational mechanism if a second exchange between the *ma-l* cistron and the centromere accompanied a first exchange between the alleles (negative chromatid interference). But, because of the location of the *ma-l* locus approximately one map unit from the centromere (1·08 map units in our attached-*X* strain), this double exchange event can be ruled out on the basis of classical recombination studies from *Drosophila*[13,14] which demonstrate high chiasma interference. Interpretations based on high negative interference[15] would have to explain the high occurrence of double exchanges taking place simultaneously with a total absence of single exchanges between chromatids attached to the same centromere.

Table 1. GENOTYPIC CONSTITUTION OF THE MUTANT HOMOLOGUE

Class	Frequency
Distal convertant or single exchange *a*	14
Single exchange *b*	0
Proximal convertant	15
Total	29

Of the 30 exceptional females, 29 appeared as a rare single female, while one appeared as a "burst" of wild type females in all of the bottles involved in the four transfers of an original bottle. These "burst" females undoubtedly reflect the occurrence of an intragenic event in the stock in the previous generation to produce a wild type female who was subsequently used as a parent. This line was lost before completion of analysis of the mutant strand.

In conclusion, the present data provide ample support for the demonstration of gene conversion in higher eucaryotes and, in the case of the *ma-l* cistron, suggest that all intragenic recombination occurs by a conversion mechanism.

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Absolute Time-of-arrival Measurements of Optical Pulses from the Crab Pulsar

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An attempt is made to understand the characteristics of pulsar slowdown in terms of various possible emission mechanisms. Though magnetic-dipole radiation seems to be dominant, the deviations from a pure dipolar decay are large compared with errors in measurement and indicate the presence of more complicated braking mechanisms, small fluctuations, and major "jumps", or period discontinuities. Some rapid-relaxation effect is evident following the discontinuous jump in period in September 1969, but there is no evidence of the quasi-sinusoidal behaviour reported before the jump by Arecibo. An alternative interpretation of the Arecibo data is given.

Soon after the discovery[1] of the pulsating optical signal from NP 0531, the Crab Nebula pulsar, we began making measurements of the absolute times of arrival of optical pulses. With the 61 inch telescope at the Agassiz Station of Harvard College Observatory and the time-keeping facility of the Smithsonian Astrophysical Observatory, we can establish the phase of the pulses to an accuracy of ± 4 μs in absolute time (UTC), with an hour's observation. We made measurements on a few nights in March 1969, with the initial purpose of comparing the times of arrival of optical and radio pulses. The measurements were then begun again in September 1969, and continued at the rate of several per month until April 1970. The comparison of optical and radio arrival times will be reported in more detail elsewhere. The continued observations were made with the purpose of measuring the pulsar period as a function of time, and we shall discuss this behaviour here.

The manner in which the pulsar period changes with time is determined primarily by the torques exerted on it through various energy-loss mechanisms, and secondarily by changes in moment of inertia. By a careful measurement of the variation of period with time one might hope first to measure the secular behaviour and thus to determine, for example, whether magnetic-dipole or gravitational radiation could be identified as the chief source of energy loss. One might then subtract this time dependence and examine the residuals for evidence of other behaviour such as sudden jumps in the period (which have indeed been noted in this and in the Vela pulsar)[2-4], periodic variation that might result from orbital motion of the pulsar and any other short-term fluctuations that might yield information about the physical conditions at the pulsar.

To do all this one must know the position of the Earth relative to the solar system barycentre with an accuracy comparable with the precision of measurement, which is a few ms of the travel time of light. The cooperation of the planetary radar group at MIT has fortunately furnished us with one of the best available ephemerides of the Earth's motion. Uncertainties in some of the parameters of the solar system are still such that one might actually gain information about these if the behaviour of the pulsar were sufficiently predictable. Also, the differential gravitational redshift resulting from the eccentricity of the Earth's orbit[5,6] is large enough (800 μs peak amplitude) to be measured accurately, provided everything else is well behaved.

As we shall show, the non-secular behaviour of the pulsar period is sufficiently complicated to make it difficult to pursue the above objectives in a straightforward manner. One application of careful time-of-arrival measurements that remains essentially untroubled by this, however, is the use of the pulsar to synchronize clocks to a few μs at any two observatories having moderate size telescopes (say, 24 inch) that can observe NP 0531.

Description of Experiment

The telescope was offset from a visible star to the position of the pulsar and guided with an auxilliary eyepiece. Unfiltered light from NP 0531, along with some nebular background and sky light, passed through an aperture (usually 6 or 8 arc seconds in diameter) at the Cassegrain focus and was detected with a cooled 1P28 photomultiplier. This signal, amplified and discriminated with a single-channel analyser, was accumulated in a 1,024 channel signal averager swept at the pulsar rate, and subsequently dumped on half-inch digital tape for analysis.

In a separate operation, both before and after each signal accumulating run, absolute timing marks, generated by dividing down from a stable quartz or rubidium standard, were also fed into the cleared multichannel scaler in order to establish accurate reference of the averager sweep relative to national time standards. Loran-C from the Nantucket synchronized slave station was used to establish regular clock synchronization to better than 1 μs, and all pulse times of arrival (TOAs) are quoted in these units (UTC).

The complete data system is shown in Fig. 1. A 1 MHz signal from the standard source is divided by 32 and used as the channel-advance signal. The scan through the memory of the analyser takes $0{\cdot}032 \times 1{,}024 = 32{\cdot}768$ ms, which is slightly less than the pulsar period, of about 33·1 ms. The analyser then resets and waits for a start pulse, as described below. This system incorporated several important refinements of the usual methods[7,8] which make possible highly precise timing measurements.

(1) Because the averager has a 50 per cent dead time at these channel-advance rates, a one-bit buffer stores signal counts for injection during the live time. With a channel width of 32 μs, the average count per channel per sweep is about 0·01, so a one-bit memory suffices.

(2) A "leap-year machine"[9] generates pulses at the predicted pulsar rate, which start the sweep through the multi-channel analyser; these pulses also reset the channel advance divider to maintain the averager sweep precisely synchronous with the pulsar, for in general the channel-advance rate is incommensurate with the pulsar period. The leap-year machine is driven by the rubidium standard at 1 MHz, giving a peak phase error of ±1·5 μs. The apparent pulsar period changes by a few tenths of a nanosecond per minute, owing to its slowdown and to the Earth's spin and orbital doppler shifts; because a period error of 1 ns would give rise to a drift in the averaged pulsar signal of 20 μs in 10 min (a typical averaging interval) it was necessary to calculate the apparent period for each minute during the observing interval and alter the leap-year machine switch settings appropriately. No jump in synthesized phase results from this operation.

(3) The peak of the signal can be located to a few μs even though it has been quantized into 32 μs bins. It is therefore advantageous to know the phase of the timing markers relative to the bins in which they are found. To do this the store-and-dump buffer is also used to gate 1 MHz into a two-digit counter during the storage interval. This figure, which is the number of μs from receipt of the pulse to injection into the averager, is noted down, along with the UTC time of the pulse, when the calibration runs are made.

To verify that all parts of the timing system were working according to plan, a test was made as follows. The leap-year machine was set to a particular pulsar period and allowed to run without interruption. At arbitrary times during the next few hours timing pulses were fed into the averager, the scan of which was synchronized by the leap-year machine, and recorded on digital tape in the usual manner. From knowledge of the synthesized period and UTC time of each of these pulses it is possible to calculate the channel number, and phase within that 32 μs channel, of each of the pulses relative to the first. Indeed, all of these test pulses were found within 1 or 2 μs of their expected locations, demonstrating that the timing system behaves as expected.

The main peak of each 10 min run was located by cross-correlating a master template with the 1,024 numbers recorded from the digital averager (this is similar to the technique described in ref. 13). From the resulting discrete correlation function the maximum was found to a fraction of a bin by parabolic interpolation. The accuracy of the peak found by this technique is limited entirely by the available signal, a result verified both by "peak-finding" experiments on computer generated noisy signals and by explicit calculation (assuming counting statistics) of the scatter of the cross-correlated peak. The standard deviation of the correlated peak is a simple function of the baseline and height of the light curve on which this template matching technique has been applied, and so we have attached an estimated standard deviation to each measured arrival time. Using the time markers at both ends of the run, the average scanning rate was calculated and the location of the light-curve peak was converted to a site TOA for a pulse close to the median time of the run.

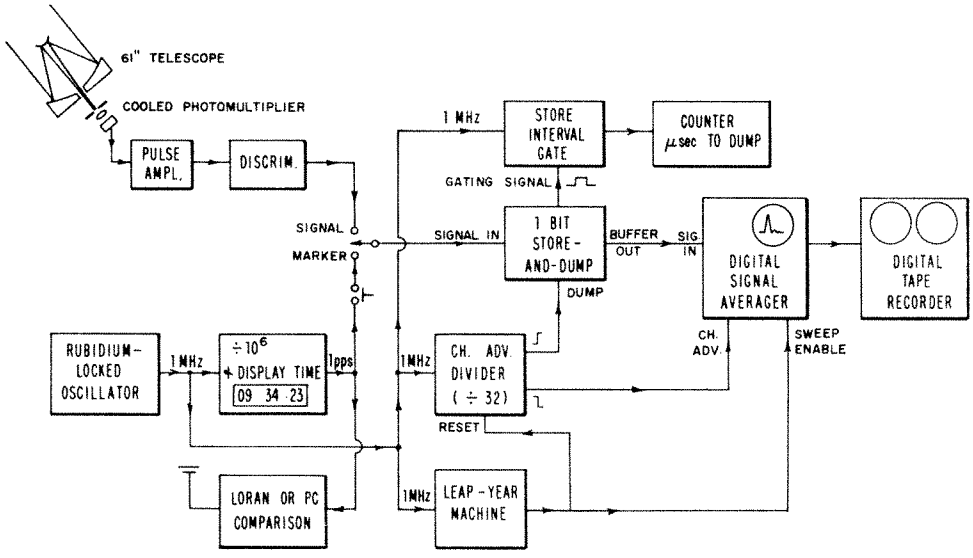


Fig. 1 Pulse time-of-arrival measuring system.

Another check was made to ensure that the timing system worked correctly. Any errors in the synthesized period supplied to the averager would result in an apparent drift of the light curve relative to the averager scan. Typically the peak was found to drift less than one 32 μs channel during 2 h of data-taking, consisting of five to ten independent runs. This gave confidence that the individual runs were accurately averaged modulo the apparent pulsar period. Taken with the test described earlier, this verified that both the pulsar light curve and the calibrating time markers were being recorded with an ultimate timing resolution limited only by the clock (1 μs) and the available signal.

A final check on the entire system consisted of reducing a set of 100 independent runs from a dozen different nights to solar system barycentric TOAs and then performing a cubic fit to phase against time. The resulting phase residuals within each night were compared with the weighted means, the deviations being expressed in units of predicted standard deviation for each timing measurement. Fig. 2 is a histogram of the result, with the curve for Gaussian fluctuations plotted for comparison. Except

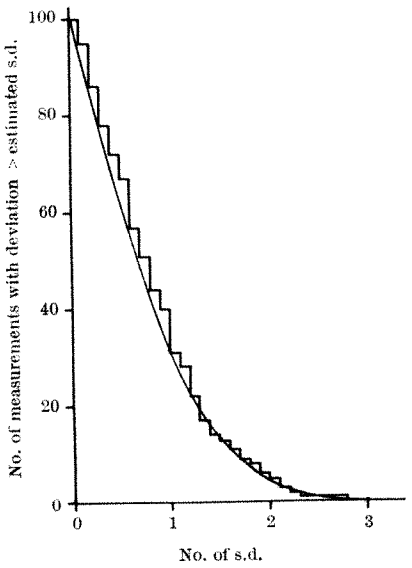


Fig. 2. Histogram of deviations of 100 individual residuals from nightly means, after a cubic fit to phase, in units of estimated standard deviations.

for possible errors of absolute clock synchronization, this result shows that pulse TOAs were being measured to the stated accuracy, and also, incidentally, that the pulsar has a greater stability (in other words predictability, for the secular slowdown has been subtracted) than can be measured in a few hours with TOA measurements of an accuracy of 10 μs.

All site TOAs were subsequently reduced to solar system barycentre TOAs by subtracting the light-time of the site-barycentre vector dotted with the unit vector in the direction of the pulsar, using the coordinates as given by Minkowski[10], and including a term for solar gravitational redshift and second-order doppler shift due to the eccentricity of the Earth's orbit. Because the scatter of measurements within each night was accounted for entirely by the limitations of available signal, a nightly representative TOA was computed and used for subsequent calculations. This was done by performing a cubic fit to phase of several months' data, then subtracting from the TOA of each night's median run the deviation of its phase residual from the weighted mean phase residual for the night. Table 1 lists the site and barycentric TOAs obtained in this way for each observing night.

Recognition of a Jump

The first calculation based on the barycentric TOA is to number each pulse. This is done by calculating the number of pulses ΔN that have elapsed from the last measured pulse by means of the formula

$$\Delta N = f_0\,\Delta t + \frac{\dot{f}_0}{2}(\Delta t)^2 + \frac{\ddot{f}_0}{6}(\Delta t)^3$$

where the parameters f_0, $\dot{f}_0$, $\ddot{f}_0$ have been obtained by a least-squares fit for all previous data, and Δt is the time difference between the present pulse and the previously measured one. The number of pulses ΔN separating these two times is rounded off to the nearest integer. Typically, even for an interval of several weeks ΔN is within 0·05 of an integer, indicating that the pulse numbering is unambiguous. The result is a set $\{(N,t_N)\}$, where t_N is the barycentric TOA of the Nth pulse. The problem is to find an analytic relationship between these two variables. If φ is a continuous variable approximating to the integer variable N, and φ is expanded as a power series in t (we have dropped the subscript N), then

$$\varphi = \varphi_0 + f_0 t + \frac{1}{2}\dot{f}_0 t^2 + \frac{1}{6}\ddot{f}_0 t^3 + \ldots \qquad (1)$$

If we truncate this series beyond the cubic term and define the residual r by $r = N - \varphi$, we can use the method of least squares to minimize these residuals, that is, find f_0, $\dot{f}_0$, $\ddot{f}_0$ such that Σr^2 is a minimum. In minimizing the residuals r we can weight each measurement by σ^{-2}, where σ is the standard deviation in the measured TOA. If, however, the calculated residuals are found to be much greater than the σ's (because of anomalous behaviour in

Table 1. PULSE ARRIVAL TIMES AT SITE AND SOLAR SYSTEM BARYCENTRE, AND CORRESPONDING PULSE NUMBER

Crab pulsar timing data

Date			Site arrival time			Sigma	Barycentric	Arrival time	Pulse number
Month	Day	Year	h	min	s	μs	Julian day	s	
3	17	1969	01	47	39·983604	50·0	2440297·5	6437·513579	0
9	12	1969	08	57	06·023401	10·6	2440476·5	32189·666495	468033096
9	13	1969	08	11	02·024519	11·5	2440477·5	29433·917910	470559950
9	16	1969	08	39	46·009152	7·2	2440480·5	31183·686258	478443104
10	9	1969	08	19	57·002321	5·1	2440503·5	30186·164151	538444366
10	10	1969	07	56	09·984035	4·1	2440504·5	28766·894782	541011518
10	17	1969	09	09	29·992356	10·6	2440511·5	33220·661799	559416162
10	18	1969	08	02	44·990390	4·6	2440512·5	29222·660511	561905392
10	19	1969	08	37	14·983562	3·4	2440513·5	31300·091969	564578148
11	16	1969	07	11	31·980315	5·9	2440541·5	26320·609244	637506568
12	5	1969	07	45	55·005933	4·4	2440560·5	28439·187459	687158492
12	12	1969	06	21	25·005616	8·1	2440567·5	23376·294043	705274534
12	18	1969	08	19	56·985649	4·4	2440573·5	30488·458890	721148376
1	1	1970	02	05	24·999442	11·8	2440587·5	7996·335239	757006265
1	3	1970	03	18	44·986356	6·8	2440589·5	12390·913015	762358574
1	11	1970	02	22	39·980689	7·1	2440597·5	8999·188371	783134278
1	14	1970	06	03	42·002578	5·3	2440600·5	22248·193239	791363730
1	16	1970	06	13	37·008305	10·6	2440602·5	22834·231771	796600923
1	31	1970	03	39	17·008171	6·1	2440617·5	13492·359294	835464572
2	5	1970	01	38	04·982081	7·3	2440622·5	6187·966638	848292409
2	8	1970	04	44	46·993842	9·7	2440625·5	17368·167257	856459147
2	24	1970	00	20	01·998223	12·2	2440641·5	1361·581707	897730144
2	27	1970	01	05	55·000236	4·5	2440644·5	4089·601008	905641422
3	4	1970	01	18	26·978656	4·1	2440649·5	1199·865754	918602218
3	7	1970	01	23	11·986946	11·7	2440652·5	5058·931412	926547588
3	9	1970	01	12	56·984122	7·4	2440654·5	4426·844686	931747690
3	28	1970	01	01	14·993929	7·2	2440673·5	3562·137620	981303336
4	4	1970	01	13	26·979862	7·7	2440680·5	4236·229829	999590399
4	6	1970	02	11	46·998164	9·7	2440682·5	7719·818813	1004914645
4	8	1970	01	34	24·999011	7·0	2440684·5	5462·152386	1010065478
4	16	1970	01	22	27·006979	14·2	2440692·5	4683·327070	1030917923
4	26	1970	01	02	21·990707	49·0	2440702·5	3409·390075	1056974148

the pulsar, or errors in the ephemeris, for example) then the measurements should be equally weighted.

The first result of such a calculation was the recognition of a sudden decrease in period similar to that observed earlier in the Vela pulsar[2,3]. This jump, which has been reported by others[4], is apparent in Fig. 3, where the residuals are plotted from a weighted cubic fit to the pre-jump arrival times. Data for the summer months, when optical observation was impossible because the Crab was in the daytime sky, were kindly provided by the Arecibo Observatory group. The dispersion delay as calculated from radio measurements alone has been removed by the Arecibo group, and the radio/optical agreement in phase, which is apparent just before the jump, indicates approximate simultaneity of the pulse arrivals at these different frequencies. A sudden change of frequency alone would result in a straight-line deviation of points after the jump; the curvature here indicates that other parameters changed also. A fit to the post-jump points confirms this. The cubic parameters before the jump and their changes (final-initial) at the jump are

$f = 30{\cdot}209\ 297\ 624$ Hz $\qquad \Delta f = 9{\cdot}3 \pm 1 \times 10^{-8}$ Hz

$\dot{f} = -3{\cdot}857\ 212 \times 10^{-10}$ Hz/s $\qquad \Delta\dot{f} = -1{\cdot}8 \pm 0{\cdot}1 \times 10^{-14}$ Hz/s

$\ddot{f} = 1{\cdot}04 \times 10^{-20}$ Hz/s^2 $\qquad \Delta\ddot{f} = 5{\cdot}9 \pm 1 \times 10^{-21}$ Hz/s^2

t = JD 2440484·5 ± 4 = Sept. 20, 1969 ± 4 days.

What can we conclude from the jump? If we assume that the frequency f of the pulses is related to the angular-rotation rate of the pulsar by $\Omega = 2\pi f$, then the jump in frequency could be the result of a sudden decrease of the moment of inertia due to a reduction of the pulsar's equatorial radius. If the neutron-star radius is 10 km, the magnitude of the jump

$$\frac{\Delta f}{f} = \frac{\Delta\Omega}{\Omega} = -\frac{\Delta I}{I} \cong 3 \times 10^{-9}$$

implies a change in equatorial radius of about 15 μm. If this contraction is a starquake of a solid crust[13] caused by the change in equilibrium shape of the slowing star, then it can easily be shown that the mean time between quakes of this magnitude, for the present f and $\dot{f}$ of the Crab pulsar, is approximately 1 week (the equilibrium equatorial radius R_e changing as $\dot{R}_e = 5\Omega\dot{\Omega}R^4/6GM$, where R is the radius for $\Omega = 0$. That no further jumps of this sort have been observed in the seven months following the September event casts some doubt on the quake

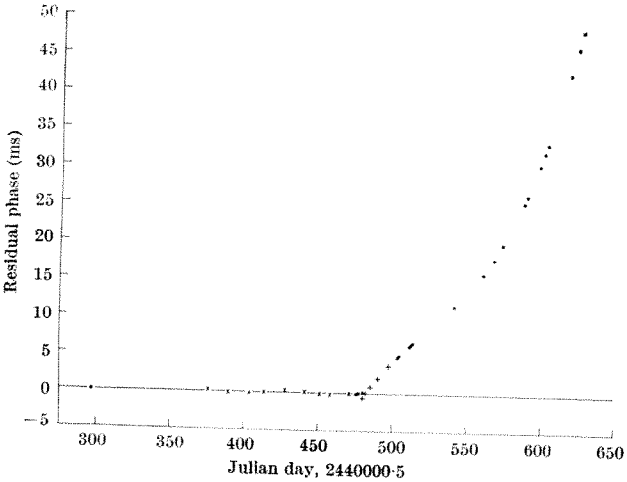


Fig. 3. Residuals against Julian day, after weighted arrival times prior to September 22, 1969 (JD 2440484·5) were fitted by the cubic form of equation (1). The phase residuals resulting from this calculation have been converted to units of time. ×, Arecibo (318 and 430 MHz); ●, SAO-Harvard (optical); +, extrapolated.

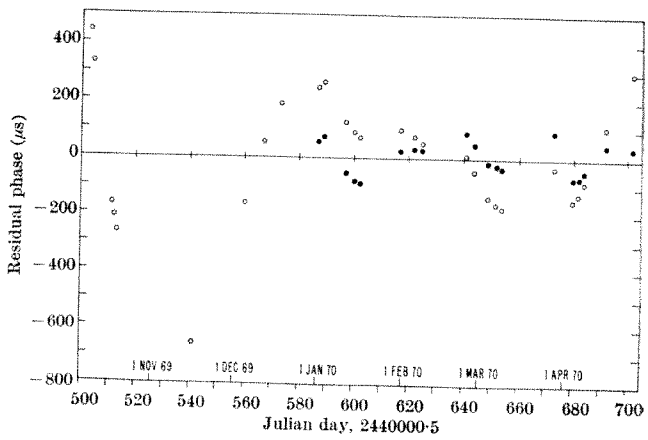


Fig. 4. Residuals against Julian day. ○, Residuals of a cubic fit from October 1, 1969, to April 26, 1970; ●, residuals of a cubic fit after January 1, 1970. The braking parameter n is 2·70 and 2·61, respectively, with an uncertainty of about 5 per cent due to possible errors of a second of arc in the alignment of solar system coordinates relative to NP 0531. The errors in measurement are too small to show in this figure but can be obtained from Table 1.

explanation, as does the observation[10] that major changes in the nearby nebula, following a period of relative quiet, coincide with the jump. The dimensionless parameter

$$Q \equiv \frac{(\Delta\dot{\Omega})^2}{\ddot{\Omega}\Delta\Omega} \quad (0 \leq Q \leq 1)$$

in the superfluid–core/rigid-shell model of Baym *et al.*[11] is approximately 0·34, implying, according to this model, that the moment of inertia of the crust is comparable with that of the core.

Fitting a cubic expression to unweighted data after the jump results in the residuals of Fig. 4. Some rapid post-jump behaviour is evident in the early points, and therefore smaller residuals result from fitting only the points beginning January 1, 1970 (solid circles); in both cases the residuals are far larger than the measurement errors, and lead us to inquire whether a more satisfactory equation relating t and φ can be obtained.

A useful general form of the slowdown law is

$$\ddot{f} = -af^n \qquad (2)$$

where n is related to the parameters in equation (1) by

$$n = f_0\ddot{f}_0/(\dot{f}_0)^2 \qquad (3)$$

If magnetic-dipole radiation is the dominant form of energy loss, $n = 3$; if gravitational quadrupole radiation dominates, $n = 5$. Because $n \approx 2{\cdot}61$ for the expression fitting the points after January 1, 1970 (Fig. 4), the dominant energy loss seems to be the result of magnetic-dipole radiation. The residuals, however, are still quite unsatisfactory because errors due to uncertainty in the Earth's position should not exceed 20 μs, and the measurement errors are only 4–10 μs. Adding one more adjustable parameter, by going to a quartic fit (truncating (1) beyond the quartic term), helps somewhat but not enough. The quartic term is also anomalously large if one compares its contribution to φ with that from the cubic term.

Perhaps a better approach would be to solve equation (2) exactly and then adjust the parameters of the solution

$$\varphi = \varphi_1 + \alpha(1 + t/L)^k \qquad (4)$$

where k is related to n by $k = (n-2)/(n-1)$, and L is a "lifetime" parameter. By expanding φ around approximately correct values for the parameters φ_1, α, L, k, equation (4) can be linearized in these parameters so that a least-squares fitting procedure can be used to minimize the residuals $\{r\}$. The set of residuals obtained by this

procedure is no better than that based on the cubic form of equation (1).

Another possibility is that the slowdown is the result of a combination of dipole and quadrupole radiation described by the equation

$$f = -Af^3 - Bf^5 \quad (5)$$

If the dipole term dominates ($A \gg Bf^2$), the solution to equation (5) is

$$\varphi = \varphi_1 + \alpha\{(t+L) + \beta[\ln(t+L) + 2]\}^{1/2} \quad (6)$$

If the quadrupole term dominates ($A \ll Bf^2$), the solution to equation (5) is

$$\varphi = \varphi_1 + \alpha(t+L)^{3/4} + \beta(t+L)^{5/4} \quad (7)$$

Each of the above solutions has four adjustable parameters and their best values result in a set of residuals identical to those obtained by using the cubic form of equation (1), which also has four adjustable parameters. The only criterion of importance in the quality of the fit seems to be the number of adjustable parameters, so that one should be very careful about the inferences drawn from the particular equation used. A good illustration of this point is the sinusoidal component introduced by the Arecibo group to minimize the residuals over the summer of 1969 (ref. 13). Their fit requires the simultaneous adjustment of seven parameters, four for the cubic part and three for the sinusoidal part of the equation relating t and φ. The cubic expression alone generates residuals as large as 300 μs, much larger than the 10 μs expected on the basis of the measurement process. It is not surprising that the addition of three more adjustable parameters allows one to reduce these residuals significantly. It is possible, however, to fit the same string of data as well with only five adjustable parameters, using an underlying theory that is even simpler than the planetary theory proposed by Richards *et al.* Fig. 5 indicates the residuals that are obtained from the data in their article by assuming a pure dipole braking law (three parameters), accompanied by a fractional increase in the dipole moment of 3·7 parts in 10^5 on JD 2440410·4 (two more parameters). A dipole fit without this jump results in residuals more than ten times as large.

We decided to analyse our data by the method of divided differences[14], because of the difficulty of deciding which function gives the smallest residuals, the desire to recognize discontinuities (jumps) quickly and the fact that none of the simple fitting functions adequately fits the data to our accuracy of measurement. Following the previous discussion, we can note that if $n = 3$, and if we expand t as a power series in φ (that is, make φ the independent variable), the cubic and higher powers of φ vanish identically

$$t = t_0 + P_0\varphi + \frac{1}{2}P_0'\varphi^2 \quad (8)$$

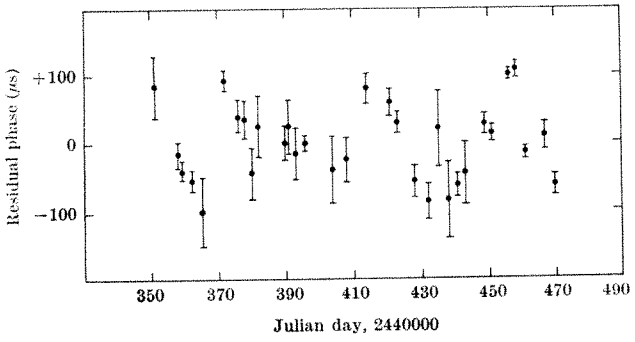


Fig. 5. Residuals against Julian day, after fitting a dipole braking law with a single jump in the dipole moment to the radiofrequency arrival times obtained during the summer of 1969 by the Arecibo group.

Because the period P is just $dt/d\varphi$, P is a linear function of φ (or N) for pure dipole braking. This simple relationship is exploited in the method of divided differences.

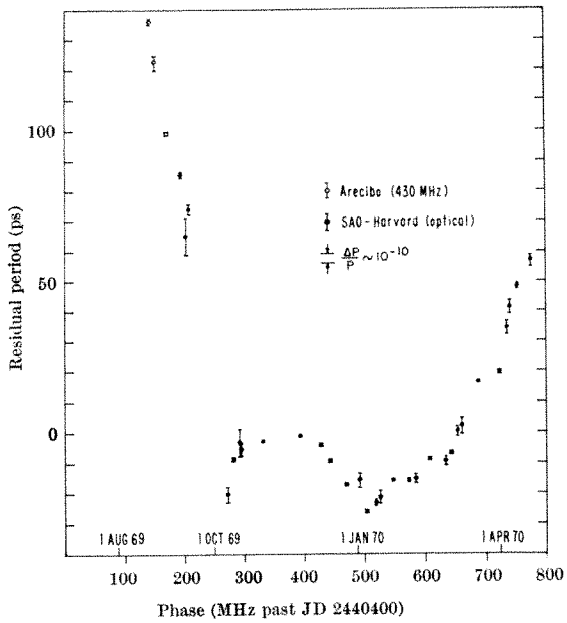


Fig. 6. Residual period against phase, calculated by divided differences, with a linear term subtracted. Error bars are omitted when less than 0·35 ps.

Method of Divided Differences

The analysis discussed before indicates that the parameter n, defined by equation (3), is close to 3. If $n = 3$, then t is a quadratic function of φ (or N) and the period P is a linear function of φ. If (t_1, N_1) and (t_2, N_2) lie on a parabola, then the slope of the parabola at $N_{12} = (N_1 + N_2)/2$ is given by

$$t_{12}' = P_{12} = (t_2 - t_1)/(N_2 - N_1)$$

By this method of calculation we can generate a new set of points $\{(P, N)\}$. Because P is expected to be a linear function of N (because $n \approx 3$), we can subtract out the best straight line (in the least-squares sense) and plot the residuals ΔP as a function of N. These residuals are shown in Fig. 6. The errors indicated are just

$$\pm \sqrt{\sigma_1^2 + \sigma_2^2}\,/(N_2 - N_1)$$

where σ_1 and σ_2 are the errors in measuring t_1 and t_2, respectively. The Ns, being integers, are considered exact. One can easily see from such a graph the discontinuous jump in P that occurred around September 20, 1969. Moreover, it is not possible to fit the data past the jump with a simple, low order polynomial. If the curvature were in one direction only, it would indicate that n was constant but that $n \neq 3$; the fact that the curvature goes both ways indicates either a varying n, an oscillatory component or the existence of long-period random fluctuations. Because the evidence for the first two is not very convincing, we prefer tentatively to accept the third conclusion, especially because a cubic fit (Fig. 4) shows similar unexplained fluctuations. In a rotating neutron star model, such "noise" could be produced by fluctuations in the braking torque (from changes in the radiating charge density, for example) of a part in 10^4, or by temporary mass redistributions that result in changes of equatorial radius of 0·1 μm. A possible cause of the latter comes from the hard-crust models. The previously mentioned failure to observe frequent large quakes suggests that the crust, if it exists, may be too weak to maintain a large distortion, and is consequently in a more or less continuous process of crumbling and fracturing to relieve the strain before it amounts to a departure of as much as 15 μm from the equilibrium shape. These effects are

extremely minute—only measurable at all because of the enormous accumulated phase (10^9 cycles per year) and the ability to measure arrival times to a small part of a cycle (10^{-4} cycles)—yet they preclude the possibility of measuring accurately the gravitational red shift of refining the Earth's ephemeris. The phase stability of the Crab pulsar, viewed as an oscillator, is described by a $Q \approx 10^{10}$.

An interesting consequence of the continuously crumbling model is that the braking parameter n, given by equation (3), is somewhat less than three even if the sole energy loss is through magnetic-dipole radiation. This results from the continuous adjustment of the equatorial radius, tending to maintain the angular velocity. The torque τ from dipole radiation is $\tau = -a\Omega^3$, and the moment of inertia about the axis of rotation of an oblate spheroid is given by $I = I_0(1 + 2\varepsilon)$, where $\varepsilon = 5R^3\Omega^2/(12GM)$, and R is the radius of the sphere when $\Omega = 0$. Setting

$$\tau = \frac{\mathrm{d}}{\mathrm{d}t}(I\Omega)$$

we find $n = 3\text{–}12\,\varepsilon$, that is

$$n = 3 - 5\frac{R^3\Omega^2}{GM} \qquad (9)$$

Using the measured value $n = 2{\cdot}61$ and setting $M = 1/2M_0$ gives $R = 50$ km. This is somewhat larger than the radius of typical neutron-star models, indicating that the deviation of the measured slowdown parameter n from the value for pure dipole braking ($n = 3$) is not the result solely of the effects of changing oblateness. In this calculation, however, we have assumed that the neutron-star material is homogeneous and incompressible and that the magnetic-dipole moment is constant.

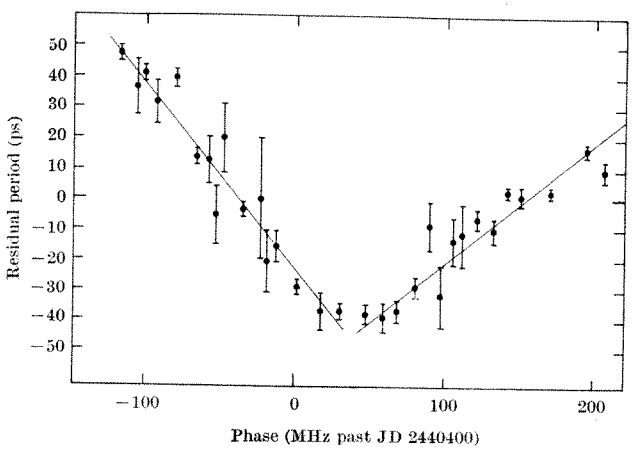


Fig. 7. Residual period against phase of the summer Arecibo data, with a linear term subtracted.

Applying the analysis of divided differences to Arecibo's summer data results in the residuals ΔP from a straight-line fit of P against N shown in Fig. 7. Richards *et al.*[13] accounted for these residuals by introducing a sinusoidal term, but two straight lines are also a possibility and permit the interpretation mentioned earlier of pure dipole radiation with a jump in the dipole moment.

Superposition of Light Curves

Because we determine the peak of each measured light curve to a typical accuracy of ~ 10 μs by cross-correlating with a template, we can easily superpose many such curves and maintain the 32 μs resolution. An example of such a superposed light curve is shown in Fig. 8, representing about 10 hours data taken throughout the previous year. It is interesting to note that the peak is still cusp-like even down to this 32 μs resolution. This implies that

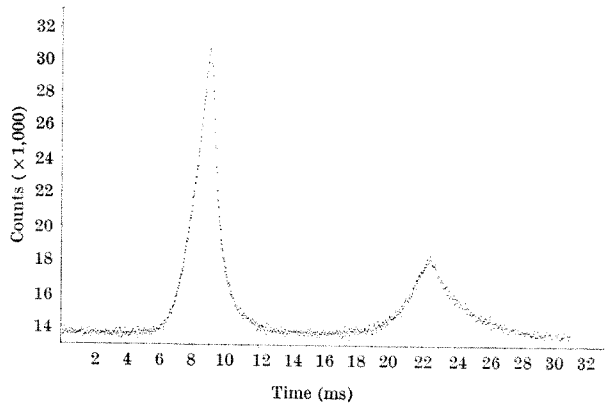


Fig. 8. Superposed light curve (32 μs per point) from ten nights of observing between October 1969 and March 1970. Total observing time is about 10 h.

the radiating region has significant detail on a scale of 32 μs of light-time, or 10 km.

The spacing between main and secondary peaks is 418 ± 1 bins, or $13{\cdot}376 \pm 0{\cdot}032$ ms. This is in excellent agreement with the spacing between the narrow spikes at 318 and 430 MHz found at Arecibo (personal communication from J. M. Rankin, J. M. Comella, H. D. Craft, jun., D. W. Richards, D. B. Campbell and C. C. Counselman, III), namely $13{\cdot}36 \pm 0{\cdot}03$ ms. The period is about 33·1 ms, so the secondary peak occurs 40 per cent through the cycle. This could not be produced by a pure dipole field configuration in a near-field model like that of Komesaroff[15], but would require a "bent dipole" with directions of peak field being no longer collinear. A curious fact about the light curve of Fig. 8 is that both main and secondary pulses are asymmetrical, but in opposite senses: the main pulse falls rapidly, but the secondary pulse rises rapidly. This is, however, just what one would expect from a bent dipolar field, for the gradients are larger on the closer facing sides of the two poles.

Details of all the computer programs, as well as additional comments on some aspects of this work, can be found in the thesis of P. H.[16].

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LETTERS TO NATURE

PHYSICAL SCIENCES

Observational Upper Limits to the Electromagnetic Energy radiated by Normal Galaxies

Low[1] has suggested that typical galaxies emit energies of the order of their present rest mass energies during their lifetime of $\sim 10^{10}$ yr. The observations[2] on which this theory is based indicate that the flux peaks sharply at an emitted wavelength of ~ 70 μm. In this report we indicate the constraints which can be put on such models from observations of the isotropic background radiation at these and longer wavelengths. It will be seen that the present upper limits on the flux still allow most of the energy emitted by galaxies to be in the infrared region of the spectrum.

In order to interpret the meaning of the background radiation measurements, we shall assume that the galactic emission is indeed confined to a narrow range of emitted wavelengths. Assuming a homogeneous and isotropic universe described by the Robertson–Walker metric, the galactic emission at λ_e leads to an observed intensity in the bandwidth λ_1 to λ_2 of

$$\Delta I = \frac{c}{4\pi} \int_{Z_1}^{Z_2} \frac{dZ}{(1+Z)^4} \left|\frac{dt}{dZ}\right| \int_0^\infty L\Phi(Z,L)dL = \frac{c}{4\pi} \frac{\Delta\varepsilon}{\langle 1+Z\rangle}$$

(see also ref. 3). Here $1+Z_1=\lambda_1/\lambda_e$, $1+Z_2=\lambda_2/\lambda_e$, and ΦdL is the number density of galaxies with luminosities (emitted near λ_e) between L and $L+dL$. Thus $\Delta\varepsilon$ is the energy radiated per unit comoving volume during the epoch corresponding to an average redshift $\langle Z\rangle$ of duration corresponding to Z_2-Z_1.

The best estimate of the present matter density in galaxies is $\rho_0=3\times10^{-31}$ g cm^{-3} (refs. 4 and 5) if the Hubble constant $H_0=75$ km s^{-1} Mpc^{-1} (ref. 6). The fraction of the present galactic mass which has been radiated electromagnetically during a given epoch is then given by

$$\Delta\varepsilon/\rho_0 c^2 = 1{\cdot}55\,\langle 1+Z\rangle\,\Delta I = 1{\cdot}55\,\langle\lambda/\lambda_e\rangle\,\Delta I$$

if ΔI is expressed in erg cm^{-2} s^{-1} sr^{-1}.

In discussing Low's model, we are interested in broad-band measurements of ΔI at wavelengths of 70–2,000 μm, corresponding to redshifts $Z=0$–30. It is unlikely that significant radiation occurs at higher redshifts, especially because the optical radiation from quasi-stellar objects seems to cut off beyond $Z\sim2$. Indeed, Low's model would indicate an effective infrared cutoff at $Z\lesssim10$, due to the short time available at large redshifts in which to radiate the maximum expected luminosity.

The observational upper limits on ΔI for various wavelength regions and the corresponding upper limits on the fractional rest mass radiated during various epochs are presented in Table 1. In computing ΔI (max), account has been taken of variation in detector response across the bandwidth. We now discuss the relevant observations, none of which are inconsistent with the expected large-scale isotropy.

Table 1. MAXIMUM ENERGIES RADIATED BY GALAXIES EMITTING NEAR 70 μm

λ (μm)	ΔI (max) (erg cm^{-2} s^{-1} sr^{-1})	Z	$\Delta\varepsilon/\rho_0c^2$
70–120	0·02 *	0–0·7	0·04
120–350	—	0·7–4	—
350–420	0·5 †	4–5	4
420–560	0·13 ‡	5–7	1·3
560–800	0·05 ‡	7–11	0·7
800–900	0·026 ‡	11–12	0·50
900–1,200	0·007 ‡	12–16	0·17
1,200–2,000	0·004 ‡	16–28	0·13

*Ref. 7. †Refs 8 and 9. ‡Ref. 10.

The intensity in the band 70–120 μm was obtained from the data of Houck and Harwit[7]. Their detector response falls off rapidly at the limiting wavelengths, with the maximum response indicating an intensity of 0·01 erg cm^{-2} s^{-1} sr^{-1} if it were concentrated near 100 μm.

The maximum intensities at wavelengths 350–420 μm were computed from the data of Shivanandan *et al.*[8] and J. R. H. and M. O. H.[9], after a detector recalibration carried out with the help of Mrs J. Pipher. The basic detector response rises from 300 μm to 800 μm. At the shorter wavelengths the upper limits have to be corrected upwards. At longer wavelengths, around 900 μm, the corrected upper limits are consistent with results obtained from the data of Muehlner and Weiss[10] and correspond to a lower limit set by Shivanandan *et al.*[8] and J. R. H. and M. O. H.[9]. The Muehlner and Weiss data are presented in Table 1 for this spectral range. It is important to realize that the corresponding upper limits on $\Delta\varepsilon/\rho_0c^2$ are obtained under the assumption that all the energy falls within each segment of the bandwidth considered.

At wavelengths greater than 900 μm, the upper limits decrease because of the weaker signal detected by Muehlner and Weiss when long-wavelength filters were inserted. It is worth noting that if the signals observed by Muehlner and Weiss are assumed to be real rather than upper limits, they could all be due to a single feature near 870 μm plus the small contribution of a spectrum similar to a 2·7 K blackbody spectrum.

The results for $\Delta\varepsilon/\rho_0c^2$ listed in Table 1 indicate that galaxies could have released on the order of their rest mass energies in photons at redshifts $4\lesssim Z\lesssim12$. The lack of useful data between 120 and 350 μm allows even higher energy releases during the epoch $1\lesssim Z\lesssim4$. The present data do not allow such significant energy release at redshifts $Z\lesssim1$, however. These results are nevertheless still consistent with Low's model in strongly evolutionary cosmologies. Because $\Delta I \propto \langle 1+Z\rangle^{-5}\Delta Z$ in steady state cosmology, however, Low's proposal is ruled out in such a universe. The data can obviously be applied to any similar model if the effective emitted wavelength is known.

It is worth noting that the data inferred by Bortolot, Clauser and Thaddeus[11], on the basis of photographic interstellar absorption measurements, further restrict the amount of energy that could have been released at epochs around $Z=4$ and $Z=7$, corresponding to wavelengths of 359 μm and 559 μm. The upper limits placed on $\Delta\varepsilon/\rho_0c^2$, however, depend on the interval ΔZ over which emission occurred. If one takes $\Delta Z(Z+1)^{-1}\sim0{\cdot}1$, the limits on $\Delta\varepsilon/\rho_0c^2$ at these respective wavelengths become $\sim0{\cdot}04$ and 0·01. If $\Delta Z(Z+1)^{-1}\sim0{\cdot}5$, one obtains corresponding values of 0·2 and 0·07 for $\Delta\varepsilon/\rho_0c^2$ at 359

and 559 μm. In that case, there would be no region beyond $Z \sim 3$ where galaxies could have expended an amount of energy equal to their present rest mass energy.

Although the upper limits obtained are not very restrictive at present, more sensitive broad-band background measurements, especially at wavelengths around 200 μm, will help to clarify the situation.

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On Pulsar Distances and Emission

IT has been suggested to us that it would be useful to extend the list of pulsar distances which two of us computed earlier[1,2] to include those pulsars which have been discovered since then. In Table VII of ref. 2 we listed distances of thirty-six pulsars. We now list the results for another eighteen, six of which were mentioned previously[2] in a note added in proof. The results are given in Table 1. Because a plot of pulsar distribution against height z above the galactic plane shows a sharp distinction between those objects for which a precise distance was found and those for which we use equation 44 of ref. 2, we have adopted $l = 200\ |\sin b|^{-1}$ pc as a lower bound distance estimate for pulsars with $n_0(\infty) \gtrsim 0{\cdot}05$ cm^{-3} whose line of sight does not intersect an HII region. For the Jodrell pulsars we have used the data of Davies, Large and Pickwick[3,4].

We want to make the following remarks concerning the data in Table 1.

(i) *MP* 0254, *MP* 1911 and *AP* 2303 seem to lie beyond the galactic electron layer, and *MP* 1857 at its edge.

(ii) As our list of HII regions does not extend beyond 1 kpc, we can only put an upper bound on the distances to *MP* 1154, *JP* 1845 and *JP* 1858.

(iii) The line of sight to *MP* 1604 just strikes the N = 2 HII region of ζ Oph at 170 pc. If this HII region is completely disregarded, we find $l \sim 300$ pc. A mean was taken in Table 1.

(iv) 70 cm^{-3} pc of the dispersion to *JP* 1946 is attributable to WR star 97 in Smith's list[5], and of the dispersion to *JP* 2003, 170 cm^{-3} pc is attributable to the same star.

(v) The line of sight to *JP* 2111 hits the HII region of 68 A Cyg.

(vi) The line of sight to *JP* 2319 hits the HII region of III Cep.

Table 1. PULSAR DISTANCES

Pulsar	l^{II}	b^{II}	$\int n_e\, dl$ (cm^{-3} pc)	$10^2\, n_0(\infty)$ (cm^{-3})	l (pc)
MP 0254	271	−55	10	5·1	> 250
MP 0818	237	12	25	3·2	700
MP 1154	297	0	270	0·0	< 3,000
MP 1359	314	11	20	2·3	500
MP 1604	8	34	10	3·5	250
MP 1706	7	15	10	1·5	200
JP 1845	29	−1	140	1·4	< 3,000
MP 1857	11	−13	35	5·0	1,000
JP 1858	37	−1	400	4·4	< 7,000
MP 1911	31	−7	75	5·8	> 1,600
MP 1944	54	−4	35	1·4	700
JP 1946	71	5	130	7·3	1,000
JP 1953	66	1	20	0·0	350
JP 2003	69	0	225	0·0	1,000
JP 2021	88	8	20	1·6	400
JP 2111	89	−1	140	1·4	1,400
AP 2303	98	−27	50	13·5	> 400
JP 2319	112	−1	140	1·4	800

Recently, Tsytovich and two of us[6] have suggested that one might perhaps find optical or infrared pulsed emission at those frequencies where the brightness temperature would become of the order of the effective temperature of the relativistic particles which produce the magneto-brems radiation. To find the frequency ν_0 at which this emission would take place, we assumed a bandwidth of about 10 ν_0 and a total emitted radiation at these frequencies of the order of the total loss of rotational energy. When writing our earlier note we had data about the rate of change of only nine pulsars. At the Jodrell Bank Crab Nebula Symposium, data were presented for seven more pulsars, and we felt that it might be of interest to repeat our calculations for those pulsars. In Table 2 we give the values of ν_0 calculated in the same way as in ref. 6, the value of the rate of change of the period (ref. 7 and unpublished data of G. C. Hunt, and ref. 8), the possible total flux in the band near ν_0 (assuming about 10 per cent of the loss of rotational energy to be converted, as seems reasonable from *NP* 0532 data). From the data in Table 2 and elsewhere[5] it seems that the most likely candidates to show pulsations in the infrared would be *CP* 0950 at about 1 μm and *PSR* 1642 and *PSR* 1929 at about 2 μm.

Table 2. POSSIBLE PULSAR INFRARED EMISSION

Pulsar	$\dot{P}$ (10^{15})	ν_0 (10^{14} Hz)	Power (10^{31} erg s^{-1})	l (pc)
AP 0823	1·66	3	0·3	> 900
PSR 1642	6·2	5	3	160
PSR 1749	8·23	4	1	1,000
PSR 1929	1·16	5	3	150
JP 1933	6·0	6	4	~3,000
AP 2015	0·13	1	0·02	300
PSR 2045	11·15	1	0·05	400

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Plate Tectonics of the Red Sea and East Africa

I WOULD like to comment on some of the assumptions and results of a recent letter by McKenzie *et al.*[1]. They reconstruct the pre-movement position of Arabia and Africa by fitting the two coast lines of the Red Sea, assuming that the entire space between the coasts is occupied by newly formed oceanic crust. This assumption ignores the existence of the Danakil horst, which consists of continental crust (Pre-Cambrian, Jurassic) and which is some 80 km wide, in between these two coast lines in the southern Red Sea depression. It seems impossible to close the gap of the Red Sea without leaving the space required for this continental block.

This reconstruction on a pole of rotation at 36·5° N, 18°, off western Greece leads (ref. 1, Fig. 4) to a considerable extensional component along the Dead Sea rift. This does not correspond to the nature of the movement along this fault line, where the strike slip movement is dominant, with minor extension alternating along it with large shortening in Lebanon[2]. This reconstruction leads finally to a crustal separation of 60–90 km across the Gulf of Suez. This is difficult to reconcile with the fact that the total width of the Gulf of Suez depression does not exceed 50 km in the north and 65 km in the south. Furthermore, there are blocks of continental material within the depression, that of Gebel Kabiliat in the west and of Gebel Zeit in the east. The extension across the Gulf of Suez cannot therefore exceed 25–30 km, according to the assumption of McKenzie *et al.* that the crust has thinned by half beneath the Gulf. This leaves a 40–60 km excess discrepancy with their results. Thus the two coast lines of the Red Sea could not be said to be matching. Instead, they may reflect the marginal faults of a rift which formed at the early stages of the opening of the Red Sea. Judging from the East African rifts, the marginal faults are usually nearly parallel, and the width of such a rift is about 60 km.

The reconstruction should therefore be carried out not by fitting the two coast lines, but by fitting two lines which are about 20–30 km seawards from each coast. This might be the place where the 2,000 m depth contour (which is the most reliable outline of the continents[3]) could be expected in such a fresh crustal separation, were it not concealed by the thick sedimentary cover.

It is true that such a suggestion leaves a wide margin of inaccuracy if a precise reconstruction of the positions of the African and Arabian plates is attempted. The pole of rotation found manually, according to the assumption that there was originally a rift 60 km wide along the Red Sea, occurs at about 32° N, 22° E, north-west of Egypt.

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Magnesium, Organic Matter and Soil Structure

SURFACE soils with good structure consist of aggregates that do not disintegrate on sudden wetting with water to form a crust when re-dried. Crusting is most severe when disintegration proceeds down to the level of clay sized particles. The beneficial effect of soil organic matter in reducing the break-up of initially dry soil aggregates when wetted with water is well known. Equally well known is the deleterious effect of exchangeable sodium in causing spontaneous dispersion of clay from aggregates if the external electrolyte concentration is reduced when the soil is wetted. Spontaneous dispersion does not occur if the exchange complex of a soil is effectively saturated with Ca ions or if sufficient organic matter is present[1]. If external work is performed on such soils when they are wet, however, then spontaneous dispersion may occur[2]. Thus a surface soil can apparently have a good structure as measured by the slaking (break-up) of dry aggregates in water and yet after shearing, as in tillage, it may still crust badly[3].

The present work was undertaken to determine, first, whether there is a difference in the ease of dispersion of a sheared surface soil when saturated with Mg rather than Ca ions; second, to show directly, by the addition of organic compounds to the oxidized clay fraction extracted from the surface soil and the corresponding untreated subsoil, that dispersion can be enhanced by organic matter.

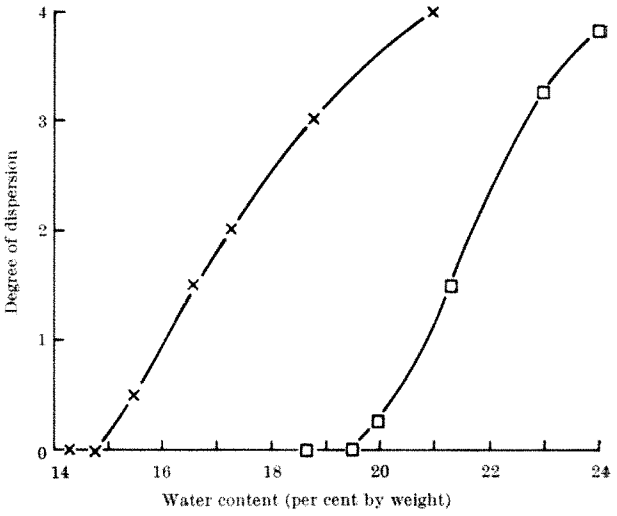


Fig. 1. The visual dispersion in water of surface aggregates of a red earth remoulded at various water contents. ×, Washed $MgCl_2$; □, washed $CaCl_2$.

The degree of dispersion in water of remoulded surface aggregates of a kaolinitic red earth[3] (C content 1·3 per cent) washed with either $CaCl_2$ or $MgCl_2$ is shown in Fig. 1 as a function of the initial water content of the aggregates. Dispersion was assessed visually using the scale 0 to 4, where 4 means apparently complete dispersion into primary particles. This simple technique is adequate for detecting the first appearance of dispersed clay. The Mg-washed soil showed signs of dispersion when remoulded at a water content of 15 per cent by weight whereas the Ca soil started to disperse at 20 per cent. The difference is significant because the field capacity of this soil is about 20 per cent by weight.

Various commonly used organic dispersants such as Na-carboxymethyl cellulose and Na-citrate were added to the peroxidized clay fraction ($< 2\ \mu$) of the surface soil and the untreated subsoil. After washing the mixtures

with $CaCl_2$ and drying, all failed to cause dispersion after remoulding wet. It was known that acid suspensions of kaolinite can be dispersed by extracts of some trees[4]. Leaves from various eucalypt spp. were slowly dried at 55° C and then extracted with water. Without added leaf extract, the water contents for dispersion of the washed clay or subsoil were greater than 200 per cent by weight. By adding the extract, not only was the water content for dispersion reduced to 30–40 per cent by weight, but the Ca-washed clay was now less easily dispersed than the Mg-washed clay (Fig. 2). Further, the respective water contents for dispersion were close to those of the natural surface soil washed with the two cations (compare Fig. 1) when multiplied by the ratio of their clay contents[2] (100/48). The subsoil plus leaf extract gave similar curves to those shown in Fig. 2 but displaced to slightly lower values according to the clay content.

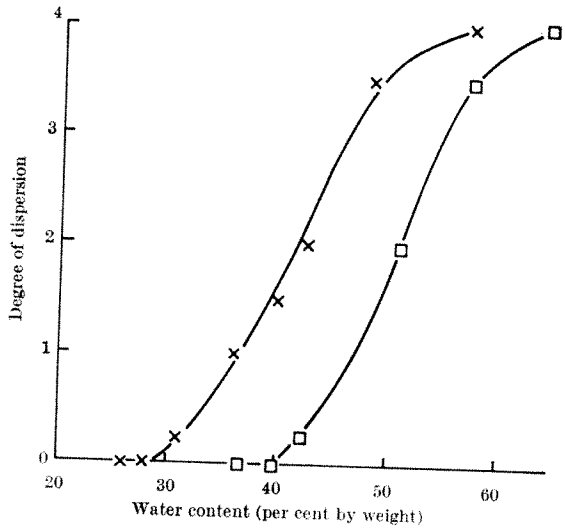


Fig. 2. The visual dispersion in water of the oxidized clay fraction of the surface soil remoulded at various water contents, after the absorption of 0·4 per cent C from an aqueous extract of *E. melliodora* leaves. ×, Washed $MgCl_2$; □, washed $CaCl_2$.

Using electro-osmosis[5], it has been shown that Al ions in the natural surface soil of the red earth are coordinated with the soil organic matter, whereas Al ions in the subsoil are free to react with added oxalate. It is suggested that the added leaf extract coordinates Al ions on the surface of the oxidized clay and on the natural subsoil, so reducing the attractive forces between the clay particles. The enhanced dispersion after Mg-washing compared with the Ca-washing is probably due to the relative solubility of the Ca and Mg salts of the added organic matter.

Until now laboratory experiments have shown little or no differential effect of Mg and Ca ions on structure, although there have been reports in the literature for a long time that the structure in the field of surface soils with a dominant percentage of exchangeable Mg ions is worse than that of similar soils where Ca is dominant[6,7]. The permeability of beds of unsheared surface soil aggregates has been measured[8,9] and experiments have been carried out with clays free from organic matter[2,10]. Where exchangeable sodium is present as well in an Mg dominant soil, the water content for dispersion can be reduced to very low values compared with field capacity.

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Tree Ring, Varve and Carbon-14 Chronologies

Carbon-14 dating has become of great importance and it is therefore necessary to determine the possible deviations between radiocarbon and actual ages. A radiocarbon age, given in radiocarbon years elapsed since the origin of the sample, is based on ^{14}C decay and is calculated from the ratio of the present ^{14}C activity of the sample to the ^{14}C activity of the standard (95 per cent of the ^{14}C activity of NBS oxalic acid). The standard ^{14}C activity is assumed to be identical to the atmospheric ^{14}C activity over the whole time interval over which ^{14}C dating is used. Thus the conventional radiocarbon age does not allow for deviations in atmospheric ^{14}C content during the past. As a result, radiocarbon years need not always be equal to solar years.

Variations in atmospheric ^{14}C have been studied in many ways, using tree rings, historically dated samples, varves, lake sedimentation rates and chronological frequency distributions[1]. Dendrochronologically dated tree rings are the most suitable for precise determinations of atmospheric ^{14}C variations during the past[2]. Each ring is formed during one year only, and ceases to accumulate or exchange carbon thereafter. By measuring the present concentration of ^{14}C in wood samples the ages of which are dendrochronologically determined it is possible to calculate the initial ^{14}C concentration in the sample at the time of formation.

There seem to be both long and short-term variations in the ^{14}C concentration of wood samples. The short-term oscillations of about one to two hundred years have been shown to correlate significantly with the solar cycle modulation of the cosmic ray flux[3–7]. The most recent figures for long-term variation in atmospheric ^{14}C obtained from tree ring data by Suess[8] are given in Fig. 1. This curve agrees in its basic features with the results obtained by Damon[9] and Michael and Ralph[10]. The time span of the tree ring chronology, at present 7,400 years, is unlikely to be extended more than a few thousand years because of the lack of suitable specimens, but varve series seem a suitable alternative for the extension of the study of ^{14}C variations.

The results of ^{14}C measurements of two extended varve series are available at present, one series being the classic Swedish varve chronology of de Geer. Although the Swedish varves contain insufficient authigenic organic material for precise ^{14}C measurements, it is possible to correlate ^{14}C dated climatic episodes of northern Europe with varve dated rates of recession and geomorphological features in southern Sweden. In addition, pollen-zone boundaries, varve dated in the Ångerman series by Fromm[11], can be correlated with ^{14}C dated pollen-zone boundaries in nearby peat bogs[12]. Using both techniques, Tauber[13] calculated the ^{14}C deviations resulting from the differences in ^{14}C age and the corrected Swedish varve age (Fig. 1).

The second varve series, from the Lake of the Clouds, Minnesota, was discovered a few years ago by H. E.

Wright and E. J. Cushing of the University of Minnesota. Parts of the ^{14}C analyses for this lake have been reported previously (for a detailed core description, see refs. 1 and 14).

Alan Craig, at the University of Minnesota, counted about 9,500 varves through the entire core, with the exception of the lowermost 40 cm where a continuous sequence was lacking. His counts are used here. All ^{14}C ages are based on the 5,730 year half-life and have been corrected for isotopic fractionation. A deduction of 340 years was made to correct for the ^{14}C deficiency of the lake water.

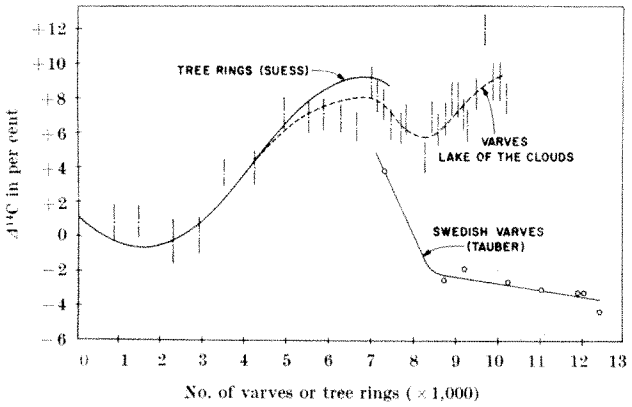


Fig. 1. $\Delta^{14}C$, in per cent of atmospheric content, plotted against Lake of the Cloud varve years (dotted line and experimental points), varve years of the Swedish varve chronology (solid line at bottom) and number of tree rings (solid line at left). Lines drawn are approximate only.

A comparison of the Lake of the Clouds varve ages with ^{14}C ages results in the calculated atmospheric ^{14}C deviations given in Fig. 1. All experimental points in this drawing are derived from Lake of the Clouds samples. The agreement between tree ring and Lake of the Clouds varve series is excellent over a 7,400 year interval. Agreement is lacking, however, between both varve chronologies before 7,000 years BP. The discrepancy may have been caused by errors in either the Swedish or North American chronology, or both.

According to the Swedish varve series, the level of atmospheric ^{14}C was about 2 to 4 per cent below the AD 1950 standard level for the 8,500 to 12,500 varve year interval. Because the tree ring chronology indicates a level of +9 per cent around 7,500 years BP, atmospheric ^{14}C activity should have increased about 11 per cent between 8,500 and 7,500 years BP (from −2 per cent at 8,500 years to +9 per cent at 7,500 years). Such a large change in atmospheric ^{14}C conflicts with the constancy in atmospheric ^{14}C indicated by a floating tree ring chronology between 7,050 and 7,350 ^{14}C BP[15], and is also not supported by lake sedimentation measurements[14,16].

If the Swedish chronology is assumed to be correct, the Lake of the Clouds varve chronology has to be in error by about 800 years. Shortening of the 7,500–9,300 varve interval to 7,500–8,500 years brings the North American chronology in agreement with the Swedish one. Such a large error seems unlikely because the agreement with the tree ring results proves that the errors in varve counting of the Lake of the Clouds series is only 2 per cent over the preceding 7,400 years. It should be noted also that such a small error has not yet been proven for the Swedish chronology. Other interpretations of the Swedish varve chronology, different from the one from which the curve in Fig. 1 was derived, are possible[17,18]. They would generally tend to increase atmospheric ^{14}C between 9,000 and 12,000 varve years above the baseline in Fig. 1.

The differences between both varve chronologies should be solved with the extension of the tree ring chronology by an additional 1,000 years. If the Lake of the Clouds chronology is shown to be correct, the following picture seems indicated. (1) Atmospheric ^{14}C concentration over the past 2,500 years was within a few per cent of the nineteenth century level; (2) between 5,500 and 2,500 years BP atmospheric ^{14}C content was reduced from about +9 per cent to the present base line; (3) for the entire 5,500 to 10,000 year BP interval atmospheric ^{14}C level was about 7 to 10 per cent higher than at present. Such a long-term trend in ^{14}C content may have resulted from a combination of: (*a*) changes in Earth magnetic dipole moment; and (*b*) changes in exchange rate between atmosphere and the oceans caused by a lower mean ocean temperature, lower sea level, and increasing ice cover.

For the Lake of the Clouds varve chronology, the change in exchange rate may explain the gradual reduction in atmospheric ^{14}C content from about +10 per cent 10,000 years ago to the present level. Changes in Earth magnetic field intensity would add a ^{14}C oscillation with an amplitude of about 4 per cent and with a period of about 7,000 years on this general trend.

The only basic assumption inherent to the calculation of the deviation of atmospheric ^{14}C from the base line is the assumption that both varves and tree rings are annual. The agreement between the Lake of the Clouds varve series and the tree rings leaves little doubt that this basic assumption is justified for the past 7,400 years. It is difficult to envisage a process that influences annuality of tree rings in Arizona and lake varves in northern Minnesota in the same manner.

In general, ^{14}C dates corresponding with 5,000 to 7,400 solar years will be 600 to 800 years too young. If the Lake of the Clouds varve chronology is accepted, a similar error is also indicated for the 7,400 to 10,000 varve year interval. On the other hand, the Swedish varve chronology indicates little deviation between ^{14}C and varve ages between 8,000 and 12,000 years. Both varve sequences show that ^{14}C ages (based on the 5,730 year half-life) are expected to deviate less than 800 years from the true age over the past 10,000 years.

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Dielectric Relaxation Studies by Time Domain Spectroscopy

DIELECTRIC relaxation has long been recognized as a powerful method of studying the physical state of a system containing polar molecules or groups. At the same time a number of inevitably time-consuming frequency domain techniques are usually required to cover a reasonably wide range of frequency, each demanding the highest degree of experimental expertise. We report here the application of one variant of time domain spectroscopy (TDS) to the study of dielectric relaxation processes in the frequency range 10 MHz–15 GHz, to illustrate the enormous potential of this method, by virtue of its simplicity of operation, for the relatively rapid estimation of the parameters usually associated with a dielectric relaxation process.

TDS may be subdivided into a number of categories (whose individual applicability to dielectric relaxation measurements will be described elsewhere) one of which is time domain reflectometry (TDR). The use of TDR for the measurement of dielectric properties was first reported by Fellner-Feldegg[1]. This method involves the generation of a step pulse containing a broad range of frequencies in a coaxial system, and monitoring the change in pulse shape after reflexion from the surface of a dielectric sample enclosed in a coaxial cell. The measured parameter is the reflexion coefficient (ρ) which is related to the complex permittivity (ε^*) by the expression

$$\varepsilon^* = \left(\frac{1-\rho}{1+\rho}\right)^2 \qquad (1)$$

Fellner-Feldegg[1] analysed his experimental data in terms of the time dependence of ρ and calculated relaxation times for a series of aliphatic alcohols.

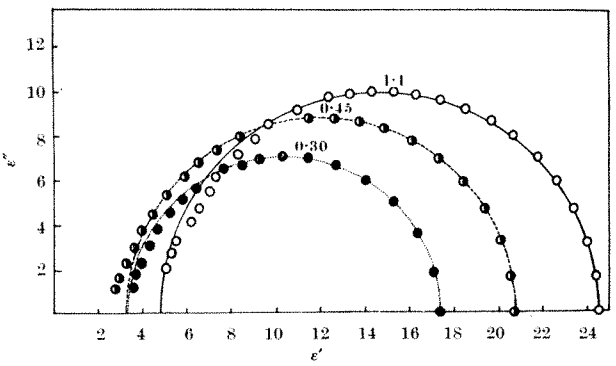


Fig. 1. Permittivity–loss data for ethanol, *n*-propanol, and *n*-butanol at 25° C displayed on the complex plane diagram. ○—○, Ethanol; ◑- - -◑, *n*-propanol; ●···●, *n*-butanol. Indicated frequencies are in GHz.

TDR has been used for several years for the qualitative analysis of transmission line systems. The paper of Fellner-Feldegg[1] is therefore of importance in that it showed for the first time the quantitative possibilities of the technique. On the other hand, the rather poor agreement between many of his estimated relaxation times and other values in the literature might be taken to indicate that TDR is too imprecise to be of much value for the study of dielectric relaxation processes. We take the view that the erroneous results of Fellner-Feldegg are largely a consequence of his method of analysing the experimental data, and do not represent the true precision of TDR. This view has been supported recently by Whittingham[2] who discussed the major limitation of the Fellner-Feldegg method.

We report here the results of an alternative approach to the analysis of TDR data. The reflected pulse and the incident pulse (in practice, the pulse after reflexion from a precision short circuit) are Fourier transformed into the frequency domain by making use of the following modification of the Shannon sampling theorem[3] due to Samulon[4]

$$F(\omega) = \frac{e^{i\omega\tau/2}}{2i \sin \omega\tau/2} \sum_{n=-\infty}^{n=+\infty} \{f(n\tau) - f(n\tau - \tau)\}\, e^{-in\omega\tau}$$

provided that $F(\omega) = 0$ for $\omega > \pi/\tau$ where ω = angular frequency in radians s^{-1} and $i = \sqrt{-1}$. The pulses to be compared are displayed on an X–Y recorder and individually sampled (n samples) at some regular time interval, τ such that the pulse under analysis has no effective frequency contribution above a cut-off frequency of $1/2\tau$. This condition can usually be met without much difficulty in practice, although for some systems it is convenient to split the waveform into two parts so that the region of most rapid change can be more densely sampled than the region of relatively slow change. The resulting Fourier analysis yields the reflexion coefficient (ρ) expressed as $re^{i\theta}$. Information about the amplitude (r) and phase (θ) may then be converted by means of the first equation to ε' and ε'', where $\varepsilon^* = \varepsilon' - i\varepsilon''$.

Experiments were performed using Time Domain equipment manufactured by Hewlett Packard Ltd. The major modification to the earlier experimental arrangement[1] is the introduction of a time marker system so that the pulses to be compared may be referred accurately to a standard point in time. This is an essential requirement of the method of analysis described here.

Table 1. COMPARISON OF RESULTS OBTAINED BY DIFFERENT TECHNIQUES FOR THE MAJOR DIELECTRIC RELAXATION PROCESS OF ETHANOL, *n*-PROPANOL, AND *n*-BUTANOL AT 25° C

Parameter	This work	TDR Fellner-Feldegg[1]	Frequency domain methods*
τ (Ethanol)	140 ps	130	150 (ref. 5), 125 (ref. 8)
τ (*n*-Propanol)	330 ps	220	385 (ref. 6), 350†
τ (*n*-Butanol)	480 ps	330	575 (ref. 6), 540†
ε_0 (Ethanol)	24·5	24·3	24·4 (ref. 5)
ε_0 (*n*-Propanol)	20·6	20·5	20·5 (ref. 6)
ε_0 (*n*-Butanol)	17·3	16·8	17·0 (ref. 6), 17·6 (ref. 7)
$\varepsilon\infty$ (Ethanol)	4·8	8·3	4·4 (ref. 5)
$\varepsilon\infty$ (*n*-Propanol)	3·0	7·2	3·3 (ref. 6)
$\varepsilon\infty$ (*n*-Butanol)	3·3	4·2	3·2 (ref. 7)

* Corrected to 25° C where necessary.

† Reference 6, recalculated from low frequency data.

A range of dielectric samples have been subjected to analysis by TDR in order to (*a*) assess the general applicability of TDR for the study of dielectric relaxation processes, and (*b*) make comparisons where possible between the results from TDR and those from the traditional frequency domain methods. We report here results for three aliphatic alcohols which have been studied fairly extensively by frequency domain methods and which were also examined by Fellner-Feldegg using TDR[1]. Fig. 1 shows permittivity data obtained by TDR for ethanol, *n*-propanol and *n*-butanol at 25° C plotted on the complex plane diagram, and clearly demonstrates the broad band nature of the technique. In Table 1 a comparison is made between the dielectric parameters obtained for the major relaxation process of the alcohols, and other values in the literature. A reasonably good agreement between the results obtained from TDR and those from frequency domain methods is thus indicated. Furthermore, it is apparent from an inspection of original experimental data that the TDR results are much more consistent with a semicircular Cole–Cole plot than the results from the different frequency domain techniques required to cover the same frequency range. The differences in Table 1 between the relaxation times derived from time domain and frequency domain methods should not therefore necessarily be ascribed to errors in the former.

A more detailed account of the experimental and analytical problems associated with time domain dielectric measurements will be discussed elsewhere, but it is pertinent in conclusion to assess briefly the major advantages and limitations of TDR at the present time. The large saving in experimental equipment (because of the broad frequency range covered) and the large saving in experimental time are, as pointed out previously[1], perhaps the most striking advantages of TDR over the traditional methods. At the same time one must set against this the lower degree of precision of TDR information, particularly for high permittivity samples, and the necessity for Fourier analysis of the data.

Taken together, these points indicate that TDR is an extremely acceptable method if information is required at other than a small number of frequencies in a limited band, provided that accurate measurements of small dielectric losses or great precision of measurement are not required. Even there, TDR offers an excellent guide to the frequencies at which the precise frequency domain measurements should be made.

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BIOLOGICAL SCIENCES

Trimodal Response of *Escherichia coli* and *Proteus mirabilis* to Penicillins

It has been generally believed that penicillins act on Gram-negative bacilli by interfering with cell wall synthesis leading to the release of spheroplasts which then succumb to osmotic damage. Such damage is usually fatal although in conditions of suitably high osmolarity the spheroplast phenomenon is known to be reversible[1]. Using turbidimetric methods supported by morphological studies in the scanning electron microscope, we have recently demonstrated that *Escherichia coli* and *Proteus mirabilis* show a variety of responses to penicillins which cannot be satisfactorily explained on the basis simply of the emergence and subsequent osmotic destruction of spheroplasts[2,3]. With the high bacterial population densities used, large numbers of "persisters" (organisms which survive exposure to bactericidal concentrations of penicillin although their progeny remain fully sensitive[4]) could be recovered after prolonged exposure to penicillins, and it seemed that this survival resulted from yet another response to the agents. In this investigation we have extended our studies and have attempted to elucidate the persister phenomenon.

A strain of *E. coli* and one of a typical swarming *P. mirabilis* previously studied[2] were used throughout. Continuous records of the opacity changes of growing cultures were obtained by means of a simple photometer[5]. Minimum inhibitory concentrations (m.i.c.) of the agents for the strains used were determined by a conventional doubling dilution tube titration method. Enough penicillin was added to the growing cultures in the mid-to-late-logarithmic phase to produce a concentration 15 times the m.i.c. To study the phase of recovery from exposure to penicillin, excess penicillinase (Neutrapen*, Riker) was added at various times. In a further set of experiments, the spheroplast fraction of the population surviving exposure to penicillin was inactivated by the addition of erythromycin (1/5 m.i.c. for intact cells[6,7]). An experiment was designed to test the penicillin sensitivity of the surviving population. The minimum amount of penicillinase which allowed growth on overnight incubation from penicillin-treated cultures was determined by preliminary titrations. In the definitive experiment, this amount of penicillinase was added to cultures which had been exposed to ampicillin overnight. When growth had again reached the point at which the original addition of ampicillin had been made, a second identical dose of antibiotic was added.

Minimum inhibitory concentrations of the agents used were: ampicillin 4 μg/ml.; erythromycin 50 μg/ml., for *E. coli*; and benzylpenicillin 4 μg/ml.; erythromycin 250 μg/ml., for *P. mirabilis*.

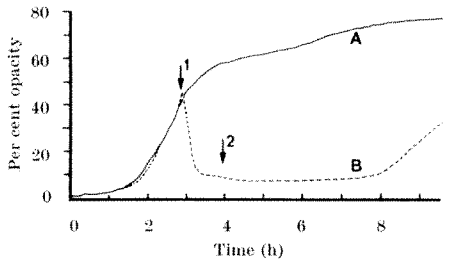


Fig. 1. *E. coli*: *A*, normal growth curve; *B*, 66 μg/ml. ampicillin added at 1; excess penicillinase added at 2.

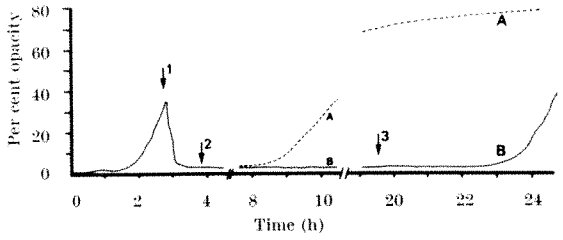


Fig. 2. *E. coli*: Two experiments with 66 μg/ml. ampicillin added at 1; *A*, excess penicillinase and 10 μg/ml. erythromycin added at 2; *B*, excess penicillinase added at 3.

The normal growth curve of the *E. coli* strain in our system is shown in Fig. 1*A*. On addition of 66 μg/ml. (15 m.i.c.) ampicillin there was a very short lag of 2–3 min before precipitate "lysis" occurred and continued over a 20 min period. During this time a hundred-fold drop in the viable count was demonstrated. Penicillinase added 1 h after the ampicillin brought about a recovery of growth which showed on the opacity trace after about 4 h. The machine will not record bacterial concentrations below about 10^6/ml. (Fig. 1*B*). This recovery was unaffected by adding erythromycin at the same time as penicillinase (Fig. 2*A*) and was observed even when the penicillinase was withheld for up to 21 h (Fig. 2*B* and 3). When an amount of penicillinase was added which just neutralized the ampicillin present, the subsequent culture showed a reaction to the addition of more ampicillin similar to that of the original one (Fig. 3), the dose of ampicillin

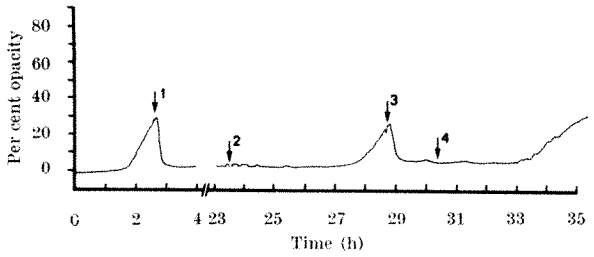


Fig. 3. *E. coli*: 66 μg/ml. ampicillin added at 1. One neutralizing dose penicillinase added at 2. A further 66 μg/ml. ampicillin added at 3. Excess penicillinase added at 4.

apparently being sufficient to induce lysis before neutralization by residual penicillinase.

The normal growth curve of *P. mirabilis* was similar to that of *E. coli*, but the response, in this case to benzylpenicillin, was different. There was only a transient lytic phase: most of the population underwent metamorphosis into spheroplasts, which accounts for the increase in opacity which followed the lytic phase (Fig. 4*A*). When penicillinase was added, fresh progressive increase in opacity was apparent after about 3 h (Fig. 4*A*). By contrast, if one-fifth m.i.c. erythromycin was added with the penicillinase, progressive increase in opacity did not take place for about 12 h (Fig. 4*B*).

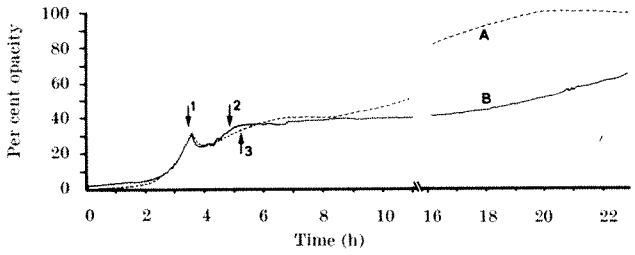


Fig. 4. *P. mirabilis*: 66 μg/ml. benzylpenicillin added at 1; *A*, excess penicillinase added at 3; *B*, excess penicillinase and 50 μg/ml. erythromycin added at 2.

Inhibition of spheroplasts by erythromycin was also directly demonstrated by microscopical examination of penicillin-treated cultures to which both penicillinase and erythromycin had been added. Unreverted spheroplasts were present and there was no sign of the bizarre morphological forms which abound during the early stage of spheroplast reversion.

Previous studies had already indicated that the traditional view of penicillin action on Gram-negative bacilli was inadequate[2,3]. Apart from transformation to spheroplasts, a proportion of the bacterial population of cultures of *E. coli* and *P. mirabilis* appeared to be rapidly "lysed", and in addition cells apparently unaffected by the antibiotic could be seen in the scanning electron microscope.

It has been shown by Taubeneck[6] that stable L-forms of *P. mirabilis* are more than 1,000 times more sensitive to erythromycin than the parent strain. In the present experiments exposure of *P. mirabilis* spheroplasts to erythromycin greatly delayed but did not abolish growth of the culture on addition of penicillinase. With *E. coli*, where spheroplast formation is typical of only a small minority of the bacterial population, recovery on addition of penicillinase was indistinguishable in the presence and absence of erythromycin. Furthermore, subsequent recovery, detectable on the opacity trace after about 4 h, was independent of the time the penicillinase was added. The surviving cells, which must retain sufficient external structure to make them insusceptible to erythromycin, are evidently inhibited by the ampicillin yet yield, on neutralization of the penicillin, progeny exhibiting the same mixed response as the parent strain. It is tempting to identify such cataleptic cells with the apparently morphologically normal survivors seen in the scanning electron microscope.

These findings are entirely analogous with those of Gunnison *et al.*[8], who concluded that staphylococcal persisters survived because at the time of first contact with penicillin they were in a state unfavourable to division. The simplest version of such a hypothesis is that penicillin exerts a bacteriostatic effect on some cells which resume growth on its removal.

So it seems that *E. coli* and *P. mirabilis* react to the presence of penicillins in three ways: rapid lysis, predominant in the case of *E. coli*; transformation to spheroplasts, predominant in the case of *P. mirabilis*; and bacteriostasis, manifest in a small proportion of both species.

Guze and Kalmanson[7] and others have demonstrated the importance of spheroplasts in the persistence of infection, where osmotic conditions are favourable. The dormancy of intact bacterial cells in the presence of high concentrations of antibiotics may offer an even more efficient survival mechanism, for it is totally independent of the osmotic ambience. Such cells might also prove to be important in being resistant to other factors, such as the bactericidal action of serum, to which spheroplasts are highly susceptible[9].

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Rapid Detection of Penicillin Sensitivity in *Staphylococcus aureus*

Conventional antibiotic sensitivity tests require many hours for accurate interpretation. We have now discovered a means of detecting the effects of the antibiotic penicillin on viable bacteria within minutes, by observing changes in the differential light scattered from bacterial suspensions. Sensitivity to penicillin in *Staphylococcus aureus* is detectable in as little as 3 min after the addition of the antibiotic.

The effect of penicillin and its derivatives on the integrity of the cell wall of sensitive bacterial strains is well known. Indeed, recent[1] scanning electron micrographs of ampicillin-treated sensitive strains have vividly shown the resulting deterioration. Differential light scattering patterns of bacterial suspensions are extremely sensitive to any changes in shape, size, structure or size distribution[2], and would therefore be expected to provide an excellent means for rapidly observing the effects of antibiotics on cells. Differential light scattering has been applied to measuring species differences in bacteria[3] and morphological characteristics of living cells[4]. The principle of the differential light scattering method depends on the unique scattering characteristics of a dilute suspension of cells of fairly uniform size.

The penicillin sensitive (pen^s) and penicillin resistant (pen^r) strains of *S. aureus* used in our studies were obtained from clinical material. They exhibited the typical haemolytic, coagulase, and mannitol fermentation characteristics of virulent strains. In addition, we confirmed by conventional means that the pen^s strain was sensitive to less than 10 units of penicillin per ml. of fluid medium, while the pen^r strain was apparently unaffected when grown in the presence of 40,000 units per ml. Light scattering measurements were made with a Science Spectrum 'Differential I' light scattering photometer equipped with a vertically polarized helium–neon laser (632·8 nm) as the light source. The relative intensity of scattered light was recorded as a function of angle with respect to the forward direction defined by the incident laser beam. For the light scattering measurements, cells were diluted in water to a concentration of about 5×10^5 cells per ml. Fig. 1 shows the differential light scattering curves obtained for the pen^s culture growing exponentially at 37° C in brain heart infusion (BHI) both (*a*) before and (*b*) after treatment with 400 units of penicillin for 10 min. The dramatic changes induced by penicillin treatment included the total disappearance of the two scattering peaks shown in (*a*) and a large increase in the scattering noise. We interpret the former change as resulting from a significant broadening of the cell size distribution[4]. In addition, the increased noise of the scattering curves such as shown in Fig. 1*b* is manifest when suspensions contain significant amounts of cellular debris. So a variety of subtle changes in morphology as well as changes in cell dispersion and fragility were detectable within minutes after antibiotic treatment.

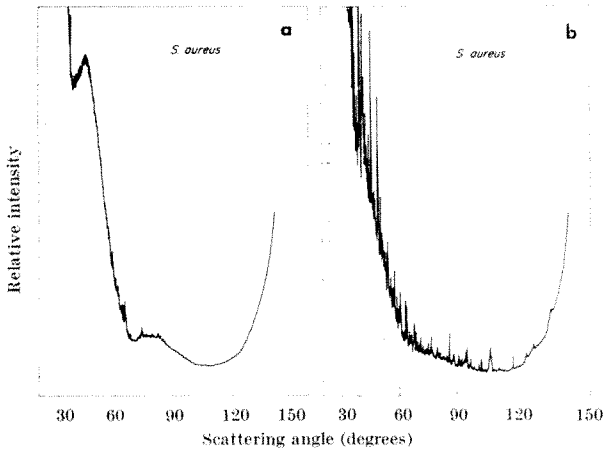


Fig. 1. Differential scattered intensity of 632·8 nm vertically polarized light from *S. aureus* (pen^s) during rapid growth in the presence of penicillin. After growth on BHI agar overnight, the culture was transferred to BHI broth and incubated for 20 min at 37° C. Light scattering measurements were made (*a*) at this time and (*b*) after another 10 min in the presence of 400 units of penicillin per ml.

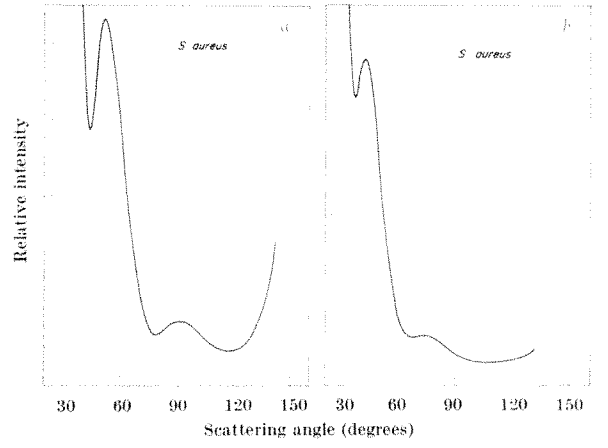


Fig. 2. Differential scattered intensity of 632·8 nm vertically polarized light from *S. aureus* (pen^s). Before the measurements, the cells were grown (*a*) in 2 per cent BHI at 37° C for 8 h, and (*b*) resuspended in fresh BHI broth for 20 min at 37° C.

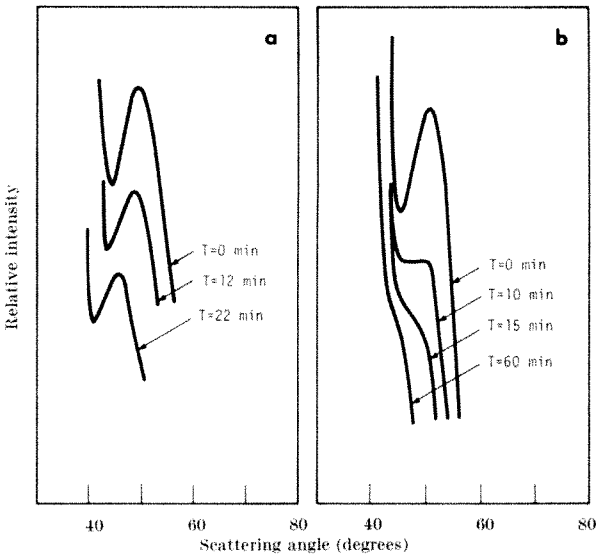


Fig. 3. Scattered intensity of 632·8 nm vertically polarized light from *S. aureus* (pen^s) in the region of the primary peak. Following a period of starvation, the culture was grown in fresh broth for 20 min at 37° C. At this time ($T = 0$), differential light scattering was measured on (*a*) a control lacking penicillin, and (*b*) a culture containing 400 units of penicillin per ml. The scattering intensities were measured at subsequent time periods as indicated.

To study in greater detail the changes in light scattering induced by penicillin, we performed experiments on pre-starved bacteria which are more uniform in size than growing cells. Strains of *S. aureus* grown overnight on BHI agar were transferred to a starvation medium which consisted merely of BHI diluted 1/50 with distilled water (2 per cent BHI). After incubation for several hours in 2 per cent BHI and in the absence of the antibiotic, the cells, which were growing slowly if at all, were transferred to normal BHI broth and incubated for 20 min at 37° C.

The differential light scattering characteristics of *S. aureus* after starvation and nutrient supplementation are shown in Fig. 2. Curve *a*, recorded at the end of the starvation period, is characterized by two well defined peaks located at angles typical of cells of approximately 500 nm average radius, and having a narrow size distribution[4]. Curve *b* shows the changes we observed in differential light scattering after the additional 20 min period in BHI broth undiluted. The marked shift in the light scattering peaks to lower angles and the decrease in the relative depths of the minima correspond, respectively, to an increase in average cell size and a broadening of the size distribution[4]. Such changes clearly indicate an acceleration in growth rate. During this period of accelerated growth the sample was divided and to one aliquot 400 units of penicillin were added ($T = 0$). The subsequent changes in differential light scattering in the region of the primary peaks are shown in Fig. 3. In the control culture (*a*), the changes which occurred during the next few minutes were small. The consisted of a decrease in the depth of the primary minimum and a displacement of the peak to lower scattering angles, effects caused by further increases both in cell size and in size distribution[4]. During the same growth period, however, light scattering characteristics of penicillin treated cells (*b*) were clearly altered after 10 min, and we observed a continued degradation of the peak for about 1 h. In Fig. 3, we have smoothed out the characteristic noise so that these changes can be seen more clearly. The interpretation of the results shown is similar to that of the earlier experiment, although for the slower growing populations the time scale obviously has been widened.

When a pen^r strain of *S. aureus* was used in identical experiments no difference could be observed between light scattering curves in the presence or absence of the antibiotic.

We specificially chose penicillin for this initial study because of its potent inhibition of cell wall synthesis, an effect which leads to gross morphological changes in growing populations. But the extreme sensitivity of differential light scattering to small physical changes suggests that it will be useful for other drug studies as well.

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Radiation Stimulated Incorporation of ^{3}H-Thymidine into Diplotene Oocytes of the Guinea-pig

UNSCHEDULED synthesis of DNA has been observed in the nuclei of HeLa cells at some stage of the cell cycle other than the S-phase when cells are exposed *in vitro* to ultraviolet light or X-ray irradiation[1]. It has since been confirmed for several other cell types[2–5]. Part of the synthesis defined in terms of the uptake of ^{3}H-thymidine —which was detected in HeLa cells as the result of large doses of ultraviolet irradiation—was non-semiconservative (repair replication), corresponding to the insertion of DNA precursors into extant parental DNA strands. Here I present evidence that X-irradiation of a 1 day old female guinea-pig with 5,000 rad is followed by a small but autoradiographically detectable incorporation of ^{3}H-thymidine into the DNA of diplotene oocytes. In the guinea-pig, the mouse and most other mammals the premeiotic DNA synthesis occurs during embryonic life[6]. After birth the oocytes have all entered prophase of meiosis during which no DNA synthesis is normally observed.

Three 1 day old female guinea-pigs received 5,000 rad of X-rays (6 meV, dose rate 385 rad/min). The animals were injected intraperitoneally immediately before irradiation with 50 μCi/g of ^{3}H-thymidine (5·0 Ci/mmole, Radiochemical Centre, Amersham) and with the same amount of precursor 15 min later in order to be sure that ^{3}H-thymidine was available during and shortly after the irradiation. Three animals served as controls and received ^{3}H-thymidine only. The animals were killed 1 h after the last injection. The histological sections of the ovaries from the experimental group and the control group were stained by the Feulgen technique, coated with Ilford 'K2' liquid emulsion, exposed for 8 or 12 weeks and developed together.

In the autoradiographs a few grains were observed over the oocyte nuclei from the irradiated as well as from the control animals. The number of grains over each oocyte nucleus was counted and the number of grains over an area of exactly the same size just beside the nucleus (representing the background labelling) was subtracted.

The grain counting over areas of equal size was performed by placing a glass slide with an engraved circle the size of an average oocyte nucleus in the ocular of the microscope. The specimens were coded before counting so that the observer was unaware of whether the specimens originated from irradiated or control animals.

Table 1. MEAN GRAIN COUNTS OVER OOCYTE NUCLEI CORRECTED FOR BACKGROUND LABELLING

	Experiment 1		Experiment 2	
	5,000 rad	Control	5,000 rad	Controls
Number of animals	1	1	2	2
Number of oocytes	241	203	197	185
Mean grain count (oocyte/background)	2·92 ± 0·20 (s.e.)	−0·21 ± 0·14 (s.e.)	1·87 ± 0·16 (s.e.)	0·09 ± 0·05 (s.e.)

In both experiments the mean grain count over the oocyte nucleus in irradiated animals is significantly higher than the background count (Table 1). In the control animals there is no significant difference. These results indicate that there is a small but significant incorporation of ^{3}H-thymidine into DNA of diplotene guinea-pig oocytes after irradiation with 5,000 rad of X-rays. By comparing the number of grains over the oocyte nuclei with the number of grains over the follicle cells that were in normal S-phase at the time of ^{3}H-thymidine injection, the radiation stimulated incorporation of ^{3}H-thymidine was roughly estimated as being less than 10 per cent of the amount incorporated during normal replication of DNA. This result is of the same order of magnitude as was observed in the radiation stimulated incorporation of DNA precursor in human blood lymphocytes *in vitro*[4].

Whether some of the ^{3}H-thymidine incorporated into guinea-pig oocytes after irradiation is the result of repair replication is not yet clear. Painter and Cleaver[5], however, have examined several kinds of cultured mammalian cells after exposure to ultraviolet light. They found that all kinds of cells in which unscheduled DNA synthesis was observed also exhibited repair replication, and there seemed to be a rough correlation of the extent of the two phenomena suggesting that they both are manifestations of the same process. The guinea-pig oocytes seem to be much more resistant to irradiation than oocytes of mice and rats[7]. The question therefore arises whether the unscheduled DNA synthesis observed after irradiation can be correlated with the higher resistance to irradiation of guinea-pig oocytes compared with oocytes of rats and mice. In a few experiments the incorporation of ^{3}H-thymidine into mouse oocytes was investigated after irradiation with 200 rad of X-rays, which kill almost all oocytes in less than 24 h. There was no autoradiographically detectable incorporation of ^{3}H-thymidine into mouse oocytes, which is in accordance with the suggestion of a correlation between the extent of unscheduled DNA synthesis and repair synthesis, and the survival of the oocytes after irradiation.

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Sarcoma Blockade *in vivo*: Rabies–Rous System in Chickens

A RECENT review[1] describes the systems involved in inhibition of viral oncogenesis by non-oncogenic viruses and emphasizes the possible use of attenuated viruses as anti-neoplastic agents. This communication describes the total *in vivo* blockade of the tumour produced by

Rous sarcoma virus (RSV), an avian subgroup A leucovirus[2,3], by a vaccine strain of rabies virus (RV), a rhabdovirus[2].

The Paris strain of live, fixed RV (Semple vaccine), prepared in rabbit brain[4] and giving a titre of $10^{6.5}$ to $10^{7.2}$ adult mouse LD_{50}/0·03 ml. (Reed–Muench), was used. Controls were a 10 per cent homogenate of normal rabbit brain (NRB) in 0·05 M sodium citrate buffer (*p*H 6·7) and ultraviolet-irradiated rabies virus (UVRV), the non-infectiousness of which was confirmed in adult white mice intracerebrally. RSV was the Bryan Master standard (strain 559). This material was injected into the wing webs of White Leghorn chickens (3–5 days old) in 0·05 ml. amounts. On days 7–10, 10 per cent tumour homogenates were prepared in citrate buffer and titrated on the chorioallantoic membranes (CAM) of 11 day chick embryos, the titres ranging between $10^{6.9}$ and $10^{7.0}$ pock forming units p.f.u./0·1 ml. Chickens were highly susceptible to the RSV, only 2·0 per cent being resistant. Daily tumour progress was recorded for at least 21 days following RSV inoculation. The criteria of total sarcoma blockade were (*a*) absence of tumour for 21 days, (*b*) absence of plasma anti-RSV neutralizing antibody.

Fig. 1 (pooled data from four experiments, each in duplicate) presents the time–effect relationship of the blockade. The maximal effect seen (44·4 per cent) was with RV at 48 h. Mean latent periods (arithmetic), although not depicted in Fig. 1, were significantly longer in RV and UVRV groups at 0, 24 and 48 h. No blockade was seen at −24 h, indicating absence of oncolysis. The possibility of its later occurrence, however, cannot be ruled out: as pointed out by Takehara *et al.*[5], it is difficult to distinguish between oncolysis and interference in such systems. Stewart and Sulkin[6] were unable to demonstrate interferon in hamsters for 24 h after rabies virus inoculation: the appearance of blockade at zero time in our system is therefore unusual. Further, the reduction in the blockade at 24 h as compared with that at 0 h leads one to wonder whether there is more than one interference mechanism.

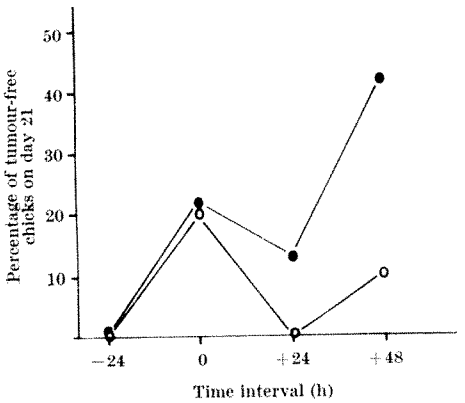


Fig. 1. Time–effect relationship. 6–8 day chicks inoculated with RV (●) or UVRV (○) or NRB* (0·05 ml. amounts per web bilaterally) and challenged with 0·05 ml. RSV at varying time intervals: RSV 24 h before RV, UVRV or NRB (each group containing 18, 8 and 5 chicks, respectively); RSV with RV or UVRV or NRB as single inocula (25, 10, 5); RSV 24 and 48 h after RV, UVRV or NRB (18, 8, 5). The RSV concentration was $10^{3.9}$ chick CAM p.f.u./0·1 ml. and RV concentration $10^{4.8}$ adult mouse LD_{50}/0·1 ml. NRB was used at the 10^{-2} dilution.

* Because all NRB controls became tumour-positive by day 7, they are omitted from the graph.

The next set of experiments investigated the RSV dose–response relationship of the blockade. Fig. 2 summarizes this relationship when the birds were treated with RV, UVRV or NRB 48 h before RSV inoculation. A graded increase in the percentage of tumour-free chicks was clearly demonstrable with increasing RSV dilution. RV and UVRV pretreatment shifted the dose–response curves in the direction of higher RSV concentration.

That the blockade was transient in nature became apparent when 33 tumour-blocked chicks, rechallenged with RSV, became tumour-positive by day 7. Here the time-interval range between the initial RSV inoculum and the rechallenge was 30–49 days. The transient nature of the blockade suggested that interferon-like substances might be the mediators. Preliminary experiments at 48 h—RV or UVRV inoculation followed by contralateral RSV challenge—indicate that the effect can be produced at a distant site. Further, the blockade seems to be reversible by hydrocortisone[7] and by actinomycin D[8]. So although at 48 h this evidence supports the hypothesis that the effect is mediated by interferon-like substances, the possibility that more than one mechanism is involved cannot be excluded for the reasons stated.

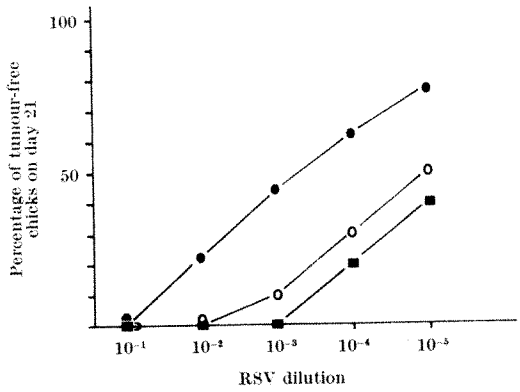


Fig. 2. Dose–response relationship. 3–7 day old chicks treated with RV (●) or UVRV (○) or NRB (■) in 0·05 ml. amounts/web in groups of 18, 10 and 5, respectively, challenged with serial ten-fold dilutions of RSV 48 h later. The 10^{-1} dilution of RSV corresponded to $10^{6.9}$ chick CAM p.f.u./0·1 ml. The concentration of RV used throughout was $10^{4.8}$ adult mouse LD_{50}/0·1 ml./chick. The NRB used was identical to that already described.

Absence of anti-RSV neutralizing antibody in tumour-blocked chicks suggests that Rous associated viruses RAV-4 and RAV-5[2] have also been totally suppressed. This seems to be unusual in a single *in vivo* system[9]. If this system is interferon mediated, and in the light of Wheelock and Sibley's work with the 17-D strain of yellow fever virus[10], it seems possible that interferonogenic, attenuated, avianized strains of rabies could be used to confer longer protection against RSV and RAV-4,5.

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Gerbich Blood Group System: a Useful Genetic Marker in Certain Melanesians of Papua and New Guinea

THE red cell antigen Gerbich (Ge^a), thought to be among the most widely distributed of blood group antigens, was first noted in 1960 when three women who lacked the antigen made anti-Ge^a in response to pregnancy[1]. Subsequently a further fourteen Ge(a−) subjects were discovered through the presence of anti-Ge^a in their serum, the antibody selecting them out of perhaps millions. The seventeen propositi were from ten different countries and were of European, Mexican, South American Indian and Negro extraction. Some of the sibs of these propositi were also Ge(a−), but outside their families no Ge(a−) subject was found in testing nearly 40,000 random people, chiefly Europeans but including more than 1,000 Negroes and more than 100 Asiatics[1,2]. The antigen was shown to be a dominant character and its locus to be genetically independent of those responsible for most of the established blood group systems[2].

A native of New Guinea, Mrs Imp., previously transfused after a post-partum haemorrhage, needed a transfusion after the birth of her second child and was found to have a strong immune IgG type antibody, which reacted with the cells of all of 700 samples from white people in Port Moresby and Brisbane: it did not react with the cells of thirteen of 371 random natives from widely spread places in Papua and New Guinea. In London, Mrs Imp. was, surprisingly, found to be Ge(a−) and her antibody to be anti-Ge^a. (Mrs Imp. does not belong to the very rare subtype[2] of Ge(a−) known as "Yus".)

Gene frequencies calculated from the 371 random Melanesian people would be misleading, for it is noteworthy that eleven of the thirteen Ge(a−) people live on the north-east coast line between Lae and Samarai. These eleven Ge(a−) coastal natives belong to at least six well recognized ethnic groups, from whom only fifty-two samples have so far been tested. Tentative gene frequencies along this strip of coast are therefore Ge^a 0·54 and *Ge* 0·46, a startling divergence from the Ge^a 1·00 and *Ge* 0·00 of the rest of the tested world.

Mrs Imp.'s husband, mother-in-law and child are also Ge(a−): she and her husband's family come from a village about 100 miles from Lae up the Markham Valley, and have no known coastal affinities. The Markham Valley is sparsely populated, however, and movement along it from the coast would have presented no problems.

We hope that this brief report may encourage those involved in surveying blood groups of remote people to include tests for antigens, such as Ge^a, known in a different part of the world to be possessed by virtually everyone and therefore thought incapable of providing any useful genetical information. Extensive testing of Melanesians is now planned, but the relatively small investigation recorded here was sufficient to bring to light a most dramatic difference in the frequencies of the Gerbich blood group genes.

We thank Dr N. Muirden and Mrs C. Brady of the Department of Public Health, Mount Hagen Hospital, New Guinea, for referring the original sample from the patient.

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Lymphocyte Stimulation by Bovine β-Lactoglobulin

In vitro stimulation of lymphocytes, by either specific or non-specific mitogens, is accompanied by increased DNA synthesis[1]. During a study of milk sensitivity in ten patients with gastrointestinal disease (including seven with coeliac disease) it was observed that β-lactoglobulin had a mitogenic activity of the same order as phytohaemagglutinin (PHA) both in patients and in five healthy controls.

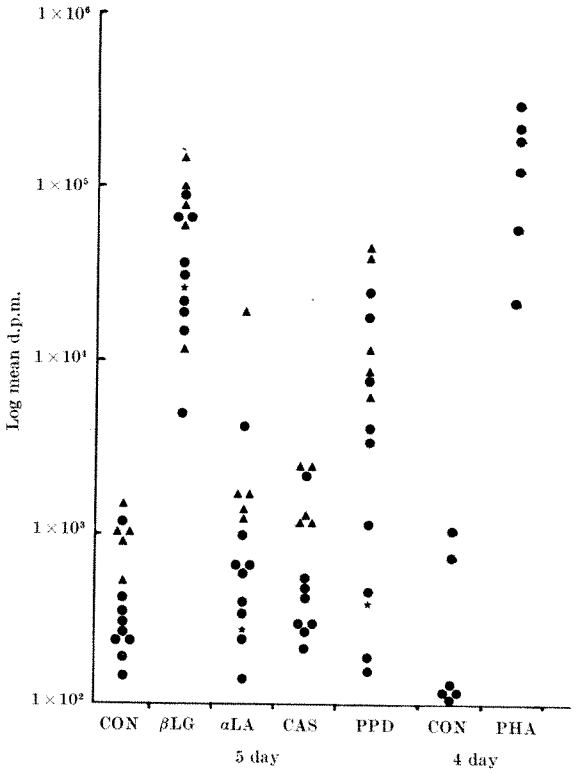


Fig. 1. DNA synthesis expressed as log mean disintegrations per min (log mean d.p.m.) for healthy controls (▲) and patients (●) with gastrointestinal disease. Control cultures (CON) containing no stimulant are compared with cultures containing 2 mg KL β-lactoglobulin (βLG), 2 mg KL α-lactalbumin (αLA), 2 mg BDH casein (CAS), 10 μg PPD or 0·033 ml. phytohaemagglutinin (PHA). The cultures of 1 patient ★ were all harvested on the fourth day.

Bovine β-lactoglobulin AB[2] and α-lactalbumin were purchased from Koch–Light (KL) Laboratories and β-lactoglobulin AB and casein hydrolysate were purchased from BDH. Bovine β-lactoglobulin AB, A and B, α-lactalbumin, casein and whole-whey protein were provided by Dr R. G. J. Lyster of the National Institute for Research in Dairying, Shinfield, Reading. Bovine β-lactoglobulin AB was prepared by Dr G. Pardoe of this department using pooled unpasteurized cow's milk within 24 h of collection. The milk was defatted by centrifugation at 4° C. PHA was purchased from Burroughs Wellcome. The contents of each ampoule were dissolved in 5 ml. of phosphate-buffered saline; 0·033 ml. was added per culture. Tuberculin purified protein derivative (PPD) was provided by the Ministry of Agriculture, Fisheries and

Food, Weybridge; 10 μg was added per culture. Staphylococcal filtrate was kindly prepared by Dr N. R. Ling in this department; 0·1 ml. was added per culture.

Peripheral blood lymphocytes were obtained from defibrinated blood by sedimentation with gelatin. The cells were spun down and re-suspended in Eagle's medium, supplemented with 15 per cent normal human serum, to give a final concentration of $1{\cdot}5 \times 10^6$ cells/ml. The serum used throughout was from two healthy individuals. Triplicate 2 ml. cultures (3×10^6 cells) were set up in 5 ml. snap topped phials containing either no stimulant, PHA, PPD, 0·1 ml. staphylococcal filtrate or 2 mg of the appropriate milk protein (the milk protein was dissolved in phosphate-buffered saline and sterilized by filtration). The cultures were incubated for 4 days in the case of PHA and 5 days for the other substances unless otherwise stated. Sixteen hours before the end of the culture period 1 μCi of ^{3}H-thymidine (150 mCi/mmole, Radiochemical Centre, Amersham) was added. When subsequently harvested two of each set of three were processed for DNA synthesis as described by Ling and Holt[3]. The radioactivity was counted in a Packard Tri-Carb scintillation counter, corrected to 100 per cent efficiency by the channel ratio method and DNA synthesis expressed as mean disintegrations per min (d.p.m.) for the two cultures. A Jenner–Giemsa stained smear was made from the third culture of each set.

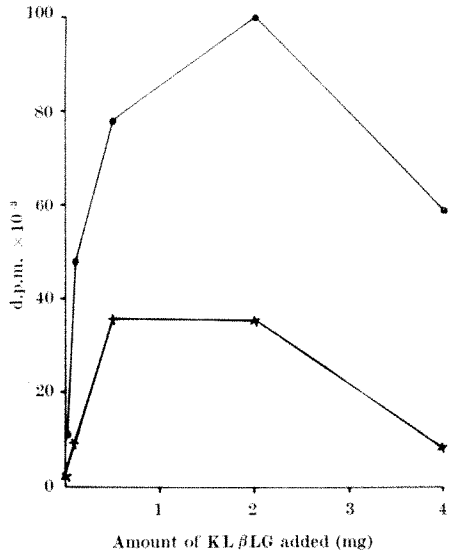


Fig. 2. DNA synthesis (d.p.m. $\times 10^{-3}$) in 5 day cultures of lymphocytes from two healthy individuals A and B produced by adding either 10 μg, 100 μg, 500 μg, 2 mg or 4 mg of KL β-lactoglobulin (KL βLG) to each culture. ×, Control A; ●, control B.

DNA synthesis in control cultures and test cultures for patients and healthy controls is shown in Fig. 1. In all cases there was marked stimulation of peripheral blood lymphocytes by 2 mg of KL β-lactoglobulin often of a similar order to that produced by PHA. By contrast, 2 mg of KL α-lactalbumin produced only moderate stimulation in one healthy individual and one patient, and either no, or minimal, stimulation in the rest, and this latter observation was also true for casein. Five healthy individuals and six patients responded to PPD to variable degrees; they were all Mantoux positive. Lymph node lymphocytes from three of the patients, obtained during diagnostic biopsy, were also tested with the same substances; the results were similar to those for peripheral blood lymphocytes.

DNA synthesis produced by different quantities of KL β-lactoglobulin AB in 5 day cultures of lymphocytes from two healthy controls A and B is shown in Fig. 2. A significant response was produced by the smallest dose used—10 μg per culture—and a maximum response by approximately 1–2 mg, beyond which increasing amounts of KL β-lactoglobulin AB seemed to be toxic.

The effect of harvesting the cultures on different days, from day 3 to day 9 inclusive, for staphylococcal filtrate, PPD and KL β-lactoglobulin AB is shown in Fig. 3. Staphylococcal filtrate produced a peak response between days 5–6, KL β-lactoglobulin AB at day 7 and PPD between days 6–8. A previous experiment with PHA showed a maximum response occurring between days 3–4.

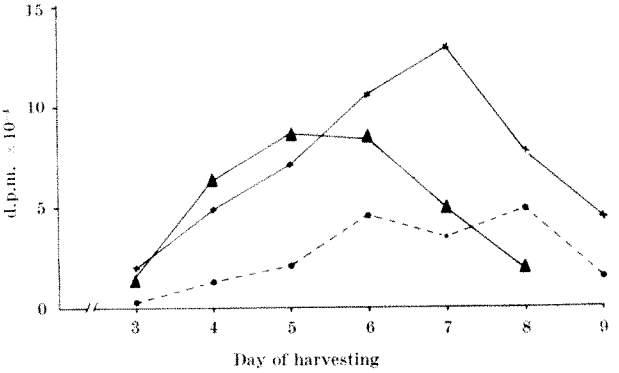


Fig. 3. The effect of harvesting cultures of lymphocytes from one healthy individual on different days. The lymphocytes were cultured in the presence of either 2 mg of KL β-lactoglobulin AB (×—×), 0·1 ml. of staphylococcal filtrate (▲—▲) or 10 μg of PPD (● - - - ●). DNA synthesis (d.p.m. $\times 10^{-3}$) on different days is shown for each substance.

In other experiments peripheral blood lymphocytes from the same five healthy controls were cultured in the presence of equal quantities of milk protein from various sources, and the results compared. The BDH β-lactoglobulin AB preparation produced very marked stimulation (mean degree of stimulation, 155× = ratio of mean radioactivities of the two test cultures to the mean radioactivities of the two control cultures). Shinfield β-lactoglobulin A, β-lactoglobulin B and β-lactoglobulin AB and our own preparation of β-lactoglobulin AB, however, produced either minimal or no stimulation (mean 9·4×, 1·9×, 1·3× and 1·0× respectively). Shinfield α-lactalbumin produced some stimulation (7× and 2×) in two individuals who also reacted to KL α-lactalbumin (66× and 8× respectively). Whole whey protein and defatted milk (sterilized by filtration), the latter in amounts varying from 0·033 ml. to 0·33 ml. (equivalent to approximately 100 μg and 1,000 μg of β-lactoglobulin), produced no stimulation. After dialysis for 24 h KL β-lactoglobulin AB retained its stimulatory capacity, while heating (100° for 10 min) reduced the degree of stimulation from 45× to 3×.

Intradermal skin tests using 100 μg of KL β-lactoglobulin AB produced a Mantoux-like reaction, maximal at 24 h in the 5 healthy controls with an area of induration ranging in size from 1·0 to 1·5 cm in diameter. There was no reaction to 100 μg of Shinfield β-lactoglobulin AB.

Cellulose acetate and polyacrylamide gel electrophoresis of the active and inactive preparations of β-lactoglobulin AB showed similar appearances and analytical ultracentrifugation produced a single peak.

Whatever the nature of the active substance it has a potency and range comparable with the strongest plant mitogens, such as PHA. The presence of such powerful mitogenic activity in two samples of demonstrably highly purified β-lactoglobulin (ascertained to be from different primary sources) and its absence from other purified or crude preparations suggest at least four possible explanations. (*a*) It may be a genuine property of β-lactoglobulin molecule lost (or gained in the case of active materials) in some processes of purification, or neutralized by inhibi-

tor substances in the inactive preparations; (*b*) it may be a mitogenic non-β-lactoglobulin bovine product in trace (< 1 per cent) amounts, which may or may not happen to be present in milk or milk preparations; (*c*) it may be a dietary constituent, such as a phytomitogen, which enters the milk only when the cows are fed on certain sorts of pasture or prepared feeds, or at certain seasons of the year; (*d*) it may be a contaminating bacterial mitogen (such as that derived from staphylococci) though it is unlikely to be a purely chance contaminant because two independent preparations contained it in much the same amount. If it is any sort of non-β-lactoglobulin contaminant (as in (*b*), (*c*) or (*d*)), it must fractionate preferentially with β-lactoglobulin and be active as a mitogen down to a concentration of approximately 0·1 μg, for KL β-lactoglobulin is at least 99 per cent pure, and 10 μg of this preparation shows appreciable activity; it is also apparently non-dialysable and thermolabile.

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Prevention of Hypercholesterolaemia in Thyroidectomized Rats by Growth Hormone

The importance of the thyroid gland in the metabolism of cholesterol is illustrated by our finding[1-3] that the rate of conversion of cholesterol into bile acids is decreased in the absence of circulating thyroid hormone and its rate of synthesis in the liver is diminished. Conversely, an excess of thyroid hormone leads to increased conversion of cholesterol into cholate, enhanced hepatic synthesis, and less cholesterol in the plasma.

Recent observations indicate that other endocrine glands are concerned in cholesterol metabolism. We have demonstrated[4] that pituitary growth hormone is important in normal cholesterol metabolism in the rat. Because the secretion of growth hormone soon ceases in the absence of circulating thyroid hormone[5,6], we wondered to what extent the hypercholesterolaemia apparently arising from a deficit of thyroid hormone was in fact a result of a secondary deficit of growth hormone. We now present results suggesting that the hypercholesterolaemia resulting after thyroidectomy is largely the result of a lack of circulating growth hormone.

Thyroid extract and bovine growth hormone were administered in various combinations to groups of both hypophysectomized and thyroidectomized male rats (Long Evans strain). Untreated hypophysectomized, thyroidectomized and normal rats served as controls. Hypophysectomized rats and their controls were each given daily 55 ml. of an egg white, milk and oil diet containing 40 mg of cholesterol/100 ml. The thyroidectomized rats and their controls were given the same volume of a previously described[7] high cholesterol diet (273 mg/100 ml.). All rats completely consumed their daily allotment of food.

Blood samples were obtained weekly and analysed for their serum cholesterol content according to the method of Martinek[8]. In addition, all blood samples obtained from the hypophysectomized animals one week after surgery were analysed in a commercial laboratory (Bay Area Pathologists' Central Laboratory, San Francisco) for their serum thyroxine content according to the method of Pileggi and Kessler[9].

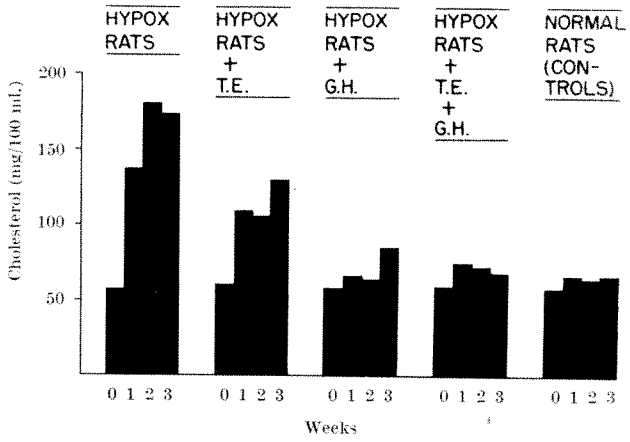


Fig. 1. Average serum cholesterol of untreated hypophysectomized (Hypox) rats (15), hypophysectomized rats (15) injected subcutaneously with 2 mg of thyroid extract daily, hypophysectomized rats (10) injected intraperitoneally with 300 μg of bovine growth hormone three times a day, hypophysectomized rats (8) injected with both thyroid extract and growth hormone, and normal control rats (10), following hypophysectomy in the first four groups.

As Fig. 1 illustrates, the average serum cholesterol of the untreated hypophysectomized rats rose significantly ($P < 0.001$) 1 and 2 weeks respectively after operation. Administration of thyroid extract to the group of hypophysectomized rats, while it did not prevent the hypercholesterolaemia entirely (see Fig. 1), did lessen it by comparison with that observed in the untreated hypophysectomized rats. The average serum thyroxine of these thyroid extract treated hypophysectomized rats 1 week after surgery was 1·9 μg/100 ml., a value not significantly different from that (2·1 μg/100 ml.) found in the normal control rats.

Administration of growth hormone to hypophysectomized rats essentially prevented the appearance of any hypercholesterolaemia during the 3 weeks of its administration, nor did injection of thyroid extract in addition to growth hormone increase the cholesterol-lowering efficacy of the growth hormone. The average serum thyroxine in these rats 1 week after surgery was normal (2·1 μg/100 ml.).

Untreated thyroidectomized rats exhibited a moderate rise in their serum cholesterol 1 week after surgery, and a much more marked rise at the end of the second and third weeks (see Fig. 2). We believe the greater rise in the serum cholesterol of these thyroidectomized rats (compared with hypophysectomized rats) was a consequence of their cholesterol-rich diet. Administration of thyroid extract to thyroidectomized rats prevented the otherwise expected hypercholesterolaemia; but when it was discontinued at the end of the third week, a marked hypercholesterolaemia ensued (see Fig. 2). Administration of growth hormone alone to thyroidectomized rats was essentially as effective as thyroid extract in preventing the occurrence of hypercholesterolaemia. Again, as with the thyroidectomized rats given thyroid extract, stopping growth hormone administration led in 1 week to a marked rise in the serum cholesterol of these previously growth hormone-treated rats (see Fig. 2).

In a previous study[4] we observed that the hypercholesterolaemia of the hypophysectomized rat could be minimized by administration of 300 μg of growth hormone per day. We now find that the hypercholesterolaemia of the hypophysectomized rat can be prevented completely by a larger dose of growth hormone (900 μg/day).

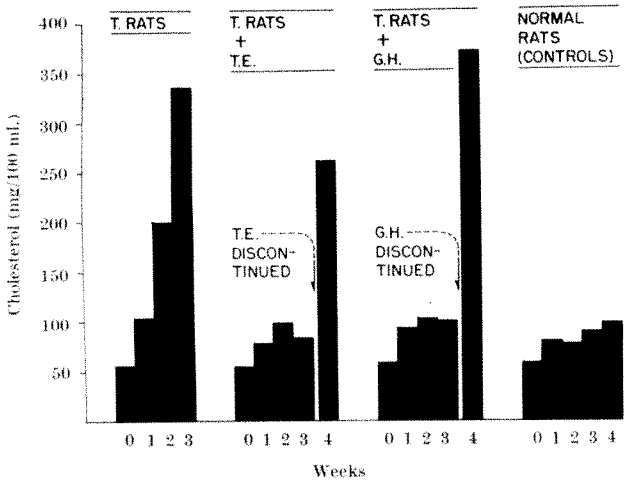


Fig. 2. Average serum cholesterol of untreated thyroidectomized (T) rats (10) thyroidectomized rats (10) injected with 2 mg of thyroid extract daily, thyroidectomized rats (15) injected with 300 μg of bovine growth hormone 3 times a day, and normal control rats (10), following thyroidectomy in the first three groups. Three weeks after thyroidectomy, the administration of both thyroid extract and growth hormone was discontinued in the two groups receiving these hormones.

These results also indicate that even in the thyroidectomized rat, growth hormone is as efficacious as thyroid hormone in preventing hypercholesterolaemia. When this fact is considered together with the observation[5,6] that secretion of growth hormone markedly diminishes after thyroidectomy, it seems possible that the chief cause of the hypercholesterolaemia following thyroidectomy may be the latter's effect on the secretion of growth hormone. Thyroxine, however, undoubtedly plays some part in the control of serum cholesterol level because its administration to the hypophysectomized rat, which is not secreting growth hormone, does mitigate[4] the animal's hypercholesterolaemia.

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Quantitative Tissue of Human Malnutrition using Scalp Hair Roots

THERE is at present no adequate way of detecting or quantifying slight degrees of protein or protein/calorie deficiency. Yet such deficiencies may affect health: malnutrition is known to increase susceptibility to infection and interferes with normal development in children. We therefore set out to find a measure of protein deficiency which would be simple to use on humans in the field and more sensitive than measurement of hair shaft thickness[1,2] or root diameter[3] or serum protein concentrations[4], which are subject to such influences as haemodilution. At best serum proteins provide an indirect measure of tissue protein metabolism. A more direct assay can be obtained from scalp hair roots which are easily accessible and are sensitive to systemic influences including drug toxicity[5,6], poisoning[7] and malnutrition[8]. Bradfield[3,8,9] has demonstrated the potential value of morphological examination of hair roots in the evaluation of protein or protein/calorie deficiencies, but experienced observers are needed for this technique, which is subjective and only semi-quantitative. These studies were performed to evaluate a method which can provide accurate quantitative objective data which reflect both hair root size and partial tissue loss in a single numerical value.

Hair roots were epilated manually by means of 'Tygon'-sheathed surgical needle-holding forceps from the parieto-occipital area. The hairs were placed immediately in physiological saline and examined with a dissecting microscope. Resting (telogen) hairs were removed. (In separate experiments resting hairs were shown not to contribute protein or DNA to the measurements used.) Total 1 M NH_4OH soluble protein/hair root was determined with groups of 10–40 hairs by the Lowry method[10]. Total DNA extracted in the NH_4OH was measured by modification of the method of Kissane and Robins[11]. (Neither DNA nor protein is extracted in significant amounts from fully formed hair shafts, so the length of shaft left attached to the root does not affect the results.) For special comparisons, individual hair root volume was measured by optical estimation of linear displacement of water in a calibrated micropipette capillary tube under a dissecting microscope, and volume/hair root values were converted from the known dimensions of the capillary tube. As with protein or DNA measurements inclusion of variable amounts of shaft does not significantly change the results. Serum albumin was determined by densitometric scanning of paper electrophoretic separations and calculation of percentage of total protein. Serum transferrin was estimated by 'Partigen' quantitative immunodiffusion plates[12].

Control protein/hair root values averaged $18{\cdot}02 \pm 5{\cdot}25$ μg (mean $\pm \sigma$) in 191 observations made on fifty-three individuals. The values were normally distributed and all fell within two standard deviations of the mean. There was no difference with age or sex between 18 and 90 years; racial differences have not yet been ruled out. Protein/hair root in several chronic mental patients, selected for their clinically evident malnutrition and histories of inadequate protein–calorie intake, ranged from 1·5 to 7·5 μg, all clearly below the control range. Similarly, hair shipped from Africa (courtesy of Dr Stewart Weegan, Communicable Disease Centre, Atlanta, Georgia) from both children and adults with severe kwashiorkor or marasmus showed very low values; hairs from well fed individuals shipped simultaneously had normal amounts of protein.

Concomitant measurement of serum albumin, transferrin and protein/hair root in a large series of hospital and clinic patients with known or suspected protein/calorie malnutrition proved that hair root protein was often more sensitive than either serum protein. Many patients showed low hair root protein with normal serum albumin or transferrin. Feeding several patients resulted in increases in hair root protein concomitantly with gain

of weight (Fig. 1). Moreover, in several instances this increase in both protein/hair root and body weight occurred with no discernible change in serum albumin and transferrin, which were already normal.

Comparison of the hair root protein with the DNA content and measurement of volume of the same hairs over the range of protein values (Fig. 2) demonstrated that all these values are directly and quantitatively related. The response of the hair root to protein/calorie malnutrition in addition to decrease of soluble protein content is therefore one of reduction in size and cell number, a phenomenon which has been noted experimentally in other tissues such as intestinal mucosa[13].

Similar suggestions were made by Short *et al.*[14] who painstakingly examined hair (wool) follicles from a single sheep during an extended period of low food intake compared with a period of high food (protein supplemented) intake. The hair roots showed a decrease in mitotic rate, follicle size and number of cells during the low intake period.

Hair root protein thus reflects hair root volume, and so can be used as a measure of the size reduction and of the loss of specific tissue elements found in malnutrition[8,9]. Morphological evaluation is therefore unnecessary except for separation of growing (anagen) from resting (telogen) hairs, a procedure which may be useful in any case for distinguishing between protein deficiency, which is said to reduce diameter of hairs, and calorie deficiency, which appears to increase the proportion of resting hairs[9]. Histological evidence of atrophied "hair papillae" in pigs on a high calorie–low protein diet[15] agrees with this interpretation.

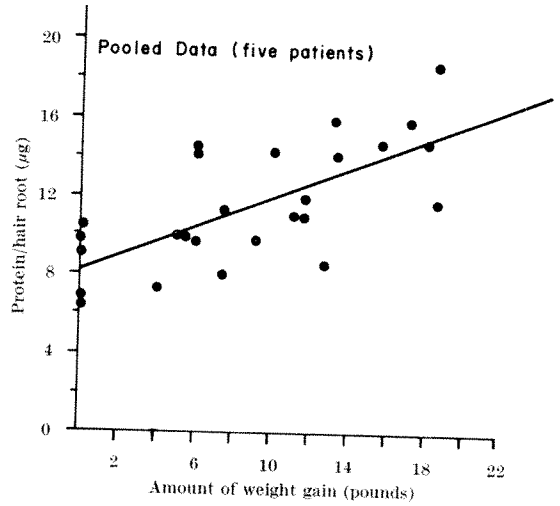


Fig. 1. Hair root protein increases significantly with refeeding of five malnourished individuals. Three of the patients had initial hair values in the "low normal" range and normal serum proteins. $n=26$; $r=0{\cdot}7040$; $0{\cdot}001<P<0{\cdot}01$.

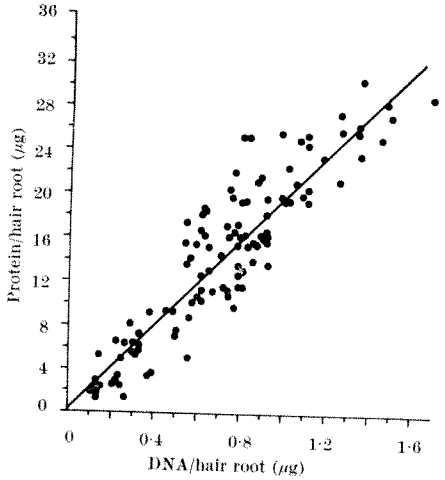


Fig. 2. The amount of DNA/hair root in plucked hairs correlates directly and highly significantly with the protein content. $n=112$; $r=0{\cdot}9176$; $P\ll0{\cdot}001$.

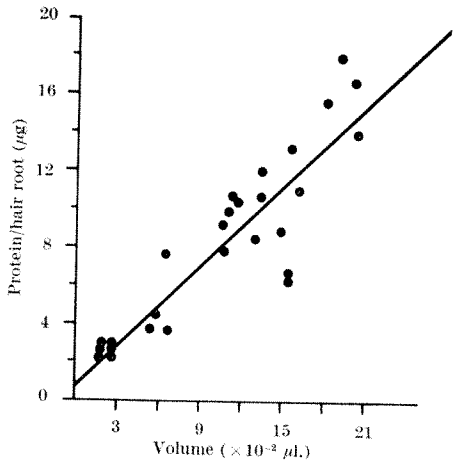


Fig. 3. Individual hair root volume and protein content are directly proportional. Because total DNA is similarly related to protein (Fig. 2) the plucked hairs from malnourished individuals are both smaller and contain fewer cells. So any of the three techniques quantify size diminution and/or completeness of hair root tissue[7,8]. $n=27$; $r=0{\cdot}9033$; $P\ll0{\cdot}001$.

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Effects of Glutaraldehyde on the Protein Loss and Photochemical Properties of Kale Chloroplasts: Preliminary Studies on Food Conversion

Ruminant animals are adapted to survival in adverse nutritional conditions. The alimentary tract has an expanded portion, the rumen or fore-stomach, where extensive microbial fermentation makes available the energy of bulky foods and fibrous materials before the food passes into the rest of the digestive tract. Extensive degradation of dietary protein, however, occurs in the rumen to give large amounts of ammonia and fatty acids.

Some of the ammonia is synthesized into microbial protein. Microorganisms are eventually digested after passing from the rumen. Large amounts of ammonia, however, pass through the rumen epithelium into the blood system and are eliminated chiefly in the urine after conversion to urea in the liver. From this point of view the ruminant is inefficient with regard to protein conversion, especially when high grade protein diets are given.

Chloroplasts are a very important source of dietary protein for herbivores. When the outer membrane of the chloroplast is ruptured the soluble stroma proteins are released, although the lamellar system is retained together with the capacity for photochemical electron transport and photophosphorylation. The degradation of soluble protein which occurs in the rumen results in a lowered nitrogen and energy retention[1,2]. Reis and Schinckel[3] and Reis[4] showed that abomasal administration of casein increased wool growth, and other work has shown[5,6] that casein treated with formaldehyde was not degraded in the rumen and increased wool growth and nitrogen retention. Zelter *et al.*[7] have prevented deamination by rumen fluid *in vitro* by treatment with tannins or aldehydes. Mangan and Pryor[8] showed that intact chloroplasts were ingested by entodiniomorphid protozoa in the rumen and degraded without producing large quantities of ammonia. During mastication of herbage, however, large amounts of soluble protein and chloroplast material may be released into the food bolus[9], revealing extensive damage to chloroplast membranes.

West and Packer[10] studied the effects of glutaraldehyde on the light-induced reactions and osmotic properties of pea chloroplasts. We wish to describe an investigation of the effects of similar glutaraldehyde treatment on the protein lost from chloroplasts after osmotic shock, and of their digestibility *in vitro*. Osmotic shock was chosen as the simplest way to rupture the chloroplast membranes and release the soluble stroma proteins which would normally be released during mastication. Measurements of the ferricyanide Hill reaction were made to establish the photochemical state of the original chloroplast preparations and to indicate the extent of glutaraldehyde fixation.

Marrowstem kale (*Brassica oleracea* L. variety *Acephala* D.C. (marrowstem)), available during the winter, gave good chloroplast preparations when ethylenediamine tetraacetic acid (EDTA) was incorporated into the grinding medium: there seems to be an excess of calcium over oxalate in this plant (personal communication from R. Hill). Walker's rapid homogenizing technique was used[11], and the chloroplasts were kept at 0° C unless otherwise stated. Leaves (80 g) freed from large veins were homogenized for 3·5 s in 500 ml. of a part frozen slurry of 0·4 M sucrose, 0·04 M sodium phosphate, *p*H 7·4, 0·01 M EDTA and 0·01 M sodium iso-ascorbate using an 'Ultra-Turrax T 45' (Janke and Kunkel, Staufen, Germany) with a 4·5 cm head (TP 45G). The homogenate was squeezed through eight layers of muslin and filtered through twelve layers. After centrifugation at 1,000*g* for 5 min the pellets were combined in 20 ml. 0·4 M sucrose. The chloroplasts which sedimented after 2 min at 3,500*g* were suspended in 0·4 M sucrose to give a chlorophyll concentration[12] of about 2 mg/ml. and used as the stock chloroplast preparation. Each 80 g kale preparation gave 10–14 mg of chlorophyll with 50–80 per cent of the chloroplasts intact. Preparations with low proportions of whole chloroplasts were always obtained after severe weather.

Oxygen production (Table 1,*a*) and protein content of the original chloroplast preparations were measured. Four equal volumes of the chloroplast stock containing 3–13 mg of chlorophyll were incubated with 0, 60, 120 and 240 mM glutaraldehyde (Polysciences Inc., Warrington, Pennsylvania) in 2–10 ml. of 0·5 M sucrose for 7 min. The glutaraldehyde was removed by diluting with 10–30 volumes of 0·4 M sucrose and sedimenting the chloroplasts at 10,000*g* for 15 min.

The treated chloroplasts were shocked osmotically to release stroma proteins by suspending them in 3–8 ml. of distilled water for 20 min. These chloroplast suspensions complete with soluble nitrogen compounds were sampled for nitrogen and chlorophyll content and digestibility. They are referred to as osmotically shocked complete preparations (Table 1,*b*).

Table 1. EFFECT OF GLUTARALDEHYDE ON PROTEIN LOSSES, DIGESTIBILITY AND THE OXYGEN PRODUCTION OF KALE CHLOROPLASTS

Experiment			Original preparation	Treated chloroplasts: mM glutaraldehyde 0	60	120	240
1	Chloroplasts about 50 per cent intact before GADH		—				
	Oxygen (μmoles/mg of chlorophyll/h)		*a*	*d*	*d*	*d*	*d*
		$-NH_4Cl$	46	67	79	28	25
		$+NH_4Cl$	109	122	95	39	26
	Per cent of total protein released on osmotic shock (from *a* and *c*)		—	35	12	3	2
2	Chloroplasts stripped of membranes before GADH		*e*	*f*	*f*	*f*	*f*
	Oxygen (μmoles/mg of chlorophyll/h)	$-NH_4Cl$	22	—	42	36	24
		$+NH_4Cl$	137	137	103	77	39
	Digestibility of treated fragments (*f*):						
	to trypsin	5 h	—	87	67	19	6
		10 h	—	100	89	41	6
3	Chloroplasts about 50 per cent intact before GADH						
	Per cent of total protein released on osmotic shock (from *a* and *c*)		—	31	6	2	2
	Digestibility of osmotic shocked complete preparation (*b*):			*b*	*b*	*b*	*b*
	to trypsin	5 h	—	89	93	90	81
		10 h	—	100	110	108	100
	to pancreatin	5 h	—	73	76	78	83
		10 h	—	100	107	111	120
	Digestibility of osmotic shocked insoluble material (*d*):			*d*	*d*	*d*	*d*
	to trypsin	5 h	—	79	78	89	65
		10 h	—	100	114	128	92
	to pancreatin	5 h	—	59	47	33	18
		10 h	—	100	91	62	31

This table summarizes the results of 3 sets of experiments. Details of the methods used are given in the text but the types of chloroplast preparation used and the general treatment are as given here.

Experiments 1 and 3

(*a*) The original chloroplast preparations had about 50 per cent of the chloroplasts intact. The chlorophyll and protein content and rates of photochemical oxygen production were measured.

(*b*) The four types of chloroplast produced after treatment with 0, 60, 120 and 240 mM glutaraldehyde (GADH) for 7 min, and its subsequent removal, were each suspended in an excess of water to give an osmotic shock. Samples of each were removed for measurements of chlorophyll and protein content. In experiment 3 the digestibility of the four types of chloroplast was also measured. The remainder of each of the four preparations was then centrifuged.

(*c*) The protein content of the supernatant liquids from the four (*b*) preparations was measured. The amount of protein released from each type of treated chloroplast by osmotic shock was found from the difference in protein content between the original preparation (*a*) and the values for each supernatant (*c*).

(*d*) Consisted of the four types of chloroplast fragments left from the (*b*) preparations after the supernatants (*c*) were removed. The chlorophyll and protein content of the particles were measured. Photochemical activity was measured in experiment 1 and the digestibility of the fragments measured in experiment 3.

Experiment 2

(*e*) All chloroplasts in the original suspension were stripped of membranes. Chlorophyll and protein content and photochemical activity were measured.

(*f*) After treatment with glutaraldehyde (0, 60, 120 and 240 mM) and its subsequent removal, the four types of chloroplasts were used to study oxygen production and digestibility.

After shocking, the chloroplasts were centrifuged at 10,000*g* for 20 min. The amount of protein released into the supernatant liquid was measured. The pellets of insoluble material containing most of the chlorophyll were suspended in 1–3 ml. of water and samples were taken for nitrogen and chlorophyll analyses and studies on digestibility and photochemical activity (Table 1, *d*).

Some chloroplasts were also stripped of their outer membranes and treated with glutaraldehyde. The fragments were used for the Hill reaction and digestibility studies (Table 1, *f*).

Protein values were calculated from: (total nitrogen − (non-protein nitrogen + chlorophyll nitrogen)) × 6·25. Analyses of total nitrogen and non-protein nitrogen after protein precipitation with 10 per cent trichloroacetic acid were made by Mr D. Jordan using an automated micro Kjeldahl apparatus. Measurements of the relative proportion of soluble stroma and insoluble lamellar protein have not been made but are probably of the order of 3 : 1 (ref. 13).

Photochemical activity was measured by following polarographically the oxygen produced during the light-induced reduction of potassium ferricyanide at 20° C in the absence and presence of 2 mM NH_4Cl (ref. 14). The 5 ml. reaction vessel contained 0·4 M sucrose, 10 mM Tris-HCl, *p*H 7·8, 2 mM $MgCl_2$, 4 μmoles of $K_4Fe(CN)_6$ and chloroplasts containing 100 or 150 μg of chlorophyll.

The digestibility of the chloroplasts was assessed by the release of non-protein nitrogen when incubated with trypsin or the pancreatin enzyme complex. Digestion mixtures of 1 ml. contained approximately 600 μg of chlorophyll from each of the four treated chloroplast preparations (0, 60, 120 and 240 mM glutaraldehyde), 80 mM phosphate buffer, *p*H 8·0, and 0·2 ml. of enzyme solution (0·25 mg twice crystallized trypsin or 1·25 mg of freeze-dried sheep pancreatic juice containing 125 μg of nitrogen). Nitrogen determinations were made on 0·3 ml. aliquots after 0, 5 and 10 h of incubation at 37° C. Values are given as a percentage of the nitrogen released (10 h of incubation) from chloroplasts treated with no glutaraldehyde.

Kale chloroplast preparations with about 50 per cent of their chloroplasts intact lost 31–35 per cent of their total protein after osmotic shock. Higher losses would be expected to occur from material with a higher proportion of intact chloroplasts originally. Treatment with glutaraldehyde reduced the losses to 6–12 per cent with 60 mM and 2–3 per cent with 120 mM. There was no reduction in the digestibility of osmotically shocked complete preparations after treatment with glutaraldehyde. Their apparent increase in digestibility may result from the binding of previously soluble proteins to the chloroplasts by glutaraldehyde treatment, thus increasing the amount of protein available, although the chlorophyll content was the same. The trypsin digestibility of the shocked chloroplast fragments after soluble material had been removed was little affected by glutaraldehyde. The pancreatin digestibility was reduced to about 30 per cent of the untreated value by 240 mM glutaraldehyde. These differences between trypsin and pancreatin are not easy to explain. Chloroplasts which were stripped of their membranes before fixation and had already lost much soluble protein were more affected by the glutaraldehyde treatment. Even in this extreme case, however, digestibility was much less affected than was the average protein loss, especially at the lower concentrations of glutaraldehyde. Results all indicate the possibility of reducing protein losses without a parallel reduction in digestibility.

Using pea chloroplasts, West and Packer found that complete fixation occurred with 200 mM glutaraldehyde. With kale, it was not possible to correlate the extent of fixation with changes in chloroplast volume after osmotic shock. It is likely, however, that the chloroplasts are similar and that fixation occurs with about 200 mM glutaraldehyde. Photophosphorylation was abolished in peas by 50–100 mM glutaraldehyde. In kale, the ATP/Pi-limited (basal) rate of oxygen production was maximal with 60 mM gluaraldehyde, after which progressive inhibition of the rate occurred. The ammonia-stimulated (uncoupled) rate of oxygen production shows the progressive inhibition which was also expected from the results with peas.

Kale seems to be promising for photochemical studies. The basal rate of electron transport was stimulated three-fold with ADP and Pi, in the rather poor preparations after severe weather. An average of 4·4-fold stimulation was given by 2 mM NH_4Cl. Oxygen production values (Table 1) for the original preparations and the treated chloroplasts were made after keeping the chloroplasts for 3–6 h respectively, indicating the stability of these chloroplasts.

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Drug-induced Release of ^{3}H-Norepinephrine and ^{3}H-Serotonin from Brain Slices

EFFECTS on the release of proposed central neurotransmitters have been reported for several psychotropic drugs, and may contribute to the influence of these compounds in behaviour[1,2]. Most of our information on the influence of drugs on release of cerebral monoamines is indirect; for example, from comparison of tissue or cerebrospinal fluid levels of amines or their metabolites before and after drug treatment[3]. The interpretation of such data is complicated by direct drug effects on the metabolism of brain amines. Use of isolated, superfused sections of brain tissue, which actively accumulate exogenous norepinephrine or serotonin in nerve terminal structures normally containing these amines[4–7], affords a direct and sensitive means of assessing the influence of drugs on central neurohumoral release. Using this technique we have found that several drugs profoundly affect the spontaneous liberation of labelled norepinephrine and serotonin from slices of rat corpus striatum.

Slices of anterior corpus striatum weighing about 20 mg were rapidly prepared from the brains of adult Sprague-Dawley rats as previously described[8,9]. Tissues were incubated at 37° C in a supplemented Krebs–Ringer medium saturated with 5 per cent CO_2 in O_2 and containing either ^{3}H-norepinephrine (7 Ci/mmole, 15 ng/ml.) or ^{3}H-serotonin (6 Ci/mmole; 15 ng/ml.). After 30 min the slices were transferred to individual chambers through which fresh oxygenated medium was rapidly circulated (150 μl./min). After 40 min, when the efflux of radio-

activity had fallen to a nearly steady level the superfusing medium was suddenly switched to one in which the investigational drug had been dissolved. Sequential samples of effluent superfusate, collected at 2 min intervals just before and during drug exposure, were analysed for total tritium by liquid scintillation spectrometry.

Table 1. EFFECT OF DRUGS ON EFFLUX OF TRITIUM FROM STRIATAL SLICES INCUBATED WITH ^{3}H-NOREPINEPHRINE OR ^{3}H-SEROTONIN

Drug	Net release (pCi/min) ^{3}H-norepinephirine	^{3}H-serotonin
Metaraminol	1,400±130*	135±34*
D-Amphetamine	665±180*	331±34*
L-Amphetamine	460±150†	158±40*
Desipramine	442±105*	19±3*
Reserpine	150±25*	174±25*
Cocaine	36±10†	−6·3±6·7
Guanethidine	17·2±7·9	3·8±8·2
Lysergic acid diethylamide	14·3±4·4	−20·3±15·8
None	−7·7±13	−8±5·6
Picrotoxin	−13±10	−17±17
Chlorpromazine	−29±14	−32±5·2†
Pargyline	−29±9	−24±7·8

* $P < 0.01$.
† $P < 0.05$ when compared with control levels.

Slices were incubated with ^{3}H-norepinephrine or ^{3}H-serotonin, then superfused as described in text. Reported net release represents the difference in tritium efflux during the 2 min interval from 6–8 min after onset of drug exposure from tritium efflux during the 2 min interval just before drug exposure. Predrug efflux from ^{3}H-norepinephrine-incubated tissues was 143±20 pCi/min; from ^{3}H-serotonin-incubated slices 170±19 pCi/min. Results are the mean±s.e. for 4 or more slices.

Metaraminol (10^{-4} M), D- or L-amphetamine (10^{-4} M), desipramine (10^{-4} M) and reserpine (10^{-5} M) produced a substantial increase in tritium efflux from slices previously incubated with ^{3}H-norepinephrine or ^{3}H-serotonin (Table 1). In all cases the enhanced efflux of radioactivity occurred within 4 min after beginning drug superfusion and continued for several minutes thereafter. In general, tritium release from slices incubated with ^{3}H-norepinephrine equalled or exceeded that from tissues incubated with ^{3}H-serotonin. Release induced by metaraminol or despiramine from slices containing ^{3}H-norepinephrine exceeded by ten-fold or more release from slices incubated with ^{3}H-serotonin. Cocaine (10^{-4} M) produced a relatively small, yet statistically significant, rise in tritium efflux from tissues containing ^{3}H-norepinephrine, but not from those containing ^{3}H-serotonin. Guanethidine, lysergic acid diethylamide, picrotoxin, chlorpromazine and pargyline (all 10^{-4} M) failed significantly to modify the spontaneous efflux of either monoamine.

The radioactive constituents in the effluent superfusate from seven pooled samples (two slices each) were determined by column chromatography[10,11]. The unchanged amine accounted for 29±9 per cent of the total efflux of tritium collected during the 2 min interval just before drug treatment, from slices incubated with ^{3}H-norepinephrine. After exposure of the slices to D-amphetamine for 6 min, the free amine was released without change in deaminated products so that the proportion of ^{3}H-norepinephrine in the effluent superfusate increased to 67±11 per cent of the total recovered radioactivity. Reserpine, applied for a similar period, resulted in a proportionately similar increase in both amine and deaminated products and failed significantly to alter the proportion of amine (31±3·3 per cent) to total tritium. These findings support previous evidence that amphetamine releases physiologically active amines[12,13], while reserpine liberates amines into the intraneuronal cytoplasm where they are converted to inactive amine metabolites[14,15].

The next efflux of labelled monoamines from superfused brain slices measured in these experiments presumably reflects the difference in the rates of spontaneous release from intraneuronal stores and neuronal re-uptake. The relative rates of release and recapture undoubtedly play an important part in the regulation of monoamine concentrations within the synaptic cleft *in vivo*. Drugs may influence these levels by one or more mechanisms. Metaraminol, an α-methylated phenylethanolamine, probably releases norepinephrine by competitive displacement from intraneuronal storage sites[16], and may compete with norepinephrine for neuronal re-uptake[17]. Similar mechanisms may account for the mild effect of metaraminol on serotonin release. Amphetamine, desipramine and cocaine have all been shown to be potent inhibitors of monoamine uptake into cerebral tissues[18–20]. Cocaine does not seem to enhance release so the increase in efflux may be a consequence of inhibition of amine re-uptake. A direct releasing action of D-amphetamine and desipramine is, however, probable, for inhibition of uptake alone does not markedly enhance release. This action may account for the greater liberation of norepinephrine observed with these two compounds than with drugs which only affect re-uptake.

It is likely that exogenous norepinephrine and serotonin enter the relatively abundant dorso-anterior terminals in striatal tissue[21–23], and that the pharmacological properties of these terminals differ from those of other monoaminergic neurones[24–26]. Nevertheless, the results of these experiments provide direct evidence for the effect of certain drugs on mechanisms subserving the release of norepinephrine and serotonin and support the contention that changed perisynaptic levels of specific monoamines are responsible for the psychotropic actions of compounds such as amphetamine and desipramine.

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Long-term Isolation in Rats reduces Morphine Response

Recently, interest has increased in the effects of previous home environments on reactions to drugs (for example, ref. 1) and experiments have been done on various species. Long-term isolation is known to induce aggressive behaviour and biochemical changes in mice[2], and it also disrupts

behavioural and physiological reactions in rats[3,4]. Isolation in both species increases their sensitivity to toxic effects of some stimulant drugs[5,6], but shortens barbiturate sleeping times in mice[7] and rats[8], and reduces the stimulant effects of amphetamine–barbiturate mixtures on the activity of rats in a Y-maze (Clarke, Rushton and H. S., to be published). It seemed important to study the effects of long-term isolation on some responses by rats to morphine.

In this experiment therefore weanling male hooded rats (average age 21 days) were ear-marked and assigned at random from their litters to either of two housing conditions: some were housed singly in cages with solid roofs and sides, and others in groups of twelve in standard colony cages. Food and water were available *ad libitum*, and the animals were not handled again until they were tested. Forty days later, each group was subdivided into two: one subgroup received 12·5 mg/kg intraperitoneal isotonic morphine HCl, a dose which pilot experiments had shown to be moderately depressant in male rats of similar age, and the other received 1 ml./kg intraperitoneal isotonic saline. The rats were replaced in their home cages, and 1 h after injection they were tested singly for 5 min in a symmetrical wooden Y-shaped runway, 13 inches high and with arms 15 inches long and 5 inches wide[9], which has been found suitable for the measurement of dose-related morphine effects on spontaneous activity (Fig. 1). The number of "entries" animals made into the arms of the apparatus (all four feet had to be inside an arm) and the number of "rears" (scored if the rats stood up on their hind feet) were counted; the total amount of time spent rearing throughout the 5 min period was measured in addition by means of an electronic timer.

As was expected from the pilot experiments, morphine markedly depressed the number of entries in the grouped

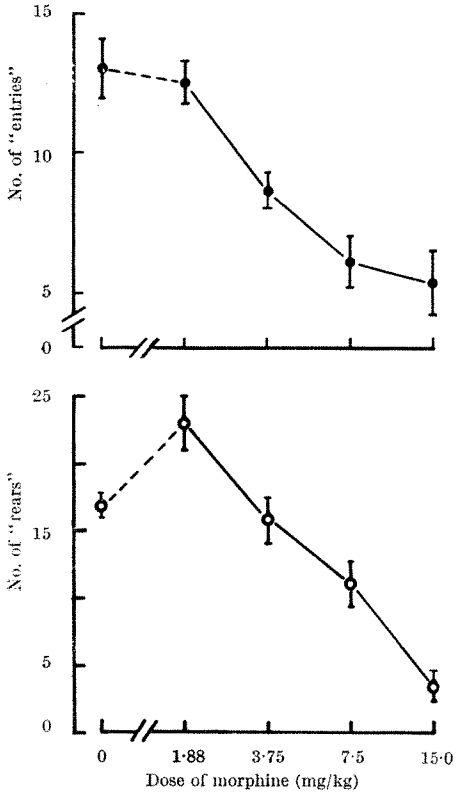


Fig. 1. Dose-related effects of morphine on Y-maze activity. The top diagram shows the mean number of "entries" ± one standard error into the arms of a Y-shaped runway made during 5 min, 1 h after subcutaneous injection of several doses of morphine, and the bottom diagram shows the corresponding results for "rears". Female rats approximately 120 days old were used, and each point represents the mean results of a different group of eight animals.

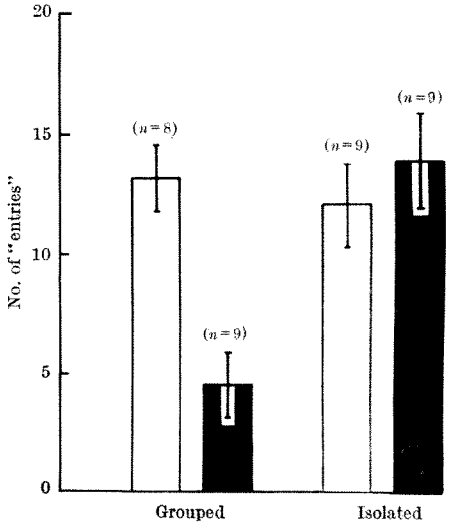


Fig. 2. Effects of morphine on grouped and isolated rats. The figure shows the mean number of "entries" ± one standard error into the arms of a Y-shaped runway made during 5 min by rats reared in groups or in isolation, which had received either saline (open columns) or 12·5 mg/kg morphine hydrochloride (solid columns). Morphine depressed activity in the grouped but not in the isolated rats.

rats and the size of the effect was also remarkably similar. In the isolated rats, however, entries were hardly depressed at all. 2 × 2 factorial analyses were performed on the results of all three measures, and the "entries" analysis revealed main effects, due to the drug and housing factors, significant at the 5 per cent level ($F = 4{\cdot}37$ and 6·07 respectively) and an interaction term, representing the different effect of morphine in the two groups, highly significant at the 1 per cent level ($F = 9{\cdot}35$). Similar trends appeared in the "rears" and "total rearing times", but only the effects due to the drug were significant ($P < 0{\cdot}001$ in both cases); for "rears", the interaction was almost significant at the 5 per cent level. The body weights of the isolated rats were significantly greater than those of the grouped rats (198·9 g ± 27·8 s.d., and 173·8 g ± 19·7 s.d. respectively; $P < 0{\cdot}01$), although there had been no difference at weaning. Examination of the activity within the 5 min period showed that the difference in response to morphine in the two groups of animals was sustained throughout the test: minute-by-minute plots showed that the two morphine groups had parallel time courses within the trial, but the curve for the grouped animals was consistently lower. In other words, the grouped animals seemed to behave as if they had received a larger dose of the drug. The "catatonic" episodes which often occur in rats treated with morphine seemed to be shorter and more sporadic in isolated than in grouped rats, and this probably accounts for the difference.

The results therefore suggest that the isolated rats were less sensitive to the depressant action of the dose of morphine used than those which had lived in groups. A number of explanations of this finding are possible. The isolated animals might have metabolized the morphine at a greater rate, for Baumel *et al.*[7] found that hepatic microsomal fractions from isolated mice metabolized hexobarbitone faster than did fractions from grouped mice. If long-term isolation reduces the rate of brain 5-hydroxytryptamine turnover in the rat in a manner similar to that observed in mice[2], then a reduction in response to morphine might be expected, for procedures which interfere with this amine can also modify responses to the drug[10–12]. It seems unlikely that the different reactions to morphine were a consequence of increased baseline "arousal levels" in the isolated rats, as the saline scores were very similar in both groups. Finally, although all animals were tested singly in the Y-maze, they did

spend the hour between injection and being tested in very different home environments, that is, singly or grouped, and this, as well as the differences in long-term housing, may have contributed to the results obtained. Experiments are in progress to investigate this possibility.

Findings of the kind reported here demonstrate how even a profound change in behaviour resulting from an opiate drug can be modified by the previous history of the recipients.

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Effect of Visual Pattern Restriction in Early Life on Brain Enzyme, Body Weight and Learning in the Rat

VISUAL pattern restriction or enrichment in early life can induce significant differences in body weight gain, performance on a visual sensory reinforcement test and brain enzymatic activity in adult rats[1]. Rats raised in an enriched visual environment, however, perform better on the visual reinforcement test than rats raised in a restricted visual environment only when light-onset is used as reinforcement; if light-termination is used as reinforcement, performance differences are completely reversed[2]. Thus the effects of early visual experience may be beneficial only in some highly specific learning situations. Our experiments test the effect of visual input in early life on learning tests other than those previously used; we also looked at possible differences in body weight and brain enzymatic activity induced by early visual experience after a period of extensive handling, a food deprivation schedule and learning experience.

Thirty-two littermate, female Holtzman albino rats, weaned at 23 days of age, were randomly assigned either to a visual pattern or to a restricted visual pattern group. The animals were raised in conditions reported previously[1] except that overhead fluorescent lights were on throughout the experiment. The visual environment of the visual pattern group consisted of a board painted with 1·94 cm thick alternating black and white stripes, whereas that of the restricted visual pattern group consisted of a board painted flat white. Each rat was housed individually in a cage with a wire mesh front and was provided with unlimited food and water.

Table 1 presents mean body weights for both groups at 23, 58, 93, and 112 days of age.

As we found before, the visual pattern group was significantly heavier than the restricted visual pattern group in the first two weighing periods after exposure to the experimental rearing condition. On the last weighing period the difference between group weights, though not statistically significant, is nonetheless large in the light of the following facts. First, both groups were subjected to extensive handling, a food deprivation schedule, and behavioural testing during the ninety-third to the hundred and twelfth days of life. Second, the visual pattern group obtained less food reinforcement than the restricted visual pattern group in the behavioural test, because of their poorer performance. Finally, rats raised in enriched environments have lower body weights than rats raised in isolation[3].

Table 1. MEAN AND STANDARD ERROR OF MEAN OF BODY WEIGHT FOR VISUAL PATTERN AND RESTRICTED VISUAL PATTERN GROUPS FOR FOUR WEIGHINGS

Rearing condition	Body weights (g)	Age (days) 23	58	93	112
Restricted visual pattern	Mean	53·5	186·5	232·2	239·7
	s.e.	1·0	2·9	4·8	5·1
Visual pattern	Mean	54·1	195·2	242·4	248·7
	s.e.	1·0	2·2	2·4	3·5
	P	N.S.	< 0·01	< 0·05	< 0·10

All animals experienced handling, food deprivation and behavioural testing between 93 and 112 days of age. Levels of significance were determined by *t*-test (two-tailed).

Starting from 93 days of age, all animals were picked up and lightly stroked for 5 min a day for four days. Next, animals were trained either on a differential reinforcement of low rate of responding (DRL) operant schedule[4] or Lashley III maze[5]. The DRL test is unaffected by the drive state of the animals, so any differences between visual pattern and restricted visual pattern groups on this test could not be caused by differences in exploratory drive or general activity of the two groups. Further, if early experience indeed produced differences in exploratory drive, they should be reflected in number of alleys entered and time taken to complete the trial in the Lashley maze.

One-half of each group was randomly assigned to the DRL test and the remaining half to the Lashley maze. For both DRL and maze training, animals were put on a 23 h food deprivation cycle and, except for training sessions, continued to live in their respective rearing conditions.

Table 2. MEAN AND STANDARD ERROR OF MEANS OF SUCCESS RATIOS

Rearing condition	Reinforcements/responses ×100	Day 1	2	3	4	5
Restricted visual pattern	Mean	23·3	37·9	53·7	52·1	50·5
	s.e.	1·2	8·1	5·5	5·9	5·3
Visual pattern	Mean	12·1	17·7	20·6	30·0	32·6
	s.e.	1·3	2·9	3·6	3·9	6·6
	P	< 0·001	< 0·10	< 0·001	< 0·02	< 0·10

Values are (reinforcements/responses ×100) for visual pattern and restricted pattern groups for the first 5 days of DRL-10 performance. Significance levels were determined by *t*-test (two-tailed).

For DRL test, each animal was trained on a DRL 10 s schedule in an operant conditioning chamber (dimensions: 28·57 × 19·68 × 23·49 cm) for food reinforcement. To obtain reinforcement in this task, an animal must not press the lever for 10 s after the preceding reinforcement. Any premature lever press restarts the 10 s interval. For DRL training, each animal was first trained to press the lever where every lever press resulted in a 45 mg food pellet. After fifty such reinforcements, the animal was shifted to a DRL 10 s schedule and was trained for an hour a day until the animal reached the criterion of at least 50 per cent success ratio for three consecutive days. As is evident from Table 2, the restricted visual pattern group maintained higher success ratios than the visual pattern group for the first five days of training: thereafter the performance of the two groups did not differ significantly.

Furthermore, the restricted visual pattern group required significantly ($P < 0·05$, two-tailed t test) fewer days to reach criterion (mean = 3·6) than the visual pattern group (mean = 7·4). There were no significant relationships between body weight and DRL performance, either in terms of days to reach criterion or success ratio, or between

DRL performance and brain enzymatic activity for either group.

For the Lashley maze training, each animal was given five trials a day, and the number of entries into blind alleys and time from start to goal box was recorded. Each animal was trained to the criterion of two successive errorless days. Further, because animals raised in isolated rather than enriched environment do not differ on initial maze learning but differ on reversal[7], animals which had reached criterion were tested for reversal (start and goal box locations reversed) for five days. But the groups did not differ significantly on initial or reversal problems in time, errors or days to reach criterion. The results of these two behavioural tests show that performance differences between visual pattern and restricted visual pattern groups cannot be attributed to differences in level of general activity.

After these tests, animals were decapitated, and their brains removed and weighed. The olfactory bulbs and cerebellum were removed, and the rest of the brain was cut into four samples (anterior cortex, posterior cortex, anterior subcortex and posterior subcortex) and frozen for biochemical assay. All samples were coded so that the analyst was unaware of the experimental condition of each sample. The acetylcholinesterase activity was determined by the rate of hydrolysis of acetylcholine perchlorate using the procedure described by Rosenzweig *et al.*[8]. As reported earlier[1], the difference in subcortical acetylcholinesterase activity between the visual pattern and restricted visual pattern groups was not significant; Table 3 therefore presents mean cortical acetylcholinesterase activity only.

Table 3. MEANS AND STANDARD ERRORS OF ACETYLCHOLINESTERASE ACTIVITY IN CORTICAL BRAIN TISSUE SAMPLES FOR VISUAL PATTERN AND RESTRICTED VISUAL PATTERN GROUPS

Rearing condition	Acetylcholinesterase activity	Anterior	Posterior	Total
Restricted visual pattern	Mean	124	76	101
	s.e.	4·4	2·0	2·7
Visual pattern	Mean	133	84	109
	s.e.	5·3	1·7	3·1
	P	N.S.	< 0·01	< 0·10

Acetylcholinesterase activity is expressed in moles acetylcholine hydrolysed $\times 10^{10}$/min/mg of tissue. Total cortical acetylcholinesterase values were obtained for each animal by calculating a weighted average. Significance levels were determined by *t*-tests (two-tailed).

The visual pattern group exhibited greater acetylcholinesterase activity in anterior, posterior and total cortex than the restricted visual pattern group. But the visual pattern group shows (statistically) significantly greater activity only in the posterior cortical sample which contains the visual cortex. Again, as in the case of body weight cortical acetylcholinesterase differences between the groups were evident in spite of 19 days of behavioural testing. Krech *et al.*[7] reported that 18 days of behavioural testing obliterates the brain acetylcholinesterase differences between enriched and isolated groups. Their animals, however, were raised in multiple environmental enrichment for 30 days and were removed from the original environment during the testing period. Finally, it should be pointed out that in the earlier article[1] we reported that acetylcholinesterase absolute cortical activity levels for visual pattern and restricted visual pattern groups were lower compared with these values. This difference in absolute values is probably because in the present study all animals were raised in continuous illumination. Our later studies have replicated reported acetylcholinesterase activity values and it seems that the duration of exposure to a visual environment is a critical variable influencing acetylcholinesterase activity.

In conclusion, the nature of visual input in early life may be the critical factor in producing changes in adult animals. The importance of visual input can also be deduced from those early experience studies[3] in which no systematic attempts are made to manipulate the visual patterns of the rearing environment. Nevertheless, the visual cortex shows the greatest effects of the environmental enrichment both in anatomical growth[9] and acetylcholinesterase activity[3] compared with the remaining neocortex. Finally, early visual experience evidently does not increase generalized problem-solving ability and its effects are determined by specific characteristics of the learning situations.

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Development of Normal Mice by *in Vitro* Fertilization

In vitro fertilization of mammalian ova has been successfully carried out in the rabbit[1-3] and the hamster[4,5], but development of fertilized ova beyond the two-cell stage has not been achieved[6]. Brinster *et al.*[7] have observed fertilization and blastocyst development in explanted Fallopian tubes using organ culture. More recently, Whittingham[8] has successfully fertilized mouse eggs *in vitro*, transplantation of which into pseudo-pregnant recipient mothers yielded 17 day old foetuses. The present studies confirm the results of Whittingham[8] and further demonstrate that apparently normal full-term progeny can be obtained from implantation of *in vitro* fertilized eggs that have been maintained in culture to the blastocyst stage.

The mice used in these studies were of the ICR/Ha (albino) and C57Bl/6 Jacobs strains (black). Large numbers of ova were obtained after super-ovulation of mature mice by an intraperitoneal injection of 5 IU of pregnant mares' serum (PMS) followed 48 h later by an injection of 5 IU of human chorionic gonadotropin (HCG). Ten to twelve hours after the second injection, the mice were killed by cervical dislocation and the oviducts were removed and uncoiled. Unfertilized ova were flushed from the oviduct into a Petri dish by injecting 0·5 ml. of normal saline into the ampullary isthmal junction with a 30-gauge needle. The eggs were then isolated from cellular debris by a finely drawn capillary micropipette and transferred to a Petri dish containing culture medium diluted with normal saline to half the original concentration. The composition of the medium was identical

Table 1. *In Vitro* FERTILIZATION AND IMPLANTATION

Donor strains*		Number of oocytes cultured	Number of two-cell embryos	Number of blastocysts obtained	Number of blastocysts transplanted	Recipient	Number of progeny obtained
Ova	Sperm						
A	A	130	34	13	13	B	5 (A)
B	B	123	33	12	10	A	6 (B)
Total		253	67	25	23		11

*A = ICR/Ha (albino); B = C57Bl/6 (black).

to that of Whitten and Biggers[9] with the exception that sodium pyruvate concentration was 0·55 mM rather than 0·33 mM. The *p*H of the medium was maintained at approximately 7·5 by intermittent administration of 5 per cent CO_2 in air.

Capacitated spermatozoa, as defined by Austin[10] and Chang[11], were obtained from the uteri of mice 3–4 h after mating with a proven fertile male. The two uteri were dissected out and their contents released into a Petri dish containing 1 ml. of half strength medium at 37° C. The Petri dish was immediately agitated to prevent coagulation of the sperm.

Approximately 0·3 ml. of sperm suspension was added to a cavity slide containing the unfertilized ova suspended in medium. The mixture of the eggs and sperm was then layered over with paraffin oil (Fisher Scientific, viscosity 125/135) so that the well was filled. The slide was incubated for 6–8 h at 37° C in a 5 per cent CO_2 atmosphere. Approximately the same number of ova without the addition of sperm were cultured as controls.

After incubation, both control and treated ova were thoroughly washed with normal saline and placed in culture using the method as described by Brinster[12] and Chang[2]. The culture was examined after 24 h for cleaving zygotes. The two-cell ova were separated into Petri dishes with fresh medium (full strength) and allowed to develop to the blastocyst stage. At this point, the blastocysts were implanted into pregnant or pseudo-pregnant females. Although some control eggs fragmented during culture, no actual cleavage was observed.

The recipient mothers were made pseudo-pregnant through mating with proven sterile vasectomized males. The vasectomized mice were always genetically similar to the recipient mother (coat colour), and therefore dissimilar to the implanted embryos. In some experiments, normally pregnant mice were used as recipient mothers instead of pseudo-pregnant animals. These matings were designed so that the progeny derived from the *in vitro* and *in vivo* fertilizations were clearly distinguishable by coat colour.

The appearance of the vaginal plug was taken as evidence of mating and counted as day 0 of pseudo-pregnancy. After 3 days of growth *in vitro*, the blastocysts were collected into a glass capillary micropipette connected to a small rubber tube. A surgical incision was made on the dorsal side of the recipient mouse, the uterotubal junction was punctured by the micropipette so that the open tip reached the lumen of the uterus, and the blastocysts were introduced by gentle blowing through the rubber tubing. Birth usually occurred 20–21 days after fertilization.

Table 1 shows the pooled results of six experiments. Of 253 eggs exposed to capacitated spermatozoa, 67 were fertilized, as appears from the two-cell ova, and 25 which developed to blastocysts. Twenty-three blastocysts were implanted into recipient mothers and eleven apparently normal offspring were obtained. Nine of these were from pseudo-pregnant mothers and the remaining two from normally pregnant females. In experiment 3, two female mice derived from *in vitro* fertilization were mated with vigorous males and delivered normal progeny.

If perfected, this technique could be applied to fertility and sterility problems or immunological relationships between the conceptus and the mother, as well as to problems in development, implantation and differentiation, by transfer of early embryos of specified ages to recipients at various stages of the reproductive cycle[13].

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Piezoelectric Effect and Growth Control in Bone

THE adaptability of bone under impressed mechanical forces has been known since the time of Wolff[1]. A possible control mechanism for the process became apparent with the discovery of the piezoelectric effect in bone[2]. In theory this effect could translate an environmental stimulus into a biologically recognizable signal controlling growth or resorptive processes. It has been recognized that the action of the piezoelectric signal may be to alter the chemistry of pertinent macromolecules such as collagen, or to influence cellular activity directly[3]. Of the two possibilities, evidence tends to rule out the importance of the former and we consider here only the latter[4].

For ordinary piezoelectric materials and for small isolated bone samples, the magnitude and sign of the charges that will appear on application of a load can be predicted. Such calculations, however, are not possible for larger bone samples, including whole bones, because of the variable architecture present. (The direction of the symmetry axis of the piezoelectric tensor becomes a function of position.) This means we have no way of knowing what constitutes a normal or abnormal charge distribution for a given bone, and therefore no basis for comparison with observed growth patterns. Alternative considerations for relating the piezoelectric effect and bone adaptability are the signal produced and the expected physical effects at the cellular level. On loading, bone will generate a bound surface charge distribution $\rho(\bar{x},t)$. In a process typically occurring in seconds, $\rho(\bar{x},t)$ is nulled by ion current in the permeating interstitial fluid. When the process is monitored macroscopically by measuring a voltage, a symmetric biphasic pulse is seen[5,6]. The symmetry of such a pulse, however, is not characteristic of the underlying process. Consider a Gedanken experiment in which there is an observer at every bone cell. In general, no two observers will record the occurrence of the same local charge distribution on loading. Similarly, they will not agree on the charge neutralization kinetics that occur

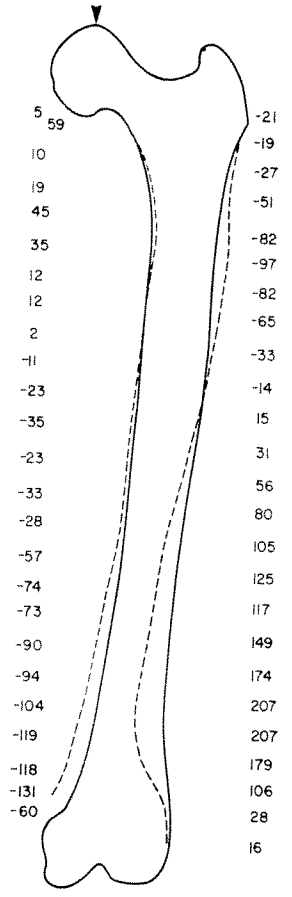
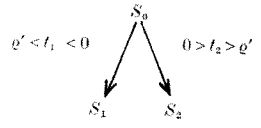


Fig. 1. Solid human femoral outline and charge distribution in pC/cm² as given by McElhaney. The medial surface is displaced at each charge location (left for growth, right for resorption) by an amount proportional to that charge. The lateral surface is similarly displaced (except left for resorption, right for growth). The dotted femoral outline is the result.

because neutralization will depend on a host of locally varying factors such as membrane shielding of the bone surface, fluid viscosity and the concentration and mobility of diffusible ions. Thus, each observer will see two processes, the creation of a $\rho(\bar{x},t)$ and its subsequent neutralization. Either process can theoretically represent a biological control signal because each possesses two of the necessary properties, variability and unidirectionality. By variability we mean that the parameters for each process will vary with cell location. For instance, for the first process some observers will note the appearance of negative regions on the adjacent bone surface, while others will see positive areas. If the former represents the biological control signal for growth, then the latter may correspond to resorption. By unidirectionality we mean that neither process generates a biphasic signal which sums to zero.

Young[7,8] postulated that the three major types of bone cells are interchangeable, the change of specialization occurring because of changes in the microenvironment which selectively activate and repress genes. We propose that either physical process described above may be responsible for switching bone cells from one kind of specialization state to another. In this case, normal bone in normal loading conditions would produce a normal $\rho(\bar{x},t)$ controlling its own remodelling, and abnormal bone (such as a healed angulated fracture) in normal loading conditions would produce an altered $\rho(\bar{x},t)$ which increases bone deposition in some areas and decreases it in others (modelling). Normal bone in conditions of no load would produce no $\rho(\bar{x},t)$ and, in the absence of this directing influence, atrophy would result.

Next we must find a relationship between one of the two processes and bone cell states. We choose the process of creation of $\rho(\bar{x},t)$. On the basis of previous work[9], polarity correlations with growth are assigned as follows



Bone cell state	Function
S_0	Resting
S_1	Building bone
S_2	Resorbing bone

where ρ' is an average over some suitable time, t_1 and t_2 are thresholds, and the rate of cellular activity in S_1 and S_2 is assumed to be proportional to the magnitude of ρ'. This scheme has been applied to the results of McElhaney[10], who subjected a whole human femur to a periodic load and measured $\rho(\bar{x},t)$. The dotted femoral outline in Fig. 1 results from connecting points plotted from the original femoral surface with a direction and magnitude proportional to each surface charge. Modelling is produced in response to $\rho(\bar{x},t)$, and the integrity of the femur is preserved. If the measured $\rho(\bar{x},t)$ was unrelated to bone adaptability, we would expect a random pattern to occur. Modelling rather than remodelling is expected here because, while the femur is anatomically normal, the loading is abnormal, for muscular effects were not included.

Further tests of these propositions require more measurements of $\rho(\bar{x},t)$ and *in vitro* studies of the interaction of charged surfaces and cells. It is clear that further study of a link between the piezoelectric effect and bone adaptability is necessary.

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Dual Innervation of the Pineal of the Fowl, *Gallus domesticus*

THE occurrence of nerve fibres in the pineal of the fowl was once thought unlikely[1], but it is now accepted that many fine neurites are present[2,3]. Although myelinated fibres have been observed[4], most are unmyelinated and many of them contain the cored vesicles[4,5] which are characteristic of adrenergic nerves. In mammals innervation is probably exclusively sympathetic from cells originating in the superior cervical ganglion[3], but sympathectomy experiments have suggested that different mechanisms of stimulation may exist in the chicken[6].

In our investigation male and female Brown Leghorns aged 2 months and 6 months were decapitated and the pineal glands were removed as rapidly as possible. Fresh frozen cryostat sections were stained by a modification[7]

of the fluorescent formaldehyde condensation method for catecholamines using 0·1 per cent alcoholic solution of sodium borohydride and water to control specificity. Some birds were injected subcutaneously 2 h before killing with 10 mg per kg body weight of reserpine, a specific, pharmacological, catecholamine depleting agent.

Frozen sections of the pineals were subjected to the Coupland and Holmes method[8] for cholinesterase, control sections being treated with 0·04 mM aqueous solution of isopropylpyrophosphoramide (iso-OMPA), an inhibitor of pseudocholinesterase, and 0·5 mM-aqueous BW284C51, an inhibitor of acetylcholinesterase. Confirmation of the histochemical results was obtained by applying the cholinesterase methods of El-Badawi and Schenk[9] and of Namba, Nakamura and Grob[10].

The formaldehyde condensation method revealed numerous strongly fluorescing nerve fibres (Fig. 1) in the trabeculae in both perilobular and perifollicular locations. Fluorescent material also seemed to be located in the parenchymal cells. Fluorescence was suppressed by the above inhibitors and by pretreatment with reserpine. Auto-fluorescence was not observed in the pineals with light of between 300 to 500 nm wavelength.

Optimum conditions of incubation in the Coupland and Holmes method were found to be *p*H 6·2 for 6 h at 37° C when this procedure caused a linear precipitation of copper sulphide in the perilobular and perifollicular septa (Fig. 2). The specificity of the method for acetylcholinesterase was confirmed by the failure of iso-OMPA to suppress the reaction and the marked inhibition by BW284C51. Because fine nerve fibres occur throughout the trabeculae, we could not entirely eliminate the possibility that the relatively diffuse reactions may have been associated with some tissue component other than the nerves, but this seems unlikely. Additional evidence of the presence of both cholinesterase and neurites in the septa was provided by the method of El-Badawi and Schenk[9], although this did not demonstrate such delicate fibres as the preceding and the silver combination procedure of Namba, Nakamura and Grob[10].

These results show that the avian pineal, like the mammalian, has an adrenergic innervation, and they substantiate what had previously been suspected from ultrastructural examinations of the gland[4,5]. The presence of acetylcholinesterase in avian pineals, however, is of considerable comparative interest because several investigations[11–13] have failed to find this enzyme in the pineals of several mammalian species. It has been suggested[14] that an evolutionary shift from the purely cholinergic innervation of lower vertebrate pineals to the adrenergic innervation of mammalian pineals occurs as a corollary to the evolution of the solid parenchymal pineal from the saccular type. Our observations suggest that this may be correct because the domestic fowl has a pineal with intermediate structural characteristics and which may be innervated by both cholinergic and adrenergic fibres.

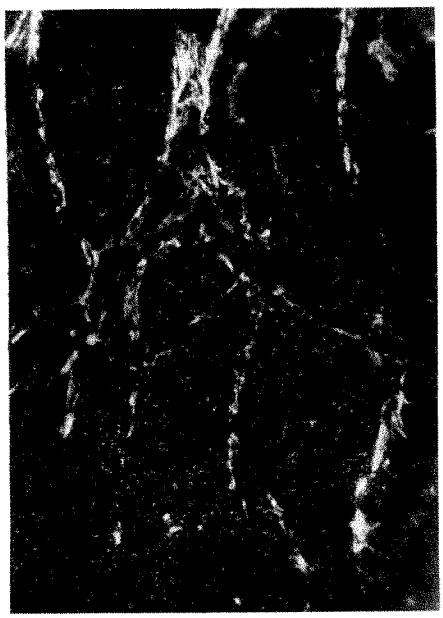


Fig. 1. Distribution of adrenergic nerve fibres in the perilobular and perifollicular septa of the pineal of the domestic fowl. Fluorescent formaldehyde condensation method. ×65.

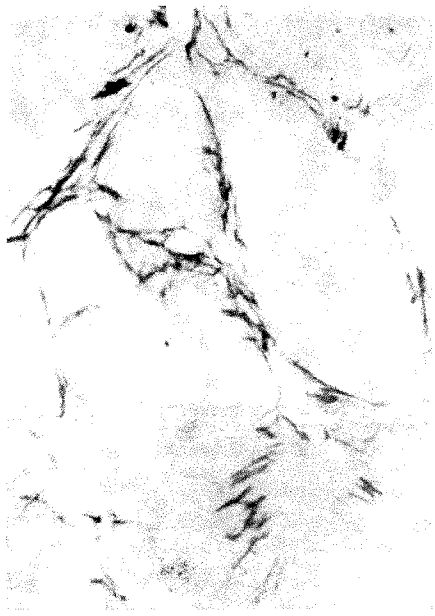


Fig. 2. Acetylcholinesterase reaction of neurites in perilobular and perifollicular locations in the pineal of the fowl. Coupland and Holmes method[8]. ×65.

Furthermore, this double innervation may have some bearing on the finding[6] that sympathetic innervation is not essential for the control of hydroxyindole-O-methyl transferase activity in the fowl's pineal. These divisions of the autonomic nerves usually produce responses opposite in direction, but in some organs they have synergistic effects. Unfortunately our findings do not explain the origin of the cholinergic fibres, for, although adrenergic fibres are undoubtedly post-ganglionic sympathetic, cholinergic fibres may represent pre or post-ganglionic sympathetic or parasympathetic efferent fibres. We have never observed neurones during the histological examination of large numbers of pineal glands from domestic fowls, so it is improbable that the cholinergic fibres are pre-ganglionic sympathetics. Elucidation of the origin and pathway of these cholinergic fibres would be of considerable interest in relation to the functional control of the pineal organ.

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Wind and the Direction of Nocturnal Songbird Migration

MIGRATING birds do not seem to be drifted off course by light to moderate unfavourable winds[1–4]. Birds tend to migrate with tail winds, and various people have proposed that the migrants reduce chances of drift by selecting following winds for their particular goal-directed flights. Some reports, however, support the view that migrating songbirds (passerines) are drifted off course by unfavourable winds[5–8]. Without adequate knowledge of the goals of the birds of a given flight, both claims are debatable. We have recently obtained, by a new technique, data that clarify the issue.

During the spring and autumn of 1969 at Athens, Georgia, and at Lake Charles, Louisiana, we used a 20×60 telescope directed up a narrow light beam to observe the passage of nocturnal migrants[9]. This technique is ideally suited for the study of low-level nocturnal migration (below 700 m). We also monitored the migrations on United States Weather Bureau WSR-57 radar[10]. Winds aloft were determined at 0000 GMT and 1200 GMT by radiosonde (radar tracked balloon) and at 0600 by pibal (visually tracked balloon). We recorded surface winds hourly. All statistical analyses of flight direction data were done according to Batschelet[11].

We have selected seven nights in spring and five nights in autumn to show the influence of different wind directions on the directions of the migrants as they passed through the light beam (Table 1). All nights had clear to partly cloudy skies. Figs. 1 and 2 show significant positive correlations between flight direction and wind direction at the surface ($r = 0{\cdot}984$, $P < 0{\cdot}001$), and at 305 m above the ground ($r = 0{\cdot}932$, $P < 0{\cdot}001$). On two occasions when the winds shifted direction during the night, the bird flight directions changed accordingly (Fig. 3). On these two nights the winds changed direction about 70°, and in both cases the flight directions of the migrants

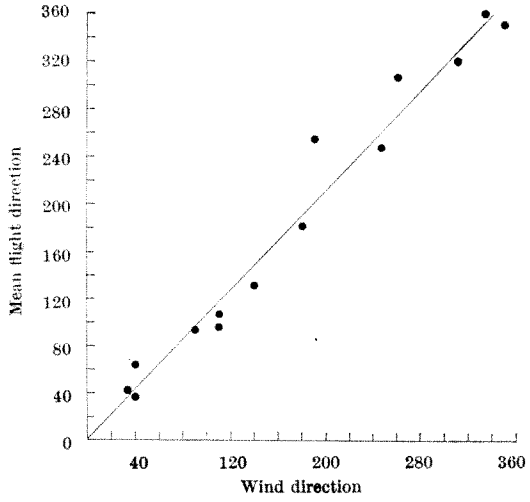


Fig. 1. A plot of the migration direction (mean vectors) on the corresponding direction toward which surface winds were blowing. See Table 1 for data.

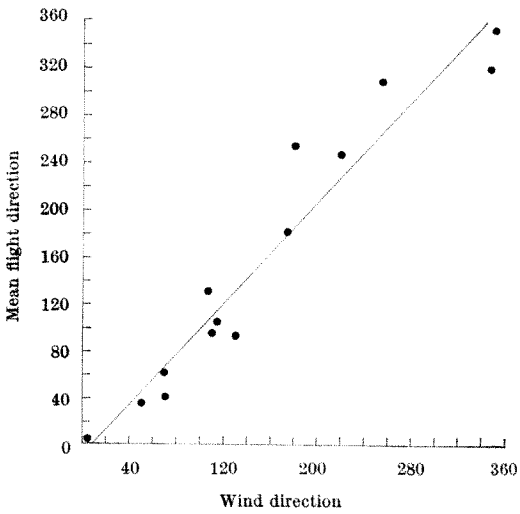


Fig. 2. A plot of the migration direction (mean vectors) on the corresponding direction towards which 305 m winds were blowing.

shifted significantly with the wind (May 10–11, $F = 0{\cdot}105$, $P < 0{\cdot}001$; September 22–23, $F = 0{\cdot}307$, $P < 0{\cdot}001$). These data show that at night songbird migrants in both spring and autumn tend to fly with the wind regardless of its direction. Movements in directions not appropriate for the season, however, are often of lower magnitude.

Table 1. INFLUENCE OF WIND ON DIRECTION OF FLIGHT

Night	Wind direction and velocity: Surface (knots)		305 m (knots)		No. of birds	Vector resultant of flight directions: Azimuth	Length (per cent)	Angular deviation
6–7 April	290°	10	295°	25	19	106°	93·0	21°
20–21 April	80	8	75	10	7	308	99·4	6
21–22 April	215	7	250	31	18	41	96·3	15
27–28 April	155	6	180	30	11	1	87·2	29
9–10 May	270	6	310	23	18	94	96·7	14
10–11 May	220	10	230	34	13	38	95·6	16
10–11 May	290	7	290	16	16	95	92·6	22
12–13 May	10	4	0	10	15	255	76·3	39
11–12 September	65	7	40	10	116	248	92·2	22
22–23 September	220	5	250	3	163	63	90·3	25
22–23 September	320	5	285	5	75	133	87·2	29
9–10 October	130	4	165	15	106	322	97·0	14
10–11 October	170	6	170	22	46	356	92·1	23
13–14 October	0	10	355	19	146	182	94·8	18

The only other direct visual studies of the direction of nocturnal migration—involving Moon watching[12,13]—also suggest that passerine nocturnal migrants tend to fly downwind. By direct visual observation we have confirmed that the misty (finely stippled) display of echoes on WSR-57 radar is produced by passerine migrants flying individually in the night sky[10]. This pattern is similar to that described by others working with radars of 10 cm wavelength[3–5,8]. The echoes from songbirds at night are nearly impossible to track on radar and, moreover, their density is usually so great during migration seasons that virtually no directional information can be obtained. In contrast, on WSR-57 and other 10 cm radars, shorebirds and waterfowl generally give strong, coherent dot echoes that can be easily tracked for several miles.

We propose that the radar evidence gathered in support of the idea that songbird nocturnal migrants compensate for wind drift might be more appropriately attributed to flocks of shorebirds, waterfowl or both. In support of this idea we give the following evidence. The proponents of the compensation-for-drift theory have not consistently employed simultaneous direct visual methods to confirm the identity of the birds responsible for the echoes they have tracked, and consequently some confusion about echo identification has resulted. Echoes from some European passerine migrants (such as thrushes) cannot be distinguished from those of shorebirds[14,15]. It is clearly stated in several reports on radar tracking

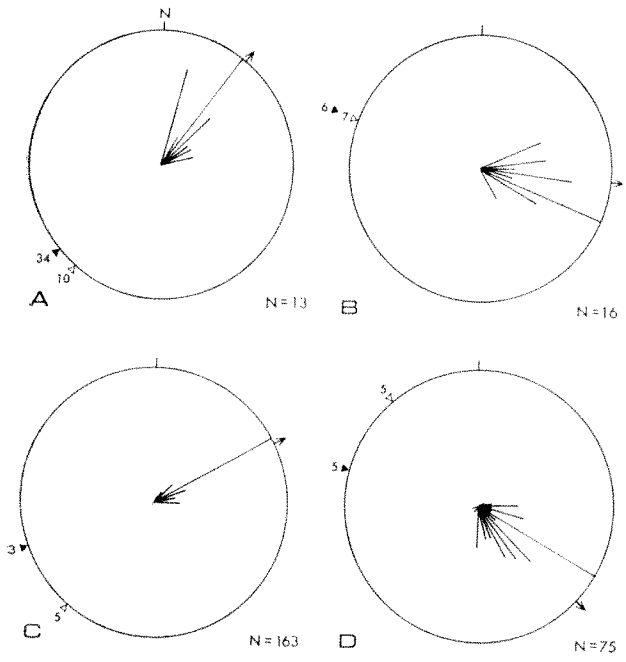


Fig. 3. Diagrams showing bird directions on two nights when winds shifted. May 10–11: *A*, 0240–0252 GMT; *B*, 0620–0645; September 22–23: *C*, 0120–0135; *D*, 0615–0645. Vector diagrams are plotted so that the radius equals the greatest number of birds in any 7·5° sector. The arrowhead is the mean direction, and the open and solid triangles represent surface and 305 m winds, respectively.

of migrants that the authors concentrated more on discrete dot echoes than on the dense pattern of fine echoes attributed to songbirds[1,16,17]. This practice has probably resulted in the selection of larger birds, flocks of birds and birds at higher altitudes. Furthermore, most radars give an inadequate representation of the migration at low altitudes (below 700 m) where most passerines fly[8]. Although our data show that small passerine nocturnal migrants often do not compensate for wind drift, preliminary results indicate that ducks, geese and shorebirds can do so at night when unfavourable winds are light to moderate. These findings support Pennycuick's assertion that crosswinds of moderate strength deflect slow birds through a large angle, but have less effect on faster birds. In general, the slower (and thus smaller) the bird, the more it is affected by unfavourable winds[18]. Our finding that songbird migrants often fly downwind regardless of the direction of the wind in the southern United States accounts for such phenomena as reversed migrations (a general movement in the direction opposite to that normal for the season) and the appearance of migrants far from their normal migration routes. The importance of proposed stellar orientation mechanisms must be re-evaluated in light of the influence of wind direction on the direction of nocturnal passerine migration.

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Development of the Brain depends on the Visual Environment

IN a normal cat, neurones of the visual cortex are selective for the orientation of lines and edges in the visual field, and the preferred orientations of different cells are distributed all around the clock[1]. Hirsch and Spinelli[2] have recently reported that early visual experience can change this organization. They reared kittens with one eye viewing vertical stripes, the other horizontal, and found that out of twenty-one neurones with elongated receptive fields all were monocularly driven, and in all but one case the orientation of the receptive field closely matched the pattern experienced by that eye.

We began a related project about a year ago and this is a preliminary report of our results. Our approach was rather different from that of the group at Stanford. We allowed our kittens normal binocular vision in an environment consisting entirely of horizontal or vertical stripes. The kittens were housed from birth in a completely dark room, but from the age of 2 weeks they were put into a special apparatus for an average of about 5 h each day. The kitten stood on a clear glass platform inside a tall cylinder the entire inner surface of which was covered with high-contrast black-and-white stripes, either horizontal or vertical (Fig. 1). There were no corners to its environment, no edges to its floor and the upper and lower limits to its world of stripes were a long way away. It could not even see its own body, for it wore a wide black collar[3] that restricted its visual field to a width of about 130°. The kittens did not seem upset by the monotony of their surroundings and they sat for long periods inspecting the walls of the tube.

We stopped this routine when the kittens were 5 months old, well beyond the "critical period" in which total visual deprivation causes physiological deficits[4]. From that time the cats were taken for several hours each week from their dark cage to a small, well-lit room, furnished with chairs and tables, where we watched their visual reactions.

At first they were visually extremely inept, whether they had been exposed to vertical or horizontal stripes. Their pupillary reflexes were normal but they showed no visual placing when brought up to a table top and no startle response when an object was thrust towards them. They guided themselves mainly by touch and were frightened when they reached the edge of the surface they were standing on. But they quickly recovered from many of these deficiencies, which were probably visuomotor problems of the kind that Held and Hein described[5]. Within a total of about 10 h of normal vision they showed startle responses and visual placing, and would jump with ease from a chair to the floor.

On the other hand, some of their defects were permanent. They always followed moving objects with very clumsy, jerky head movements and they often tried to touch things moving on the other side of the room, well beyond their reach. Perhaps most telling of all, despite their

active, and as time went on increasingly frenzied, visual exploration of the room, they often bumped into table legs as they scurried around.

There were, moreover, differences between cats reared in horizontal and vertical environments. They were virtually blind for contours perpendicular to the orientation they had experienced. They showed no startle response for an approaching 'Perspex' ('Plexiglas') sheet covered with black stripes, nor would they visually place on such a pattern, if the stripes were of the inappropriate orientation. The differences were most marked when two kittens, one horizontally and the other vertically experienced, were tested simultaneously with a long black or white rod. If this was held vertically and shaken, the one cat would follow it, run to it and play with it. Now if it was held horizontally the other cat was attracted and its fellow completely ignored it.

We moved on from behavioural studies to neurophysiology when the cats were 7·5 months old. They were anaesthetized with nitrous oxide and paralysed with succinyl choline while we recorded from single neurones in the primary visual cortex, using sodium chloride filled micropipettes. The refractive states of the eyes was corrected with contact lenses, spectacle lenses and 3 mm artificial pupils. There was no evidence of severe astigmatism, which might have explained our behavioural findings.

We used thin bright slits or edges to plot receptive fields on a screen 114 cm from the cat. The luminance of the background was about 5 cd. m^{-2} and of the bright target about 17 cd. m^{-2}. Our initial procedure was to make one long penetration deep into the medial edge of the post lateral gyrus, studying every single neurone encountered. We found units on the average about 80 μm apart, so presumably our electrode was not specially selective for the cells from which it recorded. After the long penetration we moved the electrode to other positions in area 17 and sampled just a few neurones at each place. Our exploration thus covered many cortical columns[1] and quite a large area of the visual field around the area centralis.

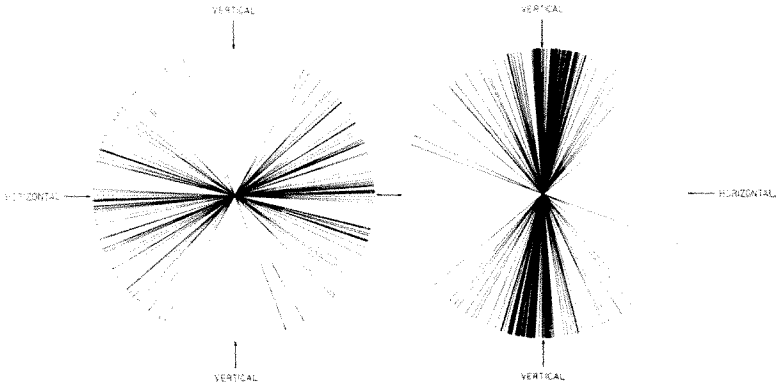


Fig. 2. These polar histograms show the distributions of optimal orientations for fifty-two neurones from a horizontally experienced cat on the left, and seventy-two from a vertically experienced cat on the right. The slight torsion of the eyes, caused by the relaxant drug, was assessed by photographing the pupils before and after anaesthesia and paralysis. A correction has been applied for torsion, so the polar plots are properly orientated for the cats' visual fields. Each line shows the optimal orientation for a single neurone. For each binocular cell the line is drawn at the mean of the estimates of optimal orientation in the two eyes. No units have been disregarded except for one with a concentric receptive field and hence no orientational selectivity.

So far we have studied 125 neurones from two cats, one horizontally, the other vertically experienced. Of all these units, only one did not have distinct orientation selectivity and it had the action potential waveform and concentric, monocular receptive field of a projection fibre from the lateral geniculate body. About 75 per cent of cells, in both cats, were clearly binocular and in almost every way the responses were like those in a normal animal. The distributions of preferred orientation, however, were totally abnormal (Fig. 2). Not one neurone had its optimal orientation within 20° of the inappropriate axis and there were, *in toto*, only twelve within 45° of it. This anisotropy is highly significant ($P < 0{\cdot}00001$: chi-squared test).

Evidently the visual experience of these animals in early life has modified their brains, and there are profound perceptual consequences. But we do not think that there is merely passive degeneration of certain cortical neurones because of under-activity. For we did not notice any obvious large regions of "silent" cortex, corresponding to the "missing" cortical columns. It seems instead that the visual cortex may adjust itself during maturation to the nature of its visual experience. Cells may even change their preferred orientation towards that of the commonest type of stimulus; so perhaps the nervous system adapts to match the probability of occurrence of features in its visual input.

We thank Patrick Jackson and Ian Creese for building the striped tubes and the MRC for research grants.

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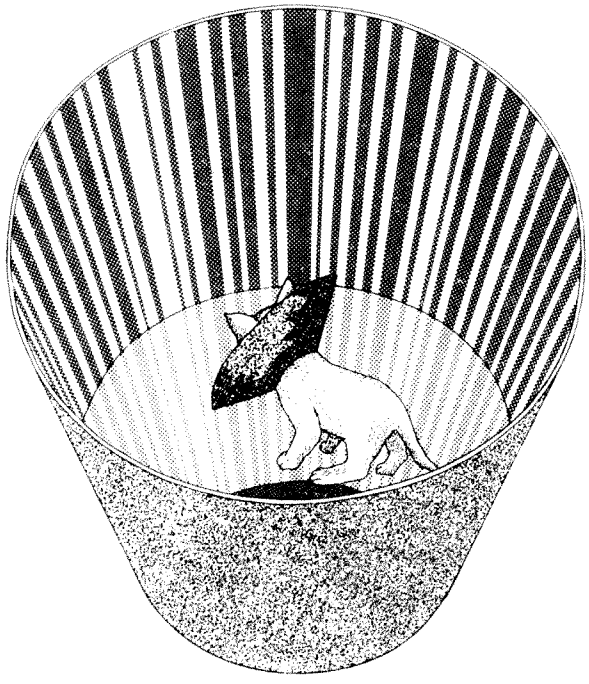


Fig. 1. The visual display consisted of an upright plastic tube, about 2 m high, with an internal diameter of 46 cm. The kitten, wearing a black ruff to mask its body from its eyes, stood on a glass plate supported in the middle of the cylinder. The stripes on the walls were illuminated from above by a spotlight. The luminance of the dark bars was about 10 cd. m^{-2} and of the bright stripes about 130 cd. m^{-2}: they were of several different widths. For this diagram the top cover and the spotlight have been removed from the tube.

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Book Reviews

BERKELEY *REDIVIVUS*

Natural Law and the Structure of Matter
By Werner Heisenberg. Pp. 45. (Rebel: London, August 1970.) 18*s*.

IN 1919, during the street fighting in Munich, while the din of shooting filled the air, a schoolboy called Werner Heisenberg, installed on the roof of the seminar building, was reading the *Timaios*—a near enough approximation to the prophet receiving the law on the mountain amid thunder and lightning. In 1964, with a prodigious career of creative work behind him, Werner Heisenberg chose to ascend another height, this time the very Hill of Pnyx, to proclaim from this hallowed ground the final triumph of Plato. This sermon has been preached before, by a famous bishop of Cloyne, among others, who thought he had routed atheism, just as Heisenberg now fancies he has liquidated its modern avatar, dialectical materialism. Berkeley's judgment was dimmed by his new theory of vision: Heisenberg is led astray by his new theory of matter.

Persiflage won't do, however, with a man of Heisenberg's stature. Every one of his pronouncements deserves close scrutiny—though not every one will bear it. In trying to expose the weak point of his plea for Platonism, I am reminded of Diderot's dictum about idealism being the scandal of philosophy, not so much because it is absurd as because it is hard to refute by rational argument. How could one find fault with Heisenberg's brilliant sketch of the course of Greek philosophy, or his penetrating characterization of the essential difference between it and modern science? Yet, on closer examination, the whole picture, dazzling as it is, shows itself utterly unreal, just because it is drawn from a radically idealistic point of view, with complete disregard of the material circumstances which alone gave the events and people substance and life. From the dramatic struggle of mankind for the control of nature he makes a shadow-play of idle philosophizing—thus demonstrating, much in spite of himself, the not very novel truth that disembodied ideas are mere *flatus vocis*, signs of things, not essentially different from material sensations.

When Heisenberg comes to modern physics, it is again hard to contradict him when he reminds us of the limitations of ordinary language for the description of atomic phenomena, or when he stresses, by contrast, the universality of an abstract mathematical description. Why, however, does he stop there? Why doesn't he adduce at this point the all-important fact that our mathematical concepts are also human inventions and accordingly bear the mark of their material origin? Why does he pretend to ignore the fact that the most abstract mathematical ideas only gain meaning from reference to sensory-motoric schemes? There is nothing, alas, in the Platonic trend he perceives in atomic physics but the pertinacious illusion of wonderful harmony in the mathematical structure of the laws of nature: a harmony that is very real, but appears less wonderful when it is realized that it only pertains to a deliberately schematized description of the phenomena. "The wonder is no wonder," said the old Stevin, who was an engineer.

I am sorry to conclude that there was really very little urgency to climb the Pnyx only to conjure up such weary ghosts. To this shadowy company, by the way, belongs as well the bogy of dialectical materialism that Heisenberg seems to have invented for the pleasure of slaying it. The conflict between materialism and idealism, under various forms, has played its historical part. If Heisenberg had been content to state that it has no longer any part to play in modern science, there would be no quarrel between us. Modern science operates with modern concepts—the most powerful of which, complementarity, is the direct outcome of the greatest discovery we owe to Heisenberg's genius.

L. ROSENFELD

REVOLUTIONARY TRAVELOGUE

Science in France in the Revolutionary Era
Described by Thomas Bugge. Edited by Maurice P. Crosland. (A Series of Monographs in the History of Technology and Culture, No. 7.) Pp. xiv+239. (MIT: London, February 1970.) 93*s*.

AT the turn of the nineteenth century the French scientific community centred in Paris was dominant in the world of science to a degree that no national scientific establishment has been since, or had ever been before, the fifty-year span of which Napoleon's rise to power marks the mid-point. The vitality of scientific life alone ought to be evidence enough to redress the picture conventionally drawn by political historians which represents the Directory to have been a time of decadence between the republican élan of the year II and Napoleon's military receivership of the revolution. So, too, must its unslackening vigour in imperial France give pause to scientists and liberals who like to believe in some functional relation between science and liberty.

Pending the day when this history may be fully written, Maurice Crosland, author of an excellent study of Napoleonic science, *The Society of Arcueil* (1967), has had the happy idea of republishing major portions of the journal kept by a Danish astronomer, one Thomas Bugge (or Bÿgge as the French chose to spell it), who spent six months in Paris in 1798 and 1799 attending the sessions of an international commission, probably the first of its kind, convoked to institute the metric system, or (in the words of an official source) "to receive the meter from the hands of France". Bugge was a well known astronomer of a practical sort, then and for many years director of Tycho Brahe's one-time Royal Observatory in Copenhagen. Everybody was interested in accounts of France in the revolutionary years, and Bugge's travelogue, published in Danish in 1800, was translated into German and English in 1801.

Crosland's edition is no mere reprinting. He has based it in the main on the original English version, but where that was obscure or incomplete, he has pieced it out from the German translation, which had been overseen by Bugge himself. Crosland has also omitted (wisely, one would imagine) the account of Bugge's journey from Copenhagen to Paris and back and certain other topics of no scientific interest. Somewhat more questionable in point of editorial policy was his decision to interpolate in his own explanations lengthy passages from other contemporary observers, particularly an acidulous Englishman, Henry Redhead Yorke. Crosland's typographical devices of boldface, indentation, and separately numbered sequences in the footnotes do not always succeed in making clear to the reader at every juncture whose voice it is that is speaking, whether Bugge's, Crosland's, Yorke's, or that of some other visitor.

A little confusion, however, is a small price to pay for so intimate and vivid a scientific memoir. Primary and secondary education under the new regime, the early école polytechnique, the other technical "hautes écoles", medical education, the functioning of the Institut de

France, the Observatory in its republican eclipse, war production and an exhibition of French industry, governmental science policy and administration, the nascent metric system itself—these are the chief sights in this tour of the most highly institutionalized scene that science had yet developed, where it was first wearing its modern dress of a professional enterprise that really mattered to the state. CHARLES C. GILLISPIE

INTRODUCTION TO SOCIOLOGY

Introducing Sociology

By Peter Worsley, Roy Fitzhenry, J. Clyde Mitchell, D. H. J. Morgan, Valdo Pons, Bryan Roberts, W. W. Sharrock and Robin Ward. Pp. 416. (Penguin: Harmondsworth, Middlesex, 1970.) 15*s*.

ACADEMIC sociology has been a long time growing up. It was a relatively minor discipline until quite recently; minor in terms of the numbers of teachers and taught, and probably minor in terms of academic achievement. Its academic pretensions were huge, for it was planned by some practitioners to be no less than the general science of society of which other disciplines, like economics and psychology, were but small particular parts. At the end of the 1950s and in the early 1960s, with the rapid expansion of higher education that then occurred, sociology became an extremely popular subject, and it is now not only a large subject in its own right among honours graduates and research students but it is also an important "minor option" in many courses, particularly in science and technology. Because it is rarely taught at school, the need for good mature introductory books is obvious.

There is a problem about introductory textbooks in the social sciences, because normally a student who takes up what is, after all, predominantly a literary study could be expected to read a considerable number of books, and five or six books in a week or two will almost always give a better idea of what sociology is about than a painstaking introductory text gone through paragraph by paragraph, as presumably introductory texts to a natural science would be. Thus, the problem of introducing this subject to intelligent students of 18-plus for the first time is accentuated by a problem of methodology.

It is fair to say that Professor Worsley's collection is much better than most. It attempts to present recent research to the students as part of a process of introducing elementary sociological concepts to them, and in this way to catch their interest and to explain exactly what it is that sociologists are up to. In particular the chapter on sociological method is a model of its kind. Two drawbacks, however, remain. The first is that which is common to almost all introductory texts of sociology, an enormous, ponderous and tedious chapter on what sociology is about, in which some of the more absurd and grandiose claims still remain, though in attenuated forms. The second is a considerable weakness on the mathematical side. In so far as social sciences have academic standing, one part of their claim to intellectual achievement rests on the application of mathematical techniques to problems of the social order. This mathematical approach is virtually absent from this book. So, too, is any serious discussion of ideas as such. A French student, for example, would, I think, find this a particularly unintellectual book. Have the students perhaps been talked down to? Seeking to avoid boredom, the writers may have achieved light weight, not light heartedness.

The strongest chapter is on the family; the weakest is on education, where, disappointingly, the impression gained from a careful reading of the chapter is of a series of dispirited slogans rather than of any attempt at rigorous analysis. JOHN VAIZEY

LAB BEHAVIOUR

The Social Behaviour of Animals

By Stuart J. Dimond. Pp. 256. (Batsford: London, August 1970.) 50*s*.

THE upsurge of interest in recent years in the social behaviour of animals has been largely based on comparative field studies of various primate species. Whether openly admitted or not the hope has been to find something of the beginnings of human society. Dr Dimond offers a review of the possibilities which lie in an analytical laboratory approach—the psychologist's rather than the ethologist's. This puts more weight on the acquisition of the elements of social behaviour through early experience, and later by social motivation. Parental and sexual behaviour, aggression, status relationships and group behaviour can all be subjects for operant techniques, thus a female monkey will, for instance, press a lever which opens a window through which she can see her own infant. The strength of lever pressing gives a measure of the strength of the urge to be rewarded in this way. Cooperation between animals in obtaining rewards was first tested by Skinner; new work shows that if a rat pressing a lever for food gives a shock to another rat, the first rat's rate of pressing will go down if it can see its effect on the second one. These are all ways of measuring relationships, though one would sometimes be happier if a parallel to behaviour in the wild was more obvious.

Evidence of the operant conditioning of the autonomic system suggests to Dimond that this may be a cause of social responses. For the autonomic changes bring about changes in endogenous processes, for example, hormone production, which in their turn cause the social responses. A theory which could explain some of the effects of early experience.

All too briefly Dimond criticizes over-facile generalization from animals to man. The strength of his critique would be greater if he took more examples from the workers he takes to task.

Perhaps brevity is a fault of this book for the discussions are short and the evidence is not abundant; nevertheless, it is a useful introduction to this approach to social behaviour. J. D. CARTHY

MODELS OF THE MIND

The Computer Simulation of Behaviour

By Michael J. Apter. Pp. 180. (Hutchinson: London, August 1970.) 35*s* boards; 15*s* paper.

THIS book is intended "to examine how far one can accept the mechanist hypothesis in psychology" and "to evaluate the usefulness of computer simulation as a technique to be used by psychologists". By "mechanist hypothesis in psychology" the author has in mind the hypothesis that a machine could exhibit consciousness or self-awareness. In the final chapter on "Consciousness", an attempt is made to discuss this issue. The most significant feature of this attempt is the almost complete failure to utilize evidence or ideas drawn from the material of the preceding 150 pages on computer simulation. Even the most primitive model—that mind is to brain as program is to computer—passes unmentioned in spite of the obvious connexion it provides between the question and the study of complex computing systems. Chomsky, who, together with colleagues such as J. J. Katz, has contributed immensely to a clearer understanding of mentalism, gets no mention under this heading save for an earlier footnote (p. 138) where the reader is warned "that Chomsky's theorizing is sometimes, especially where he uses the concept 'mind', far from rigorous"!

As to the author's second objective, again there is a singular failure to juxtapose the realities of current methodologies in experimental psychology, for example,

quantitative measures of task performance, with the computer based studies he reviews. This is perhaps not unrelated to the fact that the computer programs cited are almost all drawn from the field of artificial intelligence. In these studies the question being pursued is more like "how is it possible to recognize so many versions of an *A* ?", rather than the psychologist's formulation "how long does it take to recognize an *A* ?". Among research workers active in this area (and, unfortunately, Mr Apter is not) there are very few who find it relevant to simulate models intended to account for the results obtained from such recognition tasks.

Apter's review of these programs and of their conceptual and technical basis is extremely superficial. Genuine inquirers would be well advised to read a good introductory text on computing, write and run a simple program, and then address the literature of artificial intelligence directly. Among the misleading glosses which the author puts upon this literature is to suggest (p. 30) that it has "emerged as part of a new and exciting discipline—cybernetics". The intellectual gap between artificial intelligence and cybernetics is large precisely because the kinds of programs one is interested in cannot be usefully represented as control systems. The components of a control system, comparator, reference signal, error signal, and the like, must be represented in terms of simple variables if the mathematics of control theory (the central plank of Wiener's thesis) is to be applicable to an analysis of the behaviour of the system. But the notions of comparator and reference signal in, say, pattern recognition cannot be represented in a program in such a way if the program is to achieve an interesting level of performance.

There is little doubt that artificial intelligence provides a new way of formulating and rigorously testing theories of human behaviour and of thinking about the mind. Unfortunately this book does little to illuminate that fact.

M. B. Clowes

AMERINDIAN PREHISTORY

New World Prehistory

Archaeology of the American Indian. By William T. Sanders and Joseph Marino. (Foundations of Modern Anthropology Series.) Pp. viii+120. (Prentice-Hall: Englewood Cliffs, New Jersey, August 1970.) $4.95 boards; $1.95 paper.

The literature on the prehistory of the New World is now extremely voluminous. This includes not only a large number of books on the general or regional history of Amerindians, but also a wealth of journals of general or restricted outlook—often difficult to find in European libraries. For those wishing to refer to, or recommend to students, a short general appraisal of this research on the early cultures of the Americas the literature is a problem. For this reason, this brief but meaty survey by Sanders and Marino is very welcome. If all other volumes in the "Foundations of Modern Anthropology Series" are as worthwhile as this, then it is going to be an important series of readers for students in anthropology, and to some extent for those concerned with certain other aspects of the social and biological sciences.

New World Prehistory succeeds in reviewing in surprising detail the emergence and changing culture mosaics of the Amerindians during the past 10,000 years (though acknowledging the possibility of earlier populations extending back to 40,000 years). The ecological and evolutionary aspects of early settlement and culture variation are emphasized. Associated with the text there is a chronological chart with culture change indicated on a regional basis, and there is also an extremely valuable set of maps outlining the changing patterns of environmental adaptation and social evolution occurring in these prehistoric groups.

After the introductory chapters, the authors begin with the earliest hunting and collecting level of economy and follow with chapters indicating the multilineal evolution of early agriculturalists and thence of tribes, chiefdoms, states and empires. The final chapter is a discussion of the general significance of culture change in the early New World, and to what extent the variation to be seen through time is the result of innovation (cultural evolution) and imitation (cultural diffusion). The authors discuss these fundamental questions briefly but well. They are clearly not in favour of the introduction of "new" cultural traits by trans-Pacific movements.

For those wishing to extend their reading beyond the text, there is a select series of references. This is a well produced and valuable reference work.

D. R. Brothwell

PACKAGED EVOLUTION

Evolution

By Theodore H. Eaton jun. Pp. xi+270. (Nelson: London, August 1970.) 40*s*.

In this book the author sets out on a round trip of evolutionary theory. Chapters range from a short history of the idea of evolution through the concept of species and speciation, heredity, populations in evolution, adaptation and natural selection, to the bearing of behaviour on evolution and the relevance of geographical distribution. Later chapters treat the geological record, the origin of life and aspects of vertebrate evolution including man. So much in a comparatively small space has meant a brief and superficial treatment of each topic. This raises the question: for whom is the book intended ? The blurb states that the author assumes in the reader an understanding of the range of the animal kingdom and the principles of genetics, but the book has neither enough detail nor enough bibliographical references for most third year undergraduates. Nor does it give any leads to the most modern and controversial aspects of evolutionary theory in spite of the claim on the cover that it is a thoroughly modern textbook. For instance, there is no mention of recent ideas on neutral mutations and the possible consequences to evolutionary theory. There is no analysis of the use of statistical methods in taxonomy although the author discusses problems of taxonomy at some length. Modern mathematical approaches to biogeography are not discussed in a chapter on geographical distribution.

Evolution is not for the specialist, but it can be recommended as a quick guide to conventional aspects of evolutionary theory for undergraduates at an early stage of their university career.

Wilma George

ENERGETIC JOURNAL

Journal of Bioenergetics

Vol. 1, Nos. 1 and 2. Edited by John Avery. Bimonthly. (Plenum: London, 1970.) 210*s*; $26 per volume.

At this time when all scientists are concerned with the difficulty of assimilating the published literature, there should be some justification for the creation of another journal. This new *Journal of Bioenergetics* is an example of the move away from the "general" journal to those dealing with more specialized subjects. In the present case, although "bioenergetics" could embrace a very wide range of topics the first two numbers contain 70 per cent of their space devoted to experimental work with mitochondria. If this trend continues we shall have specialization at its most extreme.

The detailed mechanism of the process whereby energy derived from some oxidation-reduction steps in the respiratory chain may be used to move ions or to synthesize ATP is unknown. This field of research is one

in which there is now an excess of bricks of information, but nobody has yet managed to construct an acceptable building from them (*Science*, **142**, 339; 1963). The reason for this situation is that the subject is very complex; there is therefore an almost infinite combination of experimental parameters.

As is proper for a journal devoted to a subject dependent on highly complex heterogeneous catalysis, the editorial and advisory boards show a wide range of expertise in different disciplines. It is to be hoped that this will be reflected in the range of the papers published and that the high proportion of morphological papers will be balanced in future numbers by papers providing information about the chemistry of the processes involved.

The journal is well printed and the quality of the photographs is adequate. If the present policy of not giving the date of acceptance of papers is continued the speed of publication will not be able to be assessed. There is no doubt that all "mitochondriacs" will feel they must add this journal to their lengthy reading list. In my view, very specialized journals could serve a useful purpose if they publish the best work from all approaches to the problem as well as providing a forum for the critical evaluation of the earlier published work. If future numbers of this journal show that this is the case, then it will have justified its creation. W. N. ALDRIDGE

PARKINSONISM

Parkinsonism

Physiology, Pharmacology and Treatment. By Donald B. Calne. Pp. xiii+136. (Arnold: London, September 1970.) 45*s*.

THE interest of the medical profession in Parkinsonism and related disorders has been revived within the past few years following the discovery that many patients suffering from this disease may be greatly helped by treatment with L-dopa. This is one of the first chronic and disabling diseases of the central nervous system in which a form of replacement therapy based upon sound pathological and pharmacological evidence has become possible. This short monograph by Dr Calne is therefore timely and, in general, it can be warmly commended as giving a succinct and topical review of the condition.

The author's short neurophysiological introduction is, in general, clear and concise, yet reasonably comprehensive, although, like many others before him, he has tended to circumnavigate skilfully the dangerous and largely uncharted territory of basal ganglia physiology. His second chapter, in which he describes the clinical features, aetiology, natural history and pathology of Parkinsonism, is much less satisfactory, largely on account of its brevity, because occasional but important clinical features of the condition, as seen particularly in post-encephalitic cases, are not mentioned. His comments on aetiology are also rather sketchy for, in a sense, he perpetuates the myth that Parkinsonism (with a capital P) may be produced by chronic trauma (sic) or that it may result from intracranial tumours, carbon monoxide poisoning, Wilson's disease or manganese intoxication. He also avoids discussing in any depth the vexed and controversial question as to whether diffuse cerebral arterial disease causes true Parkinsonism or a syndrome resembling it. Even in a monograph which, as its title implies, purports to deal essentially with physiology, pharmacology and treatment of this disorder, the informed reader deserves a more comprehensive and carefully argued commentary on aetiology; although one accepts that a definition of what one means by Parkinsonism is all-important, and although the author attempts to define the syndrome on page 18, most neurologists would agree that many of the aetiological processes referred to earlier more often give distinctive clinical syndromes which bear only a superficial relationship to the clinical condition which most would regard as classical Parkinsonism.

There are also one or two other minor points of criticism in that in chapter three, on tremor, the author fails to distinguish between action tremor on the one hand and the intention tremor of cerebellar dysfunction on the other, and, subsequently, in an otherwise admirable short summary of present views concerning rigidity, he perhaps does less than justice to recent work on alterations in the H-reflex which have been reported in patients with this disease. He is at his best in considering the pharmacology of Parkinsonism, the methods of assessing treatment, and in his comprehensive and up to date review of the place of L-dopa in treatment. All of the important and topical questions are discussed here, though he gives little if any clear guidance as to whether patients should or should not be given standard anti-cholinergic remedies along with L-dopa. It may be that the answer to this important question is not yet known; not surprisingly he has also been unable to include in this monograph reference to reports which have appeared very recently concerning the use of decarboxylase inhibitors in combination with L-dopa in patients with this disease. Nevertheless, the book is surprisingly up to date and many references to papers appearing in 1970 are included in the bibliography. The book is clearly written, the reference list is comprehensive, there is a good index, it is inexpensive, and in spite of the criticisms mentioned earlier, it is on the whole a faithful summary of current knowledge concerning the pharmacology and treatment of Parkinsonism. JOHN N. WALTON

HORMONE REGULATION

Hormones and the Environment

Proceedings of a Symposium held at the University of Sheffield, September 2–5, 1969. Edited by G. K. Benson and J. G. Phillips. (Memoirs of the Society for Endocrinology, No. 18.) Pp. xvi+629. (Cambridge University: London, July 1970.) 140*s*; $22.50.

THE increasing awareness of the importance of environmental factors in the regulation of hormonal mechanisms prompted the symposium on "Hormones and the Environment", the proceedings of which form this, the eighteenth memoir of the Society for Endocrinology. The result is a stimulating, albeit heterogeneous, collection of the twenty-three papers presented, together with the edited discussions.

There are sections dealing with the aquatic, terrestrial and biotic environments as well as neuroendocrine mediation and temporal changes in endocrine secretions. The considerable variety of subject matter in the text precludes the possibility of a single theme emerging. One requires fertile imagination to link "Intersexuality in Fishes", with "Malnutrition and the Endocrine System in Man", or with "Pheromone-endocrine Interactions in Insects". In spite of this range, however, the smoothly edited discussions of papers reveal the ability of participants to bring their own special endocrine interests to bear on the quite different problems of others. The record of the discussions is a pleasing facet of the book.

It may be argued that several of the papers in this memoir contain relatively little about hormones; notably the account of "Mechanisms of Salt and Water Transfer across Membranes in Teleosts" by Dr J. Maetz, and "Kidney Function in Desert Vertebrates" by Professor W. H. Dantzler. These two distinguished contributors, however, clearly illustrate the need for a complete background knowledge of the phenomena over which hormones may be assumed to have control.

In a symposium concerned with the environmental influences on hormonal systems, it is inevitable that

neuroendocrinological studies should have been to the fore. Contributions by Dr J. S. Tindal on "Environmental Stimuli and the Mammary Gland", Professor L. Martini on "Hypothalamic Releasing Factors", Professor W. B. Quay on "The Significance of the Pineal", and Professor H. Heller on "The Neuroendocrine Control of Water Metabolism" are notable in this respect.

The diversity of subject matter and the comprehensive manner in which it is treated within the common framework of "Hormones and the Environment" should make excellent reading for all interested workers in the field of endocrinology. The many questions raised by the contributors will undoubtedly encourage further research.

A. THORPE

ELEMENTARY OPTICS

Optical Data Processing

By Arnold Roy Shulman. (Wiley Series in Pure and Applied Optics.) Pp. viii+710. (Wiley: New York and London, June 1970.) 260*s*.

OPTICAL data processing is one of the more exciting applications of the past decade of intensive research in lasers and opto-electronics. The publishers of this text state that it "presents a thorough introduction to this new field" with "a maximum amount of physical interpretation of the basic principles". The publication of such a book would be particularly opportune but, unfortunately, this volume is far from meeting these claims. Rather, it is a compendium of elementary optics and the associated mathematics, rarely rising above the level of a first-year university course, and spiced with some practical hints on photography and holography.

Typical examples of the general mathematical verbosity are the use of three lines on p. 121 to move from $\sqrt{2\pi}/2\pi$ to $\sqrt{1/2\pi}$ and the trivial algebraic steps on p. 284, one of the sixty-one pages devoted to the elementary mathematical treatment of Ronchi gratings. The book is cluttered with much algebraic and optical trivia, yet, curiously, while eighty pages are devoted to developing Fraunhofer diffraction, the Fresnel-Kirchoff integral is quoted (on p. 389) without any derivation at all. There is an equal profusion of trivial or repetitive photographs, such as that of a rectangular aperture on p. 624 and the thirty-three Moiré patterns in appendix 6. Circumlocution extends even to the final chapter on properties and techniques for photographic reproduction, which, like the rest of the volume, could have been drastically condensed to great advantage. This book cannot be recommended, especially at a price of 260*s*.

D. J. BRADLEY

SOUNDERS AND WHISTLERS

Plasma Waves in Space and Laboratory

Edited by J. O. Thomas and B. J. Landmark. Vol. 2. Pp. 562. (Edinburgh University: Edinburgh, April 1970.) 140*s*.

THIS volume completes the published proceedings of the Røros school of April 1968, and comprises some thirty-five papers—those classified as contributions to current research rather than as reviews. Papers concerned with space rather than laboratory plasmas predominate, on both observational and theoretical aspects.

The several contributions on top-side sounders, ionosphere resonances detected by Explorer XX and the Alouette satellites, seem somewhat contradictory and confusing, until put in perspective by Benson's review of the numerous observations. The demonstration that the resonant frequencies are displaced from the integral harmonics is important for deciding between rival theories. Allowing theoretically for non-uniformities in the magnetic field is also shown to be important. A further sizable group of papers concern themselves with whistlers, both their detection and explanation. The importance of collisions in selecting the propagating (convective) modes is brought out from two angles. There are, in addition, several papers on antenna theory—these with laboratory emphasis—and two final, isolated contributions on weak turbulence and on magnetospheric drift instabilities (as the source of micropulsations).

Through using offset reproduction of an excellent typescript, this volume gives some 50 per cent more information per £ than volume one, but at the cost of closer type and occasional clumsy format. The publishers deserve congratulations for the overall good quality production at—for these days—a reasonable price. Nevertheless, I question the sense of producing current research papers this way, at perhaps double their cost in established journals. Some of the papers will no doubt retain relevance for a few years yet, but inevitably in the two years since the Røros school, many have been superseded by more recent work. As in the first volume, abstracts are annoyingly absent, so I must again deplore the lack of adequate summaries to certain of the papers. Still, for those working specifically on top-side sounders and whistlers, this volume will provide a useful collection for reference purposes, and for would-be students it supplies a convenient introduction to these topics.

M. K. WALLIS

BETWEEN THE STARS

The Interstellar Medium

By S. A. Kaplan and S. B. Pikelner. Pp. xi+465. (Harvard University: Cambridge, Massachusetts; Oxford University: London, July 1970.) 190*s*.

THOSE of us working in the field of interstellar matter have become increasingly aware of the need for a comprehensive and up to date text on this subject. During the past decade our traditional views of the interstellar medium have undergone a significant evolution. The simple, two-component picture of an interstellar medium consisting of gas and dust clouds has proved to be as inaccurate as it is naive. In addition to gas and dust, magnetic fields, cosmic rays, radiation fields and stars must be considered together as forming a system of mutually interacting components. The authors of this book have played a major part in shaping our modern view of the interstellar medium, and in their new book they expound this view in a clear and lucid manner.

The first chapter deals with interstellar hydrogen, the most abundant component of the interstellar medium. It discusses the state of ionization of hydrogen and its distribution in space, the propagation of Lyman continuum and Lyman-alpha radiation and the emission and propagation of radiowaves in the interstellar medium. Then follows a chapter on the physical state of the gas which includes a discussion of the formation of spectral lines in the interstellar gas, molecules in space and the energy balance of the interstellar gas. Next is a chapter on interstellar dust followed by one on interstellar magnetic fields and non-thermal radio emission in the galaxy. A final chapter, devoted to interstellar gas dynamics and the evolution of the interstellar medium, discusses, among other topics, the behaviour of shock fronts and ionization fronts in the interstellar gas as well as the problem of star formation from interstellar gas clouds.

The book encompasses a remarkably wide range of topics presented in a logical order with meticulous care given to detail. Theoretical discussions are often presented from first principles, and relevant observational

data are adequately summarized throughout. This makes the book a more or less self-contained thesis which could be studied independently without frequent recourse to a bibliography. A useful bibliography is, however, provided for those who need it. On the whole the authors seem to address their writing to an audience of physicists rather than one of specialist astronomers. This makes the book an invaluable text for both the specialist research worker and beginner.

My only criticism is that in a few instances the book is already out of date by five years. Omission of recent developments in the identification of interstellar molecules and certain components of cosmic dust are notable examples. This is inevitable in such rapidly evolving fields. But it seems unfortunate that the time lag between the last Russian edition and the present translation should be as long as five years. A few of the shortcomings caused by this delay are remedied in a short addendum at the end of the volume. N. C. WICKRAMASINGHE

RIFT RESEARCH

Graben Problems

Proceedings of an International Rift Symposium held in Karlsruhe, October 10–12, 1968. By J. H. Illies and St. Mueller. Pp. 316+7 plates. (International Upper Mantle Project Scientific Report, No. 27.) (Schweizerbart'sche Verlagsbuchhandlung: Stuttgart, 1970.) $23.10; DM 82.

THIS book contains the scientific contributions presented at the third symposium of the international Rhine graben research group held at the University of Karlsruhe on October 10–12, 1968. There are four papers of a general nature on the world rift system, thirty four papers on the Rhine graben and six papers on the East African rift system.

The proceedings are introduced by Leon Knopoff giving a clear discussion of the concept and importance of rigid plate tectonics. This is followed by two excellent review articles on graben tectonics, the first of a geological nature by J. H. Illies and the second geophysical by St. Mueller. These are followed by an interesting article by J. P. Rothé on the seismicity of oceanic and continental rifts. It is often forgotten that it was Professor Rothé who paved the way to the recognition of the world rift system by his observation in 1954 that the seismic zone of the African, Carlsberg and mid-Indian Ocean rifts continues around Africa to join with the mid-Atlantic rift. Rothé here gives many more interesting pieces of information including the fact that for the period 1953–1965, only 1·5 per cent of the world's total seismic energy is liberated along oceanic rifts. This is in contrast to island arcs and deep trenches where most seismic energy is liberated, this being caused by the plunging of cold, rigid plate into the upper mantle.

These four opening chapters are followed by five papers on stratigraphic evidence pertaining to the development of the Rhine graben. These are noteworthy for their dependence on data from oil companies which have enabled the production of excellent isopach maps of the region. These are followed by five more geological papers on basement and structure.

Five geothermal papers show that the Rhine graben, like other rifts, has high heat flow. These are followed by two papers on volcanism emphasizing its basaltic nature. Next come four papers on seismotectonics; two of these are on seismicity studies and two on geodetic levelling and also plans for laser distance experiments (Kuntz *et al.*). Ahorner has collected data from 1600 to 1967 in a very comprehensive survey of the seismicity of the Rhine graben. This indicates that in the neighbourhood of Mainz, the active rift zone of the upper Rhine turns north-west towards Amsterdam rather than continuing north-north-east through the Hessian depression. For the period 1600 to 1967 the most seismically active region was between Köln and Aachen. It must be remembered, however, that the seismic activity is relatively very small, the seismicity of the whole of the Rhine area being about 1 per cent of that of southern California!

German scientists have been especially active in pursuing seismic studies including application of refraction and reflexion techniques, the use of spectral transfer ratios of long period body waves and the dispersion of Rayleigh waves. This has led to a very complicated structure for the crust beneath the Rhine graben. Emphasis is placed on a "cushion" (7·6 to 7·7 km s^{-1}) above the "Moho" beneath the rift. Above the cushion, evidence is claimed for two low velocity channels in the crust of 5·5 and 6·2 km s^{-1} respectively.

Papers on gravity, magnetic surveys and palaeomagnetism are followed by a final Rhine graben section entitled "Magneto-Telluric Deep Soundings". These reports confirm the existence of a high conducting zone beneath the rift, but there remain some uncertainties concerning its depth and temperature.

The book concludes with six articles on the East African rift system. These include three papers (by Knetsch, Tazieff and Schaeffer) giving preliminary results of recent reconnaissance expeditions to the inhospitable Afar triangle of Ethiopia. The book ends with a very useful article by Wohlenberg on the seismicity of the rift system in East Africa.

These conference proceedings show the thoroughness and intensity of the work on the Rhine graben; the German scientists are to be congratulated on the way in which they have assembled so much material and conducted new geophysical experiments, many of them at small or moderate costs. It is disappointing to see how few geologists (with the notable exception of Laubscher) interpret their observations in the light of Knopoff's opening lecture on plate tectonics. Otherwise, criticisms are minor. Many of the papers are in German; in these days when there is so much literature to be read it is regrettable that the editors did not insist on at least abstracts in English for the German papers and abstracts in German and French for the English papers. There are no lists of references at the ends of each paper; they are collected together at the end of the book. I found this irritating, especially on discovering several omissions and inaccuracies. The book is well produced, has clear illustrations and some beautiful photographs. It is somewhat expensive but is well worth it. R. W. GIRDLER

MODERN VOLCANOLOGY

Volcanoes

By Cliff Ollier. (An Introduction to Systematic Geomorphology, Vol. 6.) Pp. xiv+177. (MIT: Cambridge, Massachusetts and London, June 1970.) 68*s*.

CLIFF OLLIER has contrived to write an interesting and readable text, in which, although the subject matter is never profoundly treated, he nevertheless conveys a sense of appreciation beyond the evident limitations of length.

There are, however, some sections, for example chapters one and eight, which, although they are no doubt necessary for the sake of completeness, are unduly elementary and contribute little to a publication of restricted length. Nevertheless, the book is both useful and justified, for there is no modern text on vulcanology. The only comparable publications are reprinted or revised older works, but the considerable interest and advances in the study of vulcanology justify an entirely new publication.

The list of references is wide, representative and useful. B. C. KING

Correspondence

Restrictions on Soviet Scientists

SIR,—The depressing story of the attempts by Dr Medvedev to accept an invitation to a conference in Sheffield (*Nature*, September 19) prompts me to raise the following issues. I have been to three conferences during the past month in various parts of Europe. At each conference papers by Soviet scientists were listed in the programme and abstracts were included; on each occasion none of the Russians turned up. One of these conferences was in Yugoslavia and on that occasion neither the Russian nor a Czech contributor was able to turn up, although both were due to give major addresses. At each of these conferences the organizers were caused embarrassment as well as regret by the inability of the speakers to come, and last-minute changes had to be made to the timetable in consequence. A further important consequence of these cancellations is that Western scientists are deprived of the benefit of meeting their Soviet opposite numbers.

At one of the conferences I attended, a letter was sent to the organizer by a Russian who was unable to come (such letters are not always sent, and sometimes the speakers simply do not turn up without notice or explanation). In the letter the scientist concerned expressed his regret at his inability to come and added that he was looking forward to seeing his contribution printed in the official proceedings of the conference. The question then arose whether his contribution should in fact be printed, and it was agreed that this should not be done. It is, of course, hard on a Soviet scientist, who would have liked to attend a conference but has been prevented by the failure of his authorities to grant permission, not to see his work printed. On the other hand, as I pointed out, if the work is printed then the Soviet authorities receive, as it were, indirect kudos without even having put up the resources to enable the speaker to deliver his paper in person; the Soviet authorities would have progressively less reason to permit their scientists to go abroad if their work is published as though they had been at the conference.

I submit therefore that it would be likely to be of long-term benefit to our Soviet colleagues and make it more probable that they would more often be allowed to come in future, if as a matter of principle conference organizers were to decline to print contributions from people who have not actually turned up in person at the conferences in question.

I should be interested to know the views of other readers concerning this suggestion.

Yours faithfully,

R. W. CAHN

University of Sussex,
Falmer, Brighton, Sussex.

Accelerating Somatic Cell Genetics

SIR,—In your leading article on October 24 (*Nature*, **228**, 318; 1970), you attribute to Watkins and myself a discovery that we did not make. Watkins and I were the first to fuse together cells of human and murine origin, and we observed that in the fused cells DNA replication was markedly inhibited in the human nuclei. However, the discovery that human chromosomes are preferentially lost from proliferating man–mouse hybrid cells was not made by Watkins and myself, but by Mary C. Weiss and Howard Green (*Proc. US Nat. Acad. Sci.*, **58**, 1104; 1967).

Yours faithfully,

HENRY HARRIS

Sir William Dunn School of Pathology,
University of Oxford.

Population in Nigeria

SIR,—I wish to make some comments on your leading article of September 12 relating to the discussion at the meeting of the British Association (*Nature*, **227**, 1073; 1970). You are, of course, correct in your reporting, but there is a possible danger of misunderstanding from the comment which you attributed to me which states that I doubt whether Africa has a population problem. I would not like to leave an impression which would be wrong. What I did say was that we are sceptical of the great efforts being made on population control in Africa by some advanced countries. We are sceptical because our main problem is not that of high birth rate which would likely be followed by a great increase in population, but rather the problem of a high infant mortality rate.

To be more specific, may I explain in this way: the population of Nigeria is calculated at about 60 million. A large majority of these 60 million are young people of not more than 15 years of age and most of these young people will never live beyond the age of 15—this is a medical fact. The result is that the effective population of Nigeria as regards her manpower is certainly not 60 million but may be between 20 and 30 million. It is obvious therefore that our concern should not be, as was suggested in the question directed to me, that we should control birth rate by limiting the number of children in each family (which is the current practice in population control) but that of preventing the high infant mortality rate.

Yours faithfully,

T. ADESANYA IGE GRILLO

Department of Anatomy,
University of Ibadan, Nigeria.

Evolution of Rifting in Africa

SIR,—In his interesting comments (*Nature*, **227**, 699; 1970) on our paper[1], it seems that McConnell thinks we consider the phenomenon of sea floor spreading to be taking place in the rift zone of East Africa. This is not so. The purpose of our communication was to bring together evidence from seismicity, age of faulting, age of volcanoes and interpretations of gravity anomalies and to suggest that they together indicate that rifting in East Africa is probably associated with attenuation of the lithospheric plate. This process, we envisage to precede spreading. The rift in East Africa cannot be a site of spreading for

it is likely from geophysical and geological data that sialic Pre-Cambrian material underlies the rift floor. We stated that the positive gravity anomaly indicates the presence of an intrusive body which could be within 1 km of the rift floor . . . this suggests that extreme crustal thinning has taken place, but as yet there is no crustal separation as in the Red Sea. Unlike McConnell who uses the term "spreading" twelve times, we were careful to use it only once and then in parenthesis with the word analogous.

We agree with the comments on the age of volcanoes and McConnell's doubts are expressed by the long error bars in our Fig. 4. We plotted all the age data available at the time of writing and it remains to be seen whether the trend is born out by future data. We were careful not to use these data alone but to bring together five sets of data to support the suggestion of plate attenuation.

Yours faithfully

R. W. Girdler
J. D. Fairhead
R. C. Searle
W. T. C. Sowerbutts

School of Physics,
The University,
Newcastle upon Tyne, NE1 7RU.

[1] Girdler, R. W., Fairhead, J. D., Searle, R. C., and Sowerbutts, W. T. C., *Nature*, **224**, 1178 (1969).

Obituary

Professor A. E. Alexander

Albert Ernest Alexander, professor of physical chemistry at the University of Sydney, died on May 23 1970. In Britain and Australia, he made important contributions to colloid and surface chemistry.

Alex, as he was always known, was born in Ringwood, Hampshire. He began his university career at Reading in 1932, continuing at King's College, Cambridge, in 1935. After obtaining a first in chemistry in Part II of the Natural Science Tripos, he joined the Department of Colloid Science in Cambridge under E. K. (now Sir Eric) Rideal, a step which largely determined the direction of his future research; his fellowship thesis at King's College in 1939 was entitled "Molecular Orientation and Reactions in Monolayers". During the war, although much of his time was taken up by war research, he continued to produce papers on surface chemistry and allied topics such as liquid interfaces, interfacial tension and ageing effects, built-up films, the structure of films, the role of hydrogen bonding, and the application of the Gibbs adsorption equation. On the biological side he was interested in the penetration of membranes and surfaces and in the properties of protein films.

In 1944 he was appointed an assistant director of research in the Department of Colloid Science at Cambridge. At this time, he was very successful in fostering friendly relations with the chemical industry. The summer school on colloid science, which he organized in collaboration with the Royal Institute of Chemistry in 1945, is still spoken of warmly.

It was a great loss to Cambridge science when he accepted the Foundation chair of applied chemistry in the New South Wales University of Technology at Sydney, becoming the first head of the Chemistry School there and dean of the Faculty of Science. In 1956, having built up a flourishing department, he moved to the chair of physical chemistry at the University of Sydney, where he remained until his death. In Australia, as in England, Alexander was enthusiastically concerned with surface chemistry and its application to such things as water evaporation, the efficiency of cattle dipping preparations and the effectiveness of drugs. He was author or co-author of more than 130 papers on these subjects.

Alex married happily twice but had much more than his fair share of personal tragedy. Cathie, his first wife, died in 1965 after a long illness and in 1967 he married Giesela, the widow of a university colleague. Shortly afterwards, his daughter Naomi was killed in a car crash. With his hidden resources he was soon back in action, but tragedy struck again late in 1969, when he himself became fatally ill. He leaves Neil, the son of his first marriage, Giesela and her two children.

Announcements

University News

Professor G. C. McVittie, professor of astronomy at the University of Illinois, will be a visiting professor at the **University of Kent** during 1971.

Miscellaneous

Professor Ralph W. Chaney, University of California, Berkeley, has been awarded the **Paleontological Society Medal.**

Mr Ray Creasey, director of Advanced Systems and Technology, British Aircraft Corporation Preston Division, has been elected a fellow of the **American Institute of Aeronautics and Astronautics.**

Lord Penney of East Hendred, rector of the Imperial College of Science and Technology, University of London, has been awarded the **James Alfred Ewing medal** for his services to engineering. The **1970 S. G. Brown award and medal** has been won by **Dr D. C. Simpson** for his work on the development of externally powered artificial limbs.

The report of six new species of seaweed found in South Africa (*Division of Sea Fisheries Investigational Report No. 86*, Cape Town, 1970), which was mentioned in *Nature* (**227**, 779; 1970), unfortunately contained several errors and is to be reissued at a later date.

An **American Red Cross National Fractionation Center** has been established under the joint sponsorship of the American Red Cross and the National Heart and Lung Institute. The centre will provide facilities and staff for the large scale isolation from blood and other tissues of specialized proteins, plasma and cellular enzymes, membrane fractions and so on, which are not available from commercial sources. Further information can be obtained from Dr G. A. Jamieson, American Red Cross Blood Research Laboratory, 9312 Old Georgetown Road, Bethesda, Maryland 20014, USA.

Entries are invited for the £50 prize of the **Plastics and Polymer Group of the Society of Chemical Industry,** awarded annually for the best paper submitted on any aspect of pure or applied polymer science. The paper must be in English, and may be a description of original

work, an essay or a review. Entrants must be under 35 years old at the closing date, March 31, 1971, and if from overseas must be members of the society, although all British subjects are eligible. Further information can be obtained from the Plastics and Polymer Group, c/o Society of Chemical Industry, 14 Belgrave Square, London SW1.

ERRATUM. In the review "Ion Molecule Reactions" by P. F. Knewstubb (*Nature*, **228**, 392; 1970), the word "although" in line 7 of the first paragraph should read "even if" and the date in line 9 should be 1965 and not 1964.

International Meetings

June 7–9, 1971, **Symposium of the Association for Research on Exploration and Production Techniques,** Rueil-Malmaison (M. le Secretaire du Colloque ARTEP, Division Production, Institut Français du Petrole, 1–4 Avenue de Bois Préau, 92 Reuil-Malmaison, France).

June 9–11, 1971, **Carbon-13,** Los Alamos (E. S. Robinson, PO Box 1663, Los Alamos, New Mexico 87544, USA).

June 9–11, 1971, **Radiation-induced Voids in Metals,** Albany (Professor J. W. Corbett, Physics Department, SUNY/Albany, New York 12203, USA).

June 10–11, 1971, **Metabolism and Disease,** Ottawa (Mr R. S. Meldrum, Technical Secretariat, Food and Drug Directorate, Tunney's Pasture, Ottawa, Canada).

June 14–17, 1971, **Identification and Measurement of Environmental Pollutants,** Ottawa (M. K. Ward, c/o National Research Council of Canada, Ottawa, Ontario, Canada).

June 27–July 2, 1971, **Carbon,** Bethlehem, Pennsylvania (Professor Henry Leidheiser jun, Center for Surface and Coatings Research, Lehigh University, Bethlehem, Pennsylvania 18015, USA).

July 5–7, 1971, **Rare Earths and Actinides,** Durham (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

July 5–8, 1971, **Acetylenes, Allenes and Cumulenes,** Nottingham (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

July 5–8, 1971, **Shock Tubes,** London (Symposium Secretary, Department of Aeronautics, Imperial College, Prince Consort Road, London SW7).

July 5–9, 1971, **Crystalline Growth,** Marseilles (B. Mutaftschiev, Laboratoire des Mécanismes de la Croissance Cristalline, Faculté des Sciences de Marseille, Saint-Jerome, 13 Marseille 13e, France).

July 6–9, 1971, **Major Loss Prevention in the Process Industries,** Newcastle upon Tyne (Institute of Chemical Engineers, 16 Belgrave Square, London SW1).

July 11–15, 1971, **Health Physics Society Meeting,** New York City (Lee Gemmel, Health Physics Division, Brookhaven National Laboratory, Upton, New York 11973, USA).

July 12–16, 1971, **Society for Analytical Chemistry Conference,** Durham (Mr C. A. Johnson, Honorary Secretary SAC Conference, 9–10 Savile Row, London W1X 1AF.

July 13–16, 1971, **Synthesis in Organic Chemistry,** Cambridge (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

July 18–23, 1971, **Fluorine Chemistry,** Durham (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

July 20–23, 1971, **Mechanized Information Storage and Retrieval Systems,** Cranfield (Mr Cyril Cleverdon, Cranfield Institute of Technology, Cranfield, Bedford).

British Diary

Monday, November 2

Cryogenics and the Fawley Motor (6.30 p.m.) Mr A. D. Appleton, Institution of Electrical Engineers, in the Electrical Engineering Laboratories, University of Liverpool.

Development, Use and Requirements of STOL Aircraft (5.30 p.m. informal discussion) Institution of Civil Engineers, at Great George Street, London SW1.

Protective Coatings for Gas Pipe Lines and Storage Vessels (7 p.m.) Dr J. T. Harrison, in the Bullock Lecture Theatre, Hull College of Technology, Hull.

The Chemical Industry and Government-Sponsored Corporations (6.30 p.m.) Sir Frank Schon, Society of Chemical Industry, in the Scientific Societies Lecture Theatre, 23 Savile Row, London W1.

The Pharmacology of Neuromuscular Transmission (6 p.m.) Professor W. C. Bowman, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

The Soviet Engineer (6.30 p.m.) Mr V. S. Chousovitin, Institution of Electrical Engineers, at Byng Kendrick Suite, University of Aston in Birmingham, Gosta Green, Birmingham 4.

What is the Future for Linkage Synthesis? (6 p.m. Discussion) Institution of Mechanical Engineers, Applied Mechanics Group, at 1 Birdcage Walk, London SW1.

Tuesday, November 3

Industrial Technology and Management (6.30 p.m.) Professor C. W. Page, Institution of Electrical Engineers, at the University of Leeds.

Mycenaens and Phoenicians at Kition (5.45 p.m.) Dr V. Karageorghis, University of London, at the Institute of Classical Studies, 31–34 Gordon Square, London WC1.

The Chemical Composition of the Lunar Surface; Results from Unmanned and Manned Missions (5.30 p.m.) Professor A. A. Turkevich, University of London, at Imperial College, London SW7.

The Storage and Secretion of Hormones (5.30 p.m.) Dr A. D. Smith, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Fourth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

The Surge Transference Characteristics of Generator Transformer Installations (6 p.m.) Mr E. L. White, Institution of Electrical Engineers, at the Carlton Hotel, Edinburgh.

When Palm Trees Grew in London (1.20 p.m.) Dr W. G. Chaloner, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Wednesday, November 4

Analytical Aspects of Environmental Pollution (3 p.m.) Mr J. Robinson, Mr G. F. Longman, Mr P. G. Jeffery and Mr A. E. J. Eggleton, Society for Analytical Chemistry, at the Wellcome Building, Euston Road, London NW1.

Bench Wear Testers (6 p.m. discussion) Institution of Mechanical Engineers, Tribology Group, at 1 Birdcage Walk, London SW1.

Compensation for Death and Injury in International Air Transport (3 p.m. symposium) Royal Aeronautical Society, Air Law Group, at 4 Hamilton Place, London W1V 0BQ.

Control of Triglyceride Fatty Acid Uptake by the Tissues (2 p.m.) Dr D. S. Robinson, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

Formation of Deep Water in the N.W. Mediterranean (5.45 p.m.) Mr T. Sankey, Challenger Society, at the Linnean Society, Burlington House, Piccadilly, London W1.

High Speed Fuses for the Protection of Diodes and Thyristors (7.30 p.m.) Mr E. Jacks, Institution of Electrical Engineers, at the Assembly House, Norwich.

How Things Move (5.30 p.m.) Professor G. Porter, FRS, Royal Institution, at 21 Albemarle Street, London W1. (Lecture for Fourth Form pupils from Schools in London and the Home Counties. To be repeated on November 5, 10 and 11.)

Kant's "Universal Natural History and Theory of the Heavens", 1755 (1 p.m.) Dr G. J. Whitrow, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street. London W1.

Metrication (6.45 p.m.) Mr F. Mundy, Oil and Colour Chemists' Association, jointly with BPMA, at the Carlton Hotel, North Bridge, Edinburgh.

Microwave Generation Devices (6 p.m.) Mr K. Wilson, Institution of Electronic and Radio Engineers, Components and Circuits Group, at 9 Bedford Square, London WC1.

Performance Monitoring Techniques (2.30 p.m. colloquium) Institution of Electronic and Radio Engineers, Joint IEE/IERE Computer Group, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Recent Advances in the Chemistry of Unnatural Products (7.15 p.m.) Professor R. B. Woodward (Harvard), Pharmaceutical Society of Great Britain, in the Large Lecture Theatre, The School of Pharmacy, 29/39 Brunswick Square, London WC1.

The Value of the Computer to the Electronic Engineer (6.15 p.m.) Mr R. H. Williams, Institution of Electrical Engineers, jointly with IERE, at the University of Manchester Institute of Science and Technology, Manchester.

Thoughts on the Future of World Communications (7 p.m.) Professor E. C. Cherry, Institution of Electrical Engineers, at Dorking Hall, Dorking, Surrey.

Thursday, November 5

Airborne Microbes: Their Significance and Distribution (4.30 p.m.) Dr P. H. Gregory, FRS, Royal Society, at 6 Carlton House Terrace, London SW1.

Automation and Computer Techniques in Clinical Chemistry (5.30 p.m.) Professor T. P. Whitehead, University of London, at the Institute of Child Health 30 Guilford Street, London WC1. (Fifth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

How Much Does One Pay for High Efficiency in Fluid Plant? (6 p.m. discussion) Institution of Mechanical Engineers, Fluid Plant and Machinery Group, at 1 Birdcage Walk, London SW1.

Quantitative Microbiology (symposium) Society of Chemical Industry, Microbiology Group, at the Borough Polytechnic, Borough Road, London SE1.

The Recruitment Circus (5.30 p.m. informal discussion) Institution of Civil Engineers, Education and Training Group, at Great George Street, London SW1.

Friday, November 6

ESR and Flash Photolysis (1 p.m.) Dr K. A. McLaughlan, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Extraction and Refining (two-day residential course) Institution of Metallurgists, at the Grand Hotel, Eastbourne, Sussex.

The Open University (9 p.m.) Dr Walter Perry, Royal Institution, at 21 Albemarle Street, London W1.

Tricks of the Trade (5.30 p.m.) Dr R. A. Chalmers, Society for Analytical Chemistry, Scottish Section, in the Thomas Graham Building, University of Strathclyde, Cathedral Street, Glasgow C1.

Monday, November 9

Batteries mW to kW (6.30 p.m.) Mr C. J. Bruce, Institution of Electrical Engineers, London Graduate and Student Section, at Savoy Place, London WC2.

How Does the Introduction of SI Units Affect the Mechanical Engineer? (6 p.m. discussion) Institution of Mechanical Engineers, Thermodynamics and Fluid Mechanics Group, at 1 Birdcage Walk, London SW1.

Proofs: Positive and Presumptive (5.30 p.m.) Mr B. Gower, British Society for the Philosophy of Science, in the Joint Staff Common Room, University College London, Gower Street, London WC1.

Semiconductor Gamma Camera System (6 p.m.) Mr E. Moss and Mr W. Gore, Institution of Electronic and Radio Engineers, Joint IEE/IERE Medical and Biological Electronics Group, at 9 Bedford Square, London WC1.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Building Research Station. Current Paper 13/70: Estimation of Air-Conditioning Loads. By N. O. Milbank and J. Harrington-Lynn. Pp. 17. (Garston, Watford: Building Research Station, 1970.) *Gratis.* [169

Resources in Medicine. (A collection of papers based on a three-day seminar, *Uses of Resources in Medicine*, held at St Thomas' Hospital in 1969. Edited by Jane Collins. Pp. 76. (London: King Edward's Hospital Fund for London, 14 Palace Court, W2.) 18*s* (90*p*). [169

University of Oxford. Class and Pass Lists for Trinity Term 1970. (Supplement (3) to *Oxford University Gazette*, No. 3441.) (Oxford: The University, 1970.) 2*s*. [169

Natural Environment Research Council. Report of the Council for the period 1 April, 1969–31 March 1970. Pp. vii+108. (London: HMSO, 1970.) 13*s* (65*p*) net. [179

The Committee for Environmental Conservation. Report for 1970. Pp. 46. (London: Committee for Environmental Conservation, Council for the Preservation of Rural England, 4 Hobart Place, SW1.) [179

Twenty-one Years of Conservation. Pp. 24. (London: The Nature Conservancy, 19/20 Belgrave Square, SW1, 1970.) [179

University College of Swansea—Department of Geology. The First Fifty Years. Pp. 17. (Swansea: University College of Swansea, 1970.) [179

Countryside Recreation: The Ecological Implications. (An Investigation in the Ecological Implications of Countryside Recreation in Lowland Britain with particular reference to the Countryside of Lindsey. Pp. 125+7 maps. (Lincoln: County of Lincoln, Lindsey County Council, 1970.) [189

Bulletin of the British Museum (Natural History). Entomology, Vol. 25, No. 4: A Revision of the World Species of *Chilo Zincken* (Lepidoptera: Pyralidae). By Stanislaw Bleszynski. Pp. 99–195+5 plates. (London: British Museum (Natural History), 1970.) 82*s*. [219

Jacksons' Millboard and Fibre Co., Ltd., Directors Report and Accounts 1970. Pp. 11. (London: Jacksons' Millboard and Fibre Co., Ltd., 1970.) [219

Building Research Station. Current Paper 24/70: Some Results of Exposure Tests on Durability of Calcium Silicate Bricks. Pp. 14. (Garston, Watford: Building Research Station, 1970.) *Gratis.* [219

PEP. Broadsheet 520: The Economics of Television. By C. F. Pratten. Pp. 71. (London: PEP, 1970.) 16*s*. [219

Planners *versus* People? (Proceedings of the 1969 Annual Conference at the University of York.) Pp. 58. (London: Pemberton Books, Ltd., 1970. Published for the Rationalist Press Association.) 5*s* (25*p*). [219

Greenwich Time Report. Royal Greenwich Observatory—Time and Latitude Service, 1969, October–December. Pp. 109–122. (London: Science Research Council, 1970.) [219

The Health Education Council. Annual Report 1969/1970. Pp. 51 (18 plates). (London: The Health Education Council, 1970.) [229

University of Oxford. Schedules of Lectures Authorized by Boards of Faculties, Michaelmas Term 1970. (Supplement (2) to *Oxford University Gazette*, No. 3441.) Pp. 1441–1511. (Oxford: The University, 1970.) 2*s*. [228

Department of Education and Science. Statistics of Education 1968. Vol. 2: School Leavers, G.C.E. and C.S.E. Pp. xxvii+101. (London: HMSO, 1970.) 30*s* net. [239

Field Studies Council. Oil Pollution Research Unit, Orielton Field Centre. Annual Report 1969. Pp. 51. (Pembroke: Eric Cowell, Field Studies Council, Orielton Field Centre, 1970.) [239

Countryside Commission. Coastal Preservation and Development. The Coastal Heritage: a Conservation Policy for Coasts of High Quality Scenery. Pp. viii+99+34 plates. 80*s*. The Planning of the Coastline: a Report on a Study of Coastal Preservation and Development in England and Wales. Pp. vi+94. 17*s* 6*d* (87½*p*). (London: HMSO, 1970.) [249

Ministry of Transport: Road Research Laboratory. RRL Report LR 345: The Full-Scale Reinforced Concrete Experiment on the Grantham By-Pass, Performance During the First Six Years. By J. R. Nowak. Pp. 31+25 figures. (Crowthorne, Berkshire: Road Research Laboratory, 1970.) *Gratis.* [249

Three-Fifths of Medicine—One Year's Work of the Chest and Heart Association 1969/1970. Pp. 32. (London: The Chest and Heart Association, Tavistock House, WC1, 1970.) [249

British Standards Institution. Annual Report 1969 to 1970. Pp. 96. (London: British Standards Institution, 1970.) [249

Ministry of Public Building and Works—Directorate of Research and Information. Construction Research: A Report on the Work of the Construction Research Advisory Council and the Construction Industry Research and Information Association. Pp. iii+65. (London: HMSO, 1970.) 15*s* (75*p*) net. [259

Ministry of Transport: Road Research Laboratory. RRL Report LR 323: The Effect of Tread Pattern Depth on Skidding Resistance. By G. C. Staughton. Pp. 9+6 plates. (Crowthorne, Berkshire: Road Research Laboratory, 1970.) *Gratis.* [259

Mullard Jubilee 1920–1970—Photographic Record of some of the Highlights of the First Fifty Years. Pp. 24. (London: Mullard, Ltd., 1970.) [259

Other Countries

Fonds National de la Recherche Scientifique. Quarante-deuxième Rapport Annuel, 1968/1969. Vol. 1: Pp. 211. Vol. 2: Pp. 127. Vol. 3: Pp. 255. (Bruxelles: Fonds National de la Recherche Scientifique, 1970.) [219

US Department of the Interior: Geological Survey. Bulletin 1294-C: The Shenango Formation (Missippian) in Northwestern Pennsylvania. By Grant E. Kimmel and George R. Schiner. Pp. 13. $0.15. Bulletin 1312-N: Some Rare-Element Mineral Deposits in Mainland China. By K. Y. Lee. Pp. iv+34. $0.25. (Washington, DC: Government Printing Office, 1970.) [219

Proceedings of the 4th Triennial Conference of the European Association for Potato Research (EAPR), Brest, 8/13. IX. 1969. Pp. 298. (Wageningen, Netherlands: European Association for Potato Research; Paris: Institut National de la Recherche Agronomique, 1970.) 48.37 francs. [219

Modelli in Biologia (Cancerogenesi ed Immunita). By Orlando Gregorini. Pp. 83. (Bologna, Italia: Dott Orlando Gregorini, 1970.) [229

New Zealand. Report of the Department of Scientific and Industrial Research for the year ended 31 March, 1970. Pp. 62. (Wellington: Government Printer, 1970.) $0.40. [229

The Australian National University. The Research School of Physical Sciences. Annual Report 1969. Director's Report 1969. (Canberra: Australian National University, The Research School of Physical Sciences, 1970.) [229

US Department of the Interior: Geological Survey. Water-Supply Paper 1899-K: A Glossary of Karst Terminology. Compiled by Watson H. Monroe. Pp. 26. (Washington, DC: Government Printing Office, 1970.) $0.25. [239

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Another Round of SALT

THE resumption of the SALT negotiations in Helsinki this week is an important occasion. In the past year, the governments of the United States and the Soviet Union have been able at least to bring out into the open some of the issues which must attend any attempt to regulate strategic weapons systems. In the previous meetings, the United States representatives have put forward a number of suggestions as to how the deployment of strategic weapons systems might be controlled by international agreement. From the proceedings at Vienna earlier this year, it is clear that many important issues will have to be hammered out in the weeks ahead —the extent to which short range missile systems based in Europe, or tactical strike aircraft, should be included in a SALT agreement, for example. At Helsinki, however, the immediate interest will be the response of the Russian government to the various suggestions so far put forward, chiefly from the American side. From the tenor of the Russian reply, it should be possible to glean some inkling of the outcome of the SALT negotiations. The hope is that the Russian reply will be constructive, which is not to suggest that there can be a quick settlement—the technical problems are too complicated for that. By the same test, a negative reply to the American suggestions put forward earlier this year could easily bring the negotiations to a halt—and there are some who argue that a quick ending to the talks would be preferable to a long drawn out and unproductive negotiation.

Would protracted talks be a danger in themselves? This is one of the arguments put forward in the past few weeks by those in the United States who fear that the continuation of the negotiations will provide opportunities for the Soviet Union to steal a march on the United States in the deployment of strategic weapons. This reflects the anxiety now prevalent in the United States about the rapid growth of the Russian long range rocket system, particularly in the deployment of the very large SS-9. By now, the chances are that the number of launching sites for SS-9 missiles is greater than the number of Minuteman sites in the United States. At the same time, the carrying capacity of the new Russian missile is thought to be large enough to accommodate either a large number of independent warheads or the kind of explosive power needed to deliver damaging attacks on underground missile silos. In short, many Americans fear that underneath the umbrella of the SALT negotiations, the Soviet Union is acquiring a capacity to make a successful attack against the retaliatory strength of the United States. This, the argument goes, would upset the present balance between East and West. It is therefore only proper to acknowledge that this fear would be real only on a much longer time scale. If the deployment of SS-9 missiles continues for much longer at the present rate, and if the warheads are able to accommodate independently manoeuvrable missiles capable of reaching missile silos with precision, there will be reason to fear that the balance of terror as it is called has been upset. But is not that state of affairs a long way in the future? For one thing, the accuracy of the Russian independent warheads is unlikely, at this early stage of the game, to be great enough to ensure a first strike capability. And, after all, only when a nation is almost sure that an attack against its opponent's missiles will succeed will it seriously contemplate the risk of retaliation against its population centres. In this unpleasant game, a first strike is feasible only if it is literally devastating. And there is at present no prospect that the deployment of the Russian force of SS-9s will be effective against the missile launching submarines. It will be the late seventies before countermeasures against submarines are a threat to their strategic credibility, and only then will there be reason to fear that the American strategic force is vulnerable to a pre-emptive attack. By then, no doubt, the development of independently manoeuvrable warheads in the United States will have done much to redress the balance. There is even the possibility that the anti-ballistic missile system will by the late seventies be efficient enough to give American rocket silos an appearance of immunity against attack. In short, the complaint that the SALT talks may serve as a cloak for a destabilizing development of the Russian force is probably exaggerated or, more accurately, premature.

Another danger is that protracted talks will create a climate in which a meaningless or even a dangerous agreement may be produced. It is therefore important that the great difficulty of negotiating a treaty to limit the deployment of strategic weapons should be widely appreciated. So far, the guiding principle behind the American proposal has been the wish to conserve a measure of stability. Restricting the numbers of delivery systems of various types deployed by

each of the two powers is not an objective in itself although it may be a means to such an end. To the extent that such restrictions may form part of a SALT agreement, it must also be anticipated that both parties will continue with research and development intended to make the fullest use of the launching platforms (the only verifiable components in the system) permitted. This is why the American proposals (not made public) seem also to have included a limitation on the sizes of the warheads to be deployed, but here it must be acknowledged that such strictly physical considerations will tell against the Russians, not the Americans. The issue of verification is also, of course, a serious obstacle to any easy negotiation. To be sure, the United States government seems to have overcome its traditional rigour about the need for accurate verification to the extent of supposing that external methods of inspection (including satellites) will suffice to warn of potential violations. It is, however, improbable that Congress—the ultimate arbiter in the United States of the acceptability of international treaties—will be easily assured that all is well. In other words, for a variety of reasons, many of them technical, a workable agreement will not easily be reached and may even be unattainable.

So is Helsinki bound to be merely a talking shop? The preoccupation with strategic missiles and their deployment, which goes back to the early sixties, may in the long run be misleading. If the objective is to reduce the cost of weapons development, to preserve the strategic balance and even to enhance its stability by spelling out its essential characteristics, it should also be acknowledged that other methods may be useful. An extension of the nuclear test ban treaty to underground tests would be a useful step to consider, even though there is something in the view that a certain amount of routine testing is necessary to ensure that warheads on the shelf have not deteriorated. In this sense, the isolation of the SALT negotiations from the broader discussions on arms control at Geneva may be a misfortune if it continues for too long. Attempts to limit the destabilizing effects of multiple warheads and of anti-ballistic missile systems by prohibiting the testing of such devices are probably unrealistic, if only because they cannot be verified. It might, however, be of great value to think of other methods of increasing the stability of the strategic balance—even outrageously unorthodox schemes such as the requirement that both sides should publish fuller information about the weapons systems which exist. And there is probably much in the view that the potential disputes within SALT about the restrictions to be placed on short range missiles would disappear if there were a European agreement for the control of weapons systems, coupled if necessary with inspection.

Suicide by Pinprick

THE treatment now being meted out to scientists in Czechoslovakia is probably no worse, on a personal level, than that which other citizens have to endure. But science, more than other professions, depends on free communication between its practitioners and stands to suffer directly in proportion to the extent that communication is impeded. Everybody deplores the Lysenko affair, but few stop to consider how far it may be responsible for the present standing of Soviet biology, mediocre both on an international basis and in comparison with what Soviet scientists have achieved in other fields. The connexion between the persecution of scientists and the decadence of their trade is by no means a cause and effect relationship, but it is sufficiently credible to make the more thoughtful commissar pause a little before he tightens the noose.

Exactly what is happening in Czechoslovakia cannot yet be known, although it seems that scientists along with other members of the academic community are being compelled to submit to the anachronism of a loyalty test and several of the heads of institutes of the Czechoslovakia Academy of Sciences have been forced to resign. Two recent episodes, though trivial in themselves, are distinctly gloomy indications of the level of personal vindictiveness that now seems to govern the relationship between rulers and ruled. According to the letter on page 589 of this issue from Dr Zemlicka and Dr Chladek, it is now official policy to prevent Czechoslovak authors resident abroad from having their papers published in Czechoslovak journals. Whether or not the refusal to publish a scientific paper for purely political reasons is unprecedented in the history of science, it is hard to conceive of any self-inflicted insult that further epitomizes the qualities of pettiness, pointlessness and pusillanimity.

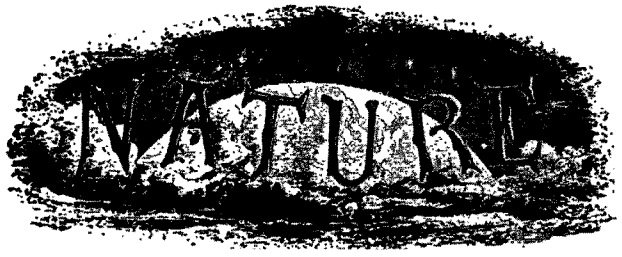
The second incident is not unprecedented—it is unfortunately a habit all too common in the Soviet Union—but for the rest is much like the first. Earlier this month the International Union of Biological Sciences held its general assembly in Washington DC. Among the list of speakers was Dr Ivan Malek, the director of the Institute of Microbiology of the Czechoslovak Academy of Sciences. A few days before the conference began Dr Malek sent a telegram to the organizers confirming that he would come. Then followed a second telegram saying that because of "new difficulties" he could not come.

There are, of course, a score of possible reasons for Dr Malek's absence, but it is hard to conceive that he would have allowed anything other than a government veto on his journey to force him to disappoint at one blow the IUBS, of which he is a vice-president, the scientists who had come to hear his talk, and the

National Academy of Sciences which was host to the meeting.

These practices, and the policies that lie behind them, do not amount to an inquisition, but nor are they mere irritations. They are weeds that if allowed to flourish may block up the channels of scientific communication and turn Czechoslovak science into a backwater. It is one thing to humiliate the members of the Writers' Union and force the signatories of the *Two Thousand Words* to recant—the connexion between literature and economics is remote and in any case the masterpiece suppressed today may be published tomorrow. It is another matter to impede, however slightly, the flow of information and ideas among scientists, because that circulation is vital to the body it sustains. The officials responsible for these pinpricking restrictions, should consider whether the injury they do to their own scientific community, and the dismay they evoke among scientists abroad, are really a price worth paying for whatever goal they have in mind.

100 Years Ago



NOTES

In stating that we believe that the English Eclipse Expedition is now finally arranged, it is due to the Government to add, and we do so with the greatest pleasure, that it is now quite clear that only a small part of the blame, which certainly attaches to some one, can be laid at their doors. In fact, explanations certainly are due from those who have had the management of the now famous Joint Committee. It appears that a deputation was named, and accepted the trust of representing the requirements of Science to the First Lord of the Treasury, which trust they neither fulfilled nor handed back to the Committee in order that another deputation might be appointed. We next hear of a letter written to the wrong Government department; and last of all, we are informed that the letters of the Government department—the last, we believe, written not later than the beginning of September—stating, among other things, that an application should be made in October, when the possibility of granting ships could be better discussed—have not yet been brought before the Joint Committee, which has just been summoned by the Secretary for the 4th of November, that is, to-morrow. It is not for us to censure such conduct as this, but it is our clear duty to point it out, and we hope the matter will be taken up. In spite of this mismanagement, however, we hear that the Government are prepared to aid both by money and ships when an application shall be made, and we cannot doubt that application will be made. There is still ample time to organise an expedition which shall do much good work, though perhaps it is too late to send out and erect the largest class of instruments. Large instruments, however, will be in the hands of the members of the American Government Expedition, so that this is the less to be regretted.

From Nature, **3**, 13, *November* 3, 1870.

OLD WORLD

DEFENCE

World Armaments

In spite of the slow progress being made in the Strategic Arms Limitation talks which reopened in Helsinki this week, the latest yearbook of the Stockholm International Peace Research Institute (SIPRI) offers some hope for arms limitation. The yearbook (Gerald Duckworth and Co., 125*s* bound; 63*s* paper) reports that total world military expenditure did not rise in 1969, and probably fell a little in 1970. This improvement arises chiefly from American policy in Vietnam which has reduced direct expenditure on the war by increasing the part played by the Saigon government forces. But as a direct result of this policy, sales of weapons to underdeveloped countries increased to $1,500 million in 1969—the third highest figure ever.

While the number of intercontinental ballistic missiles and submarine launched missiles continues to rise rapidly both in the USA and USSR (see *Nature*, **227**, 1077; 1970), SIPRI suggests that both sides now have second strike capability but neither has a first strike capability. This is a position in which the nuclear deterrent really does deter, and provides the most stable situation except for total disarmament. The SIPRI yearbook suggests that the best possibility for progress in disarmament now lies in Europe, where, although the Warsaw pact countries have superiority in numbers over the NATO countries, the NATO strategy of defence (which requires less force than an offensive strategy) and the marked superiority of its air forces should encourage NATO not to allow numerical disparity alone to become an immovable block to any moves towards disarmament. Any such moves should be welcomed as an opportunity to reduce the very large stockpiles of tactical nuclear weapons in Western Europe.

The least encouraging aspect of the confrontation between the USA and USSR lies in their response to the relatively new types of warfare, notably chemical and biological warfare (CBW) and the opening up of the sea bed. Although the US Senate is expected to ratify the Geneva protocol on CBW, like many governments it chooses to exclude the use of defoliants and tear gases from the restrictions of the protocol. Chemicals dangerous to plants but not human life are not specifically banned by this agreement because, when it was drafted in 1925, no such chemicals existed; but it is clear that they fall within the spirit of the agreement. Official US figures give the total amount of defoliant used in Vietnam as over 50 thousand tons, spread over some 20 thousand square km, so that the ratification of the Geneva protocol in the form proposed is rather an empty gesture.

Development of the joint USA/USSR treaty on the use of the sea bed, which is now being drafted, also gives some cause for concern. Originally proposed by the USSR as a treaty banning the use of the sea bed for any military purposes, it has been emasculated to the stage where it now bans only mass destruction weapons. No such weapon systems capable of operating on the ocean bed exist, and in any case the most likely military use of these regions lies in the provision of submerged bases for servicing and arming nuclear submarines.

Only the success of the Treaty of Tlatelolo, signed in

1967 to ensure that Latin America remains free of nuclear weapons, provides any real hope in this somewhat gloomy picture. This treaty has now been in operation for three years, which shows that adequate supervision is possible, and encourages the hope that other similar zones may be established elsewhere.

Medvedev Reinstated

Dr Zhorez Medvedev, the Soviet scientist who was arrested last May (see *Nature*, **226**, 887; 1970), has been allowed to resume his scientific research. He has recently been appointed Senior Scientist at the Laboratory of Proteins and Amino Acids of the Research Institute of the Physiology and Biochemistry of Agricultural Animals. The institute is situated about 18 miles from Obnisk, where Dr Medvedev is now living. He plans to continue his research into molecular aspects of ageing.

Dr Medvedev's arrest and subsequent detention in a mental hospital sparked off a storm of criticism and protest within the Soviet Union and among members of the scientific community in other countries. This eventually led to Dr Medvedev's release, but until his appointment last week, he had been effectively barred from carrying on his scientific research.

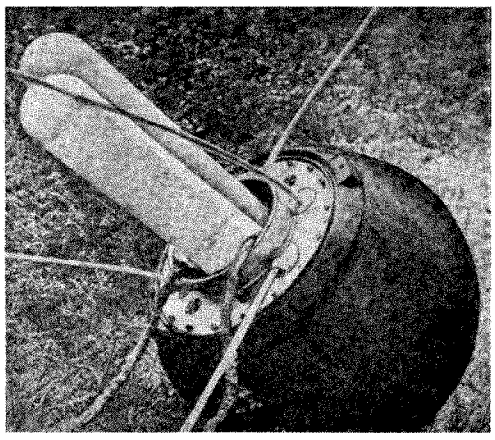
MOON ROCKS

Luna-16's Cargo

from our Soviet Correspondent

The first results of the Luna-16 flight were announced last week at a special conference organized jointly by the Academy of Sciences of the Soviet Union and the Soviet Ministry of Foreign Affairs. The highlight of this conference was the information presented by Academician A. P. Vinogradov, a leading Russian geologist, about the lunar rock recovered by Luna-16.

The rock was recovered from a point in the Sea of Fertility, at 0° 41′ S and 56° 18′ W (some 900 km from the landing site of Apollo 11), and it consists of friable surface material (regolite) only. It was reported at the conference that after the drill struck against what was either solid bedrock or a large detached fragment of bedrock, further penetration "did not exceed 5 mm". The total weight of the specimen was a little more than 100 g, and it represents a sample of the top 35 cm of the lunar surface.



Container used for transporting Moon rocks in Luna-16.

The specimen is a dark grey, almost black, powder which has been divided into six zones of different granularity. For identification purposes, these fractions have been retained separately along the length of the coring device. The first two zones (0–15 cm along the coring device) consist of fine-grained material with small amounts of the coarse fraction. The next zones (from 15 to 33 cm) consist of material of varying granularity, containing inclusions of bedrock and other particles more than 3 mm in diameter, and the final zone (from 33 to 35 cm) consists of large-grain material, with fragments of the bedrock, which the drill hardly penetrated.

The average size of small particles (less than 1 mm) varied along the coring device from 70 μm at the surface to 120 μm at the deepest point. The mean specific gravity was 1·2, and the mean porosity at a depth of 35 cm was 50–60 per cent.

The composition of the specimen may be divided into basalt-type rocks, feldspar-type rocks, grains of various individual material (including plagioclase, olivine, pyroxene and ilmenite) belonging to the basalt group, brecchia, metallic iron particles and small vitreous spheres.

Some 70 chemical elements, including a number of isotopes and short-lived radio-nucleids, have been identified in the Luna-16 specimen and its composition, compared with that of the Apollo-12 specimens, is given in Table 1.

Table 1

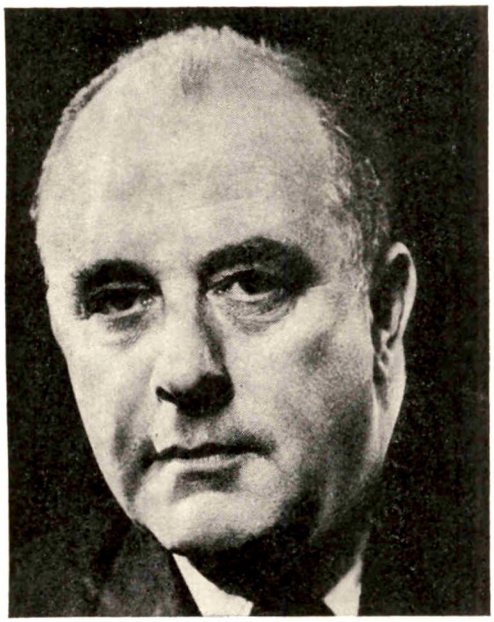
	Basalt rock Luna-16	Fine fraction Luna-16	Basalt rock Apollo-12	Fine fraction Apollo-12
SiO_2	43·8	41·7	40	42
TiO_2	4·9	3·39	3·7	3·1
Al_2O_3	13·65	15·32	11·2	14
FeO	19·35	16·8	21·3	17
MgO	7·05	8·73	11·7	12
CaO	10·4	12·2	10·7	10
Na_2O	0·33	0·37	0·45	0·40
K_2O	0·15	0·10	0·065	0·18
MnO	0·2	0·21	0·26	0·25
Cr_2O_3	0·28	0·31	0·55	0·41
ZrO_2	0·04	0·015	0·023	0·09

The preliminary conclusion, according to Academician Vinogradov, is that the rocks of the surface of the lunar seas, as exemplified in this specimen, are basaltic, but differ somewhat in the content of their individual chemical elements. They are closely related to the primitive basalts of the Earth. He also suggests that the lunar seas have at some time been subjected to inundation by volcanic lava, and the basaltic rocks were therefore formed because they were the fraction most easily melted in the conditions which existed within the Moon. He argues that "the general course of differentiation of the substance of the Earth and Moon, and, probably, of the other planets of terrestrial type will follow converging paths, although they will have reached different stages of development".

GOVERNMENT

Zoologist in the Hot Seat

LORD ROTHSCHILD, whose appointment as head of the Central Policy Review Staff was announced last week, is a welcome, if somewhat surprising, addition to the small band of scientists holding senior positions in the higher echelons of the Civil Service. The news of his appointment has provoked widespread interest and comment for two reasons. First, the Policy Review Staff is a new venture whose success is by no means guaranteed, and it would seem to demand an economist rather than a zoologist to steer it through the inevitable early storms. Second, Policy Review is an important and controversial item in the Conservative Party's plans for reforming the apparatus of government, and so it is curious to find it left to the tender mercies of a Labour peer.



Lord Rothschild.

But Lord Rothschild is no stranger either to government or to administration. He was chairman of the Agricultural Research Council for ten years, and has been coordinator of the research activities of the Royal-Dutch Shell group since 1965. These positions are a fitting apprenticeship for public office, and he was already on the Treasury's select list when Sir William Armstrong, head of the Civil Service, came to choose his new troubleshooter.

Lord Rothschild will not find himself academically isolated when he takes up his new appointment, for his old friend and colleague Sir Solly Zuckerman, chief scientific adviser to the Government, is also a zoologist. Lord Rothschild's research, which earned him Fellowship of the Royal Society before he was 45, was concerned with the physiology of spermatozoa, both in marine animals and mammals. In particular, he was interested in the way in which spermatozoa move and derive energy for swimming, and in the process of fertilization.

Scientists and politicians seem to agree that the appointment of Lord Rothschild is an enterprising gamble which deserves success. Certainly there are few men better equipped for the job, but time alone will tell whether he can add the role of inter-departmental peacemaker to his multifarious talents.

CHEMICAL WARFARE

Wraps Off at Nancekuke

THE question of what is going on at Nancekuke was answered to the satisfaction of almost everybody when the Chemical Defence Establishment (CDE) outstation above Portreath Bay, Cornwall, opened its gates to the press last week. In all the rumours of nefarious and arcane activities, it seems that the establishment is doing no more than it has always claimed; it manufactures the riot control chemical, CS; it tests the feasibility of manufacturing and storing new chemical weapons; it produces minute quantities of nerve gas for research purposes, and it is Britain's sole source of P2S, an essential component of the only effective remedy for nerve gas poisoning.

Nancekuke houses the Process Research Division of CDE Porton Down, the hub of Britain's chemical and biological warfare activities. Public anxiety about the outstation has centred chiefly around the nerve gases which are manufactured there and transported to Porton for field testing. Nerve gas has not in fact been produced in any quantity since 1955, and the equipment for its manufacture now stands derelict and partially dismantled. A small plant which can be used to make minute quantities of nerve gas when required is still functional, but was last used two years ago when approximately 20 pounds of the nerve gas VX was produced. The remains of this batch of gas, claimed to be Britain's entire stock of nerve agent, reside in three steel canisters in the locked and sealed nerve gas store at Nancekuke. The report of a working party chaired by Professor R. H. S. Thompson of the Middlesex Hospital Medical School published recently (HMSO, 1970, 1*s* 6*d*) was reassuring about the risk to the public and to the environment from the manufacture and transport of these small quantities of nerve gas.

The chief role of Nancekuke is to determine whether novel chemical weapons developed at Porton can be manufactured and stored cheaply and efficiently. This is claimed to be a defensive and not an offensive measure. Before any new substance can be regarded as a realistic threat in the hands of an aggressor, it must be known whether large scale production is feasible—milligrams of a nerve agent may prove lethal in laboratory trials, but on a field scale the dose must be measured in kilograms. Throughout 1969 and 1970, only one such substance, the nature of which was not disclosed, has been undergoing production trials at Nancekuke.

The real question hanging over Nancekuke is whether its productivity justifies its existence. The buildings are shabby, and they are rearranging the chemical plant once used to make nerve gas to manufacture the antidote P2S. The staff is much under strength, and finance is a mere £330,000 annually. All the station's activities—such as the manufacture of P2S antidote and CS—could, at a pinch, be moved elsewhere; perhaps to safety, for the vast expanse of the outstation represents an ideal target. The continued existence of CDE Nancekuke seems not so much sinister as merely inefficient.

SPACE

New Satellite Consortium

On November 3, the day on which the European Space Conference reopened to discuss the Lefevre mission to Washington, the setting up of a new European satellite consortium was announced. The British partner in the linkup, which involves seven companies, is Marconi Space and Defence Systems Ltd, who recently won a British government contract for the Skynet 2 military communications satellite programme.

The consortium is planned to be a loosely grouped concern with little or no central organization and the chairman of the managing board, appointed for a year, is Commander D. W. Malim, of Marconi Space and Defence Systems. The leadership of any particular project will be undertaken by whichever firm seems to have the most appropriate expertise; capabilities range widely through aerospace technology, telecommunications and electronic systems.

Although the new group will obviously look towards Europe and possible European association with the American space shuttle plans, it also hopes to tender for possible satellite contracts with countries such as India, Australia and Canada. Several of the companies involved in the consortium have considerable experience in launcher work which could be particularly useful for smaller satellite projects. In particular, the French company Société Nationale Industrielle Aérospatiale (SNIAS) has been actively involved in both the launcher and satellite programmes of ELDO and ESRO.

The other five members of the consortium are Etudes Techniques et Constructions Aérospatiales (Belgium), Société Anonyme de Telecommunications (France), Messerschmitt-Bölkow-Blohm and Siemens (Federal Republic of Germany) and Selenia (Italy).

PHYSICS

Particles and Telescopes

from our Soviet Correspondent

Within the past two weeks, two "unique" achievements in physics have been announced in the Soviet press. The first of these concerns the Joint Nuclear Research Institute at Dubna, where there is a synchrophasatron, now accelerating deuterium nuclei to energies of 11 GeV. This is claimed to be the first accelerator in the world to accelerate complex atomic particles to velocities approaching the velocity of light. The director of the Dubna Institute, Academician N. N. Bogolyubov, said in an interview in *Pravda* that the "transformation of the synchrophasatron into a 'relativistic' nuclear accelerator" is an "outstanding achievement". It penetrates into a new field of research in which it is hoped that many important and interesting data will be accumulated.

At the same time, the new wide-band UTR-2 radio telescope of the Ukrainian Academy of Sciences went into operation. Operating in the 10–20 MHz frequency band, this telescope, with an area of 150,000 square metres, comprises 2,040 linked antennae. It is T-shaped, extending 1,860 m in the north-to-south direction and 900 m east-to-west.

This instrument is particularly important because it is designed to work in the low frequency band where very little information has been gathered. There are similar arrays in British Columbia and at Clark Lake in the USA, but the development of radioastronomy lately has been towards higher frequencies where observations are less troubled by ionospheric disturbances.

So far, the general level of Soviet radioastronomy has not been highly regarded in the rest of the world. This does not detract from the valuable contributions which are being made to this field in the USSR, but their first claims are usually treated with even more caution than those of western radioastronomers. It is to be hoped that with the opening of this new telescope and the expected rapid completion of another very large instrument now being built in the Caucasus and designed to operate at high frequencies, Soviet radioastronomy will achieve a reputation comparable with that of the best western work.

COAL

Smokeless Fuel Fiasco

Years of patient Parliamentary work, starting with the first clean air legislation in 1955, seem in danger of falling temporarily by the wayside this winter as shortages of solid smokeless fuels place more and more smokeless zones in jeopardy. Among the alternative smokeless fuels, only gas (now produced chiefly from oil or piped from the North Sea) looks reasonably certain to be dependable throughout the winter. The supply of electricity, which now accounts for about 50 per cent of the national coal output of 160 million tons a year, could all too easily suffer because of the Electricity Generating Board's disappointing prediction that there will be only a 9 per cent surplus over the estimated maximum demand, coupled with low stocks and the threat of industrial unrest within the coal industry.

This solid fuel shortage, now running at about 300 to 400 thousand tons a year, will cause the lifting of at least 127 smoke control orders and probably many more. This is, however, by no means a return to the murky days of the nineteen fifties; coal consumption in London, for example, will remain well below 10 per cent of the figure for 1952—the year of the great smog. The situation would be even worse if central and local government had not adopted a definite policy of looking to other smokeless fuels. This alone is expected to save about 450,000 tons of smokeless fuel a year, and in addition the gas boards expect to have supplied large concerns with about 7,000 units during 1970 for conversion of solid fuel boilers to gas.

The annual output of smokeless coal is now running at about 8 million tons of which about 4·5 million comes from the National Coal Board and about 2·3 million from private firms. The remainder has in the past been a byproduct of gas production from coal, but supplies from this source have dwindled from 1·6 million tons in 1969/70 to 0·97 million tons in 1970/71 and the Coal Board claims that this method of gas production has been

phased out much more quickly than expected. Some uneconomic gasworks are being especially retained at an annual cost of £300,000 to produce 170,000 tons of coke.

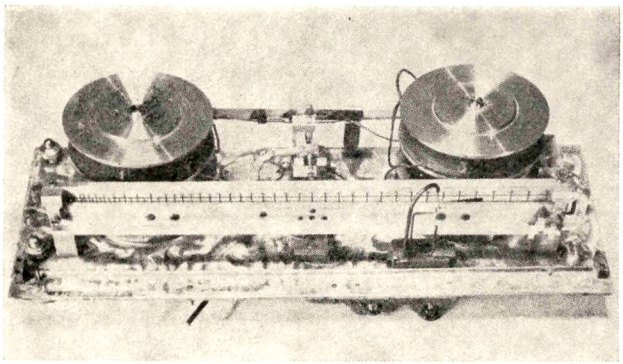
The National Coal Board's contribution, which includes the naturally occurring smokeless anthracite, has been marred by some conspicuous failures, the most notable of which is the production of so-called Bronowski bullets by the treatment of a variety of types of coal to form a fluid which could be cast into blocks. Even now, 15 years after the idea was first conceived, only 130,000 tons a year are produced by this method, compared with the millions of tons originally envisaged. Other efforts to bolster up supplies are exemplified by the purchase of 75,000 tons of briquettes from France produced from otherwise useless South Wales anthracite dust.

HISTORY OF SCIENCE

Kelvin Collected

It is appropriate that a scientist of the stature of Lord Kelvin (formerly Sir William Thomson) should be remembered in some permanent way by the University of Glasgow where he held the chair of natural philosophy for more than fifty years until 1899. A book (*Kelvin's Instruments and the Kelvin Museum*; University of Glasgow; 1970, £1) has recently been published as a complement to the collection of Kelvin's instruments in the Department of Natural Philosophy and it completes a fitting memorial to his experimental talent which ranged over the field of electrical measurements and even extended to the production of marine instruments.

Professor P. I. Dee took up his post as head of the Natural Philosophy Department at Glasgow in 1945 to find "a positive aura of Kelvin everywhere in the place" which he decided should be preserved in the shape of a small museum. By 1954 the Kelvin Museum had a permanent home in the new research block and the result was an almost complete collection of more than one hundred Kelvin instruments, among them the famous current balance, together with several pieces of equipment used by his co-workers Joule and Kerr. Although Mr J. T. Lloyd, one of the authors of the book, was chiefly responsible for collecting and displaying the exhibits, it was the late Dr George Green, scientific secretary to Lord Kelvin in the three years before the latter's death in 1907, who has provided the personal link with the man and his work which enabled a full account of the development of Kelvin's instruments to be produced and included as the kernel of the book.



Current balance (length 46 cm).

Parliament in Britain

Concorde

Another £95 million has been added to the estimated research and development costs of Concorde. Mr Frederick Corfield, Minister of Aviation Supply, announced last week that the latest estimate is £825 million at June 1970 prices. The previous estimate, made in October 1969, put the research and development costs at £730 million and the increases are made up of £35 million for improvements to the propulsion system, £20 million for other adjustments to the programme and £40 million for pay and price increases since October 1969. The British and French governments have already spent £460 million between them on Concorde and the new estimates therefore provide for a further £165 million to be spent by the British government and £200 million by the French government.

Mr Corfield also announced that the flight tests of Concorde should be completed by the middle of December, and evaluated by March or April 1971. The British and French governments will then review the project thoroughly, taking into account the commercial prospects of the aircraft. So far, airlines have taken options on seventy aircraft, and no firm orders are expected until the flight tests have been evaluated. (Oral answers, October 28.)

Teacher Training Enquiry

Lord James of Rusholme, Vice-Chancellor of the University of York, has agreed to be chairman of the Committee of Inquiry into the Training of Teachers. This was announced by Mrs Margaret Thatcher, Secretary of State for Education and Science, who also said that she is consulting all interested parties about the composition and terms of reference of the committee. The committee of inquiry should start work early next year, and it will have available the evidence taken last session by the Select Committee on Education and Science. (Written answers, October 22.)

Warfarin Resistance

Mr Anthony Stodart, Parliamentary Secretary to the Minister of Agriculture, gave an assurance that it is not difficult to control Warfarin resistant rats if occupiers and local authorities follow advice available from the Ministry of Agriculture. He also said that the spread of Warfarin resistance should not increase the number of rats or the dangers to public health. Warfarin resistant rats are confined chiefly to Shropshire and Montgomeryshire, but they have also been found on farms elsewhere in Great Britain. Mr Stodart also said that intensive use of Warfarin in some towns has resulted in large increases locally of the proportion of mice resistant to the poison. (Written answers, October 27.)

Industrial Reorganization Corporation

Mr John Davies, Secretary of State, Department of Trade and Industry, gave further details about the winding up of the Industrial Reorganization Corporation. The corporation will undertake no new commitments and its existing commitments will eventually be taken over by the Government. Loans will be recovered according to the terms of the various agreements and equity investments will be realiezd when such opportunity offers. (Oral answers, October 30.)

BAC 3-11 and European Airbus

THE British government's request for more time to consider the issues involved in rejoining the A-300B European Airbus project is a good indication of the fierceness of the battle which is going on in the aircraft industry. Ostensibly, the government is faced with a choice between rejoining the European aircraft project or backing a rival airbus, the BAC 3-11, which is being developed by the British Aircraft Corporation (BAC). The situation is, however, complicated by the financial position of Rolls-Royce, whose involvement in the BAC 3-11 may be critical for its future, and by the government's obvious desire not to blot its copybook in its negotiations for entry to the Common Market. There is also little doubt that a decision either way will have important implications for the future of the British aircraft industry.

The offer from the governments of West Germany, France and Holland for Britain to rejoin the A-300B project was timed almost to perfection. On the one hand, it gives the British government an opportunity to demonstrate its willingness to participate in European projects and thus, by implication, if the offer were turned down it could be used as an argument against Britain's entry into the Common Market. And, on the other hand, the initial capital outlay involved in rejoining the A-300B is considerably less than that involved in backing the BAC 3-11. The European course is therefore likely to appeal to a government which is committed to reducing public expenditure.

The European offer, which was first made two weeks ago, is for Britain to rejoin the project which she left in 1968 when it seemed to be getting too expensive. West Germany, France and Holland then decided to carry on with development of the A-300B and are now offering Britain a stake in development of a new B-7 version of the airbus, for an outlay of about £20 million. In return, the European partners, and in particular West Germany, are willing to put about £25 million into Rolls-Royce for development of the RB 2-11 engine which could be offered to customers of the A-300B as an alternative to the scheduled General Electric engine.

The A-300B is a 250-seat airliner, which will be powered by twin General Electric CF6-50A engines, and sales are expected to reach more than 350 aircraft. The airliner could be in production by 1974, about a year ahead of the BAC 3-11, but the time difference is not crucial because it is widely accepted that the demand for such aircraft will not reach its peak until towards the end of the 1970s.

About £45 million has already been spent on development of the airframe and West Germany, France and Holland have guaranteed a sum of £180 million for research and development of the airframe and another £300 million for full-scale production costs. If Britain entered the project, the £20 million entry fee would therefore be devoted almost entirely to development of the B-7 version which has been tailored chiefly for British European Airways.

The alternative airliner—the BAC 3-11—is a short to medium haul aircraft powered by Rolls-Royce engines. The proposal which the government is considering is that the cost of research and development of the airframe would be shared equally by the government and the British Aircraft Corporation. At the last estimate, these costs were put at £150 million, and the company would give an undertaking, under any agreement, to meet cost overruns. BAC has already spent about £3 million on research and development on the 3-11, and it has now reached the point where it must abandon the project if government backing is not forthcoming.

But that is only half the cost which the government would face if it decided to back the BAC 3-11. Development of the Rolls-Royce RB 2-11 "Dash 61" engines which have been proposed to power the aircraft could cost about another £75 million and the government would be expected to provide all the money necessary for this development.

But it is paradoxically the position of Rolls-Royce in the airbus debate which could sway the government in favour of backing the BAC 3-11. Rolls-Royce has in the past year been feeling the pinch, chiefly because the company has sunk a large amount of capital into development of engines for the Lockheed Tristar, and it has yet to see any return on its investment in the research and development of the RB 2-11. The BAC 3-11, with its expected sales of some 280 aircraft, would therefore provide Rolls-Royce with a much-needed long and profitable production run. In addition, if the company does not develop the "Dash 61" follow-up to the RB 2-11, it would find itself slipping badly behind in production of civil aircraft engines towards the end of the 1970s.

The offer of £25 million to Rolls-Royce from the partners in the Eurobus is much less attractive than participation in the BAC 3-11 for several reasons. Firstly, General Electric has already been involved in the A-300B project, and even if its participation in the production stage is not guaranteed by contractual commitments, the company would be expected to pick up much of the engine sales. The BAC 3-11, on the other hand, would be built around the "Dash 61" from the start, and would virtually ensure Rolls-Royce of a substantial return on its investment.

Another important factor which the government must take into account is the effects that a decision to back the European Airbus may have on the future balance of payments. Sir Anthony Milward, chairman of British European Airways, has already declared his support for the BAC 3-11, and has hinted that if the government decides not to support the 3-11, BEA would not have the European Airbus forced down its throat.

On the face of things, the economic arguments for joining the A-300B project seem to be attractive, but the government is obviously under great pressure from the British aircraft industry to back the 3-11. It would be difficult, however, for the government to justify supporting the high costs of Concorde and at the same time sink more money into the BAC 3-11. On the other hand, it would be politically unwise to pull out of the Concorde project in addition to turning down the A-300B offer.

NEW WORLD

Call to Remake Federal Science Policy

by our Washington Correspondent

A MAJOR restructuring of the federal support for scientific research is advocated by the House Subcommittee on Science, Research and Development, in a report issued this week. The chief target of the report* is the Office of Science and Technology (OST) which is held to be understaffed, misused and inadequate. To remedy this and other structural weaknesses the subcommittee calls for the Administration to set up a task force which would draft a national science policy for the United States.

The background to the subcommittee's recommendations is the hearings on national science policy held this summer during which some sixty witnesses gave their views on the present parlous state of the national science effort. But the subcommittee and its chairman, Representative E. Q. Daddario, are also drawing on the experience of previous (and still unheeded) hearings in their plans for remoulding the present organization of science. By way of preface Mr Daddario calls attention in the new report to the way in which the annual rate of increase in the federal support of science has plunged from about 12 per cent in the mid-1960s to a negative 5 per cent for the present financial year. Allowing for inflation, this represents a decline in present purchasing power of from 20 to 25 per cent compared with the peak years of 1965–68.

The Daddario subcommittee lays much of the blame for this state of affairs at the door of the Office of Science and Technology. The Office was charged in 1962 by President Kennedy with the duty (transferred from the National Science Foundation) of developing "the pursuit of a national policy for the promotion of basic research and education in the sciences". But neither the National Science Foundation nor the Office of Science and Technology has ever occupied a sufficiently commanding post in the federal hierarchy to implement such a policy, the report observes. Part of the trouble is that the Office of Science and Technology, which should be the focal point for the government's sponsorship of scientific activities, is "fragmented as to mission and badly overburdened as to operation". Its presiding officer has also to serve as the President's science adviser, chairman of the President's Science Advisory Committee, and chairman of the Federal Council for Science and Technology, and many of the staff of the Office have similarly diffuse responsibilities.

The Office itself, the report observes, seems often to be used by the Executive Office for tackling immediate crises, whether scientific or otherwise, which leaves little opportunity for planning the welfare of federal science. The result is that the Office "does not fulfil even its few statutory obligations". In particular it has failed to take the lead in formulating basic government science policy and has also neglected the duty of evaluating and reporting on overall government research activities. The better to fulfil these existing obligations, as well as becoming patron of the nation's scientific effort, the Daddario subcommittee recommends that the Office of Science and Technology should be strengthened both in statutory backing and in staff and money, as well as being separated from any direct administrative connexions with the President's science adviser or the President's Science Advisory Committee (PSAC). "This is not to say that OST should be removed entirely from the PSAC arena, if the President wishes to call upon it; but it should not be one of the chief components through its director and staff. . . . It should be relieved of the time-consuming, often delicate, often classified, support function it now provides the President's science adviser".

As part of its trusteeship for the national scientific endeavour the Office of Science and Technology should submit an annual report to the President and Congress reviewing the status of research and development in the United States and recommending a programme of research and development for the coming year. The Daddario subcommittee also recommends that the Office should have the job of setting the criteria by which mission oriented agencies might decide whether to support particular areas of basic research. In other words OST would become the arbiter of the concept of relevance by which until recently the Department of Defense was bidden to eschew all basic research not obviously relevant to its military mission.

Besides a revamping of the OST the Daddario subcommittee urges several other institutional changes in the federal support of science.

- Setting up of the proposed National Institutes of Research and Advanced Studies (NIRAS). The proposal, not yet given substance by the Administration, was put forward by the subcommittee last year as a means of bringing under one organization the federal programmes for the support of academic research that are now scattered among several agencies.
- Setting up of the Office of Technology Assessment, another of the subcommittee's projects that has yet to be taken up by Congress. Mr Daddario recently tried without success to introduce the necessary legislation in the form of an amendment to the Congressional reform bill.
- Creation by Congress of a Senate committee having centralized jurisdiction over science and technology, as does the Committee on Science and Astronautics (the parent of Mr Daddario's subcommittee) in the House of Representatives.
- Action by the Office of Management and Budget to ensure that the funding of scientific research is maintained at a stable level and preserved from precipitate cutbacks.
- An increase in block grants to universities and other institutions, to be channelled through the National Science Foundation.

* *Toward a Science Policy for the United States* (US Government Printing Office, 1970). n.p.

The subcommittee's proposals have been discussed informally with Dr David, the President's science adviser, and with the staff of the OST, but official reaction has yet to come from the Administration. If, as is possible, the only reaction is silence, the subcommittee proposes to hold hearings next spring in lieu of the task force it has asked the Administration to set up. The hearings would discuss science policy in a more directed way than those held in July to September this year. Meanwhile Mr Daddario, who is leaving Congress to become (if fortune and the polls favour him) governor of Connecticut, has this parting reproach for those who over the last few years have let science starve: "Research teams have been decimated or disbanded. Promising projects have been slowed or shelved. Science and engineering enrolments at academic institutions are down. Medical schools, particularly the non-State schools, are in serious financial straits. Graduate student-teachers have had to postpone their schooling and often abandon their research fields. Planning of any kind for the future by the research community is difficult and in some instances impossible. Research industry stocks are down disproportionately. Small companies are disappearing. Most serious of all, many of the nation's brightest young people who would otherwise enter the science arena are passing by without any real exposure to its attractions and potential. There is little question that the morale of the scientific community is at a low ebb."

Is the picture really as black as Mr Daddario paints it ? Has science been seriously wounded by the budget cutbacks or is there still more fat, as the saying goes, to be squeezed out of the system ? In a speech given on October 13 to mark the sesquicentennial of the University of Indiana, Dr Philip Handler, president of the National Academy of Sciences, delivered a more guarded review of the status of science. He too alluded to a 20–25 per cent reduction in federal funds over the last four years, a trend that "most commentators agree . . . reflects principally growing public disenchantment with science and the technology it makes possible". Dr Handler also admitted that United States leadership in certain areas of science is now slowly moving abroad, but "in view of the international character of science, that cannot be seriously objectionable, of itself". But he was unable to point to any gaping wounds on the scientific body politic: "Relatively few academic laboratories known to me have yet been really seriously injured. Only a few major national facilities have been closed and I presume these to have been only marginally productive."

If Dr Handler was presenting a more hard-nosed analysis than might be expected from one in his position, that may reflect in part the fashion of the times. For example, an Assistant Secretary of the Treasury, Mr Murray L. Weidenbaum, told a recent meeting of the American Institute of Aeronautics and Astronautics in Houston that he had often been struck by the absence of "that objectivity and hard, factual, quantitative analysis that I associate with the core of the scientific method. I am amazed when scientists say we should embark upon a major technological project on faith . . .". Mr Weidenbaum was not one of those who sees a cutback of research dollars as no less a sin than the suppression of truth; to his mind a professor asking for $100 million of the taxpayers' money to build a linear accelerator should have to justify it "not in the soft, theological terms so often used by the natural scientists in these matters, but in the hard, objective manner of the social scientist".

Mr Weidenbaum explained his paradoxical inversion of the hard/soft scale by saying that natural scientists should justify their works in relation to criteria such as expected benefits versus costs, whether important elements of cost such as pollution have been included in the analysis, and whether alternative uses of public funds would not be profitable. These prescriptions, if followed, would demand a new order of ingenuity in the filling out of grant application forms but there is nothing necessarily wrong in that.

NASA

Is Space Worthwhile?

by our Astronomy Correspondent

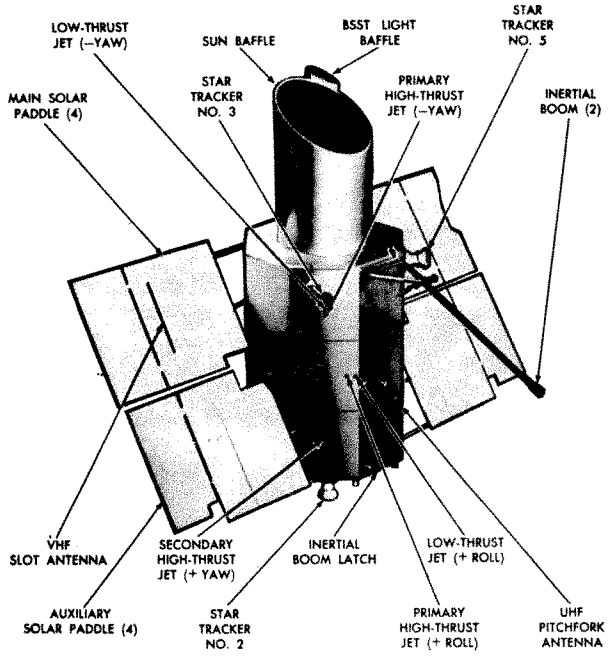
ASTRONOMY in the United States is having a thin time. The most urgent need continues to be the building of new radio telescopes. But the most severe blow that has been dealt this year is widely considered to be the cancellation in September of two Apollo missions, conceivably because the cancellation brought home how much the new astronomy has been scientific spin-off from the American space programme. It must therefore rankle that many astronomers are refusing to endorse the new projects in manned spaceflight that NASA considers vital if it is to survive with anything like its present strength. To whip up support for schemes such as the shuttle and the twelve-man space station was the chief aim of the NASA contingent attending a conference in New York last week on the planning of space missions. Much of the justification of the meeting, organized by the New York Academy of Sciences, was to relate current questions in astronomy to the hardware that NASA would like to have available in the seventies. At the same time the supporters of manned spaceflight ingeniously grasped every opportunity for shedding light on the problems of the day. Communications satellites are now being justified not for their superfluity of circuits but by favourably comparing the pollution from rocket motors with that from cable-laying ships.

Only Dr Norman Baker of Columbia University surprised his audience by launching a protest against the NASA programme, after delivering his review paper on variable stars. There is not much evidence that NASA cares about astronomy, he said, and what is really needed is a few per cent of the space budget to be diverted to the construction of ground-based optical and radio telescopes. At that stage of the meeting most of the NASA representatives had already left, and Dr Zdenek Kopal of the University of Manchester was the first on his feet to dissociate himself from Baker's views. Baker's firecracker soon fizzled out, indeed, leaving an overall impression of dumb acquiescence with the agency's programme.

Dr Wernher von Braun staked out his new philosophy for NASA. An era in space is coming to an end now that the enthusiasm that characterized the Moon landings is subsiding, and from now on the space effort must stand on its own feet. In this light the space shuttle, he claimed, is a major step maximizing the return for space applications and science. The burning

question continues to be whether the shuttle should have a delta wing, giving manoeuvrability and a consequent wider choice of landing sites, or a more straightforward design with stubby straight wings imparting a lower lift to drag ratio at hypersonic speeds, a narrower choice of landing sites, but easier handling for the pilot. But von Braun agreed that in the present budget situation it is impossible that NASA will be able to tackle the shuttle and the space station simultaneously, and it would be best to tackle the shuttle first.

The meeting had its lighter moments. Dr T. B. Taylor, president of International Research and Technology Corporation, wanted to see a resurrection of the scheme whereby a space vehicle rides the shock waves from a series of nuclear bombs exploded behind it. A project along these lines was one of the panic reactions to the first sputnik, and survived until 1965 after a surprising $10 million had been spent. The test-ban treaty and an understandable lack of confidence in the technology finally finished off the project, but Dr Taylor appeared convinced that its potentiality could be demonstrated using the work already done and a limited number of underground tests. Oddly enough, he thought that a vehicle along these lines would be a fine medium to begin international cooperation in space. On the scientific side Dr F. Johnson of Electro-Optical Systems, Inc., delighted the meeting with his theory that the diffuse interstellar absorption lines are due to the magnificent molecule $MgC_{46}H_{30}N_6$—one of the porphyrins.



The Orbiting Astronomical Observatory to be launched on November 17. Its height is ten feet and weight 4,680 pounds, making it the heaviest scientific satellite so far.

It was hard to see what the new NASA programme had to offer the astronomers while so much remains to be done with conventional rockets. The development of electric propulsion, for example, would open up the solar system to a new generation of probes that could be launched by the Titan class of rockets. Typically a payload of 500 kg would reach Pluto in five years without the need for the gravitational boost on which the grand tour missions depend, and 1,000 kg to Jupiter would take less than one year.

In the circumstances the agency must be hoping that the launch this month of a massive satellite devoted to astronomy will redeem some of its tarnished image. The satellite is the latest of the Orbiting Astronomical Observatories which began with the expensive failure of OAO 1 in 1966, and the highly successful OAO 2 launched nearly two years ago. Although few of the data from OAO 2 have yet found their way into the scientific literature, what has been released makes it clear that important discoveries are being made. OAO 3, to be launched on November 17, is basically a 38-inch Cassegrain telescope with a spectrometer covering 1000 to 4000 Å, and Dr Albert Boggess of the Goddard Space Flight Center is the principal investigator. The pointing accuracy is expected to be one arc second and the telescope should be able to record down to twelfth magnitude.

ANTIBIOTICS

Swann Report Exported

from our New York Correspondent

THE immense and often indiscriminate use of antibiotics around the world has led in recent years to increasing fears that bacteria are developing resistant strains faster than scientists can turn out new antibiotics. Scientists from eleven countries met last month under the auspices of the New York Academy of Sciences to discuss these problems, especially as they relate to infections from drug resistant bacteria in hospitals and to the increasing number and kinds of drug resistant bacteria found in food animals.

It has been known for a number of years that disease-causing bacteria have built up resistance to antibiotics that were originally able to control them. A few years ago, Professor Tsutomu Watanabe of Keio University School of Medicine, Tokyo, discovered that this resistance, or R-factor, can often be transferred to bacteria that are not already resistant to drugs and in fact have not been exposed to antibiotics.

There was general agreement at the conference that many hospitals are now a major source of contamination of R-factor bacteria. Studies have shown that patients are more resistant to antibiotics after being in hospital for several weeks than they are when they enter. A more controversial area of concern is the widespread use of antibiotics in animal feed. Since the 1950s antibiotics have been added to feed both for disease prevention and for increasing weight in food animals. It is estimated that 80 per cent of the animal protein consumed in the United States comes from animals fed medicated feed during part or all of their lives.

Soon after antibiotics began to be used to prevent disease, it turned out that they also increased growth by acting against growth-depressing micro-organisms in the intestinal tracts. An even more important result of feeding antibiotics to domestic animals is in preventing the effect of so-called stressful conditions, such as crowding, shipping and vaccination. These uses have had a tremendous impact on both the drug and the food industries. It has been estimated that from 20 to 25 per cent of all the anti-

biotics produced in the United States are used in animals, a $400 million a year market. As for the farmers themselves, chicken breeders alone have saved an estimated $240 million from 1950 to 1965 by using antibiotics.

The Federal Food and Drug Administration, the agency that is responsible for approving all animal drugs as safe and effective, has always monitored the use of antibiotics in feed. The increase of antibiotic resistant strains in animals and the fear that such strains can be transferred to man, thereby increasing the already dangerous level of resistant strains produced by man himself, has, however, put pressure on the FDA to follow the recommendations of the Swann Commission report which was published in Britain ten months ago and should soon be put into effect. The Swann report recommended that the use of therapeutic antibiotics be limited to treating diseases and be available only on prescription by veterinarians, not automatically added to feed as preventive medicine, as has been the case. The growth antibiotics do not usually develop multiple drug resistance and are not known to cause the transfer of resistance, and so were not included in the restriction.

The FDA responded last April by setting up a task force to study the problem and report back to the commissioner, probably by this December. The charge of the task force is to take an "in-depth review of the usage and actual value of low-level antibiotics in feeds, the acquisition of the R-factor by organisms through animal feeding practices, the possibilities of transference of R-factor to human pathogens, and the economic impact of restricting the use of antibiotic feed additives on both the manufacturers and the users". Dr C. D. Van Houweling, director of the FDA's Bureau of Veterinary Medicine and chairman of the task force, pointed out last week that the restrictions already imposed by the FDA on the use of antibiotics are far tighter than they were in Britain at the time of the Swann report, that the general animal inspection and sanitary standards are higher in the United States, and that much of the outcry in Britain was caused by an outbreak of disease from natural overspill of organisms from calves to humans not directly related to the R-factors.

The conference participants generally agreed that it is too early to say that the presence of R-factors in animals is related to R-factors in the human population or that animal R-factors are themselves dangerous. Professor Thomas H. Jukes of the University of California at Berkeley argued that while all feed additives need continuing surveillance, "if antibiotics in animal feeds produced an increase in resistant pathogens, the effectiveness of antibiotic feeding would have disappeared long ago". Dr John R. Walton of the Animal Health Trust Stock, England, noted that scientists have been looking for a connexion between animal and human R-factors for ten years without success. Such lack of information, he added, must not be allowed to give rise to complacency, but from our present knowledge of drug resistant bacteria in human foods "one can only assume that antibiotics are being used carelessly and that hygiene is being neglected at many levels of food production".

Professor Watanabe believed that while animal R-factors are probably responsible for some of the R-factors in humans, they account for only a very small proportion. He pointed out that in Japan antibiotics have not been used until very recently for cattle, poultry and fish raising, yet R-factors are present in humans to a much greater extent than they are in the United States, due to the excessive amounts of antibiotics used by the Japanese themselves.

Considering the present state of uncertainty that surrounds the transfer of R-factors from animals to humans, coupled with the probably considerable pressure from the agricultural and drug lobbies against any sort of ban, it seems fairly certain that the FDA will not take strong measures to curtail the use of antibiotics in animal feed merely as a safety precaution. Dr Van Houweling stated at the conference that "food animal drug uses are largely a matter of economics. If animal drugs make it possible to produce more food more efficiently and, therefore, economically, it is advantageous to all consumers for these drugs to be utilized. Proposals to curtail, restrict or prohibit drug uses must therefore be supported by evidence that the drugs are not effective, are not safe. . . . Decisions are always subject to review." The review will probably be a long time coming.

STRATEGIC ARMS

Fears of a Sham at SALT

by our Washington Correspondent

THE third round of the Strategic Arms Limitation Talks (SALT) opened this week in Helsinki to the fears as well as hopes of their well-wishers. Apprehension that the talks might be leading up to a sham treaty was voiced this week by the Strategic Weapons Committee of the Federation of American Scientists, a small but intellectually heavyweight lobbying group concerned with arms control problems. A treaty that limited only the total numbers of strategic weapons without reference to their quality, the committee said, "would legalize an accelerated arms race directed towards qualitative improvements and the deployment of ever more dangerous weapons".

One method of limiting qualitative improvements in a verifiable way, the committee suggests, would be to agree to allow only such changes as do not alter the external appearance of a weapon or launching vehicle; such an agreement, though it would by no means halt the arms race, could at least dampen it enough to effect major savings. The members of the committee are Dr Herbert Scoville, assistant director of the Arms Control and Disarmament Agency under President Johnson, Dr Marvin L. Goldberger, chairman of the physics department at Princeton and a former member of the President's Science Advisory Committee, and Dr Morton H. Halperin, a former staff member of the National Security Council under President Nixon.

Other features of a SALT agreement the committee would welcome are a restriction on anti-ballistic missile systems which either ban them entirely or limit them to the defence of Moscow and Washington, and a halt to the testing, production and deployment of missiles carrying multiple, independently targetable warheads (MIRV). But by all accounts there seems to be little hope of bolting the stable door on MIRVs at least, in part because of the stage of development now reached on these weapons, but also because of the intractable problems of verification in deciding whether a launching vehicle carries one or many warheads.

NEWS AND VIEWS

Artful Model Building

IRREVERENT people who scoff at model building in molecular biology will have their opinions reinforced by two articles in this issue of *Nature*. They will be comforted to see that two teams of model builders have arrived at contradictory theses to explain how DNA is transcribed into RNA. Building models of nucleic acids has been in vogue ever since Watson and Crick successfully proposed that DNA is a double helix, but the foundations of such model building are perhaps more brittle now than at any time since 1953.

Molecular biologists of a more experimental turn of mind will maintain that the platonic relationship of the model builder with his subject makes this approach more art than science. After all, they will argue, you can construct innumerable models for, to cite an example, the transcription of DNA into RNA, but the crucial question remains unanswered: what mechanism is used in the cell? Thus it will be held that only those workers who dirty their hands to collect data by experiments in the laboratory can hope to resolve this problem.

How far is this cry supported by reason rather than prejudice? Certainly, even the most obsessed model builder must admit that nucleic acid models have been conspicuous recently for their lack of success, compared with the renowned spectaculars which elucidated the structure of DNA and formulated the clover leaf model for tRNA. The latter was a triumph of model building which is acknowledged by even the most grudging critics. Several possible models for secondary structure were constructed from the primary sequence of nucleotides and these models gave different predictions for how various parts of the tRNA molecule would react to enzyme or chemical attack. The use of these criteria then singled out the clover leaf model from the others. But now, with incompatible models abounding for tRNA tertiary structure, the model builders seem to have all but suspended their activities in despair, to await the experimental data expected from X-ray crystallography.

Compared with more recent attempts to construct models for DNA and RNA, the elegant formulation of the double helical structure of DNA shines out as though derived from the supernatural. No convincing model has yet been produced to explain the mechanism of replication of DNA or of its transcription into RNA, chiefly because of the lack of sufficiently detailed information about these processes and in particular about the way in which the replicating and transcribing enzymes interact with their templates. A formidable difficulty is the extensive ignorance of how DNA unwinds (see *Nature*, **227**, 1294; 1970).

Undeterred by this, however, Florentiev and Ivanov present on page 519 a model to account for how a DNA duplex might unwind to allow RNA chains to form by complementary base pairing with one of the exposed DNA strands. But on page 522, Riley resuscitates an old idea that DNA need not unwind to transcribe RNA, and postulates that the new RNA strand could grow in the wide groove of the DNA double helix.

Both models attempt to explain how a region of local distortion could move along the DNA template at the growing point where RNA is being synthesized. The Russians take as their starting point what is known (organochemically speaking) about the details of how phosphodiester bonds are made and broken. They propose that first the 3′ hydroxyl of the last nucleotide added to the RNA chain attacks the 5′ phosphate group of the incoming nucleotide; then pyrophosphate is released by breakage of an ester bond of the triphosphate.

Because the first step requires energy but the second liberates it, Florentiev and Ivanov suggest that the two processes may be coupled together. This demands a change in the stereochemical conformation of DNA from its usual B-form to the normally unstable A-form, which could be stabilized at the site of RNA synthesis. One way to achieve the B to A transition might be to extrude water from the section of DNA bound to the polymerase.

Riley circumvents the problem of how DNA unwinds by proposing that the duplex retains its integrity in transcription. He suggests that bases of the RNA chain could specifically hydrogen bond with the base pairs of the DNA template if the conformation of the sugar-phosphate backbone is distorted so as to tilt the nucleotide bases in the helix. This could be achieved by a perturbation of the DNA progressing in a wave-like manner, with the maximum distortion at the growing point of the RNA chain.

That it is possible to propose two such conflicting models illustrates the level of ignorance about the mechanism of transcription. Such models risk achieving the remarkable distinction of being at once too complicated and too naive. They are too complicated, because, allowing that the available data can be interpreted to support either model, any number of models can be propounded, all consistent with what is at present known about transcription. And they are too naive, because transcription is enzymatically catalysed and is not merely a chemical process; so little is known about the details of how RNA polymerase interacts with the DNA template

that it is safe to say that the environment of DNA surrounded by protein is virtually unknown.

Should yet more model building be encouraged, therefore, to define the limits of what little is known and perhaps to suggest further experiments, or is this approach at present premature? It is clear, for example, that one (at least) of the two models presented here for transcription must be wrong. But it is hallowed scientific tradition that it is useful to construct models—be they of a theoretical or more practical nature—which make predictions that can be put to experimental test. The trouble in this instance, however, is that it is difficult to suggest practical experiments which will sort out even the bedevilled question of whether DNA unwinds in transcription. It is tempting to enter a plea for less speculation and more experimentation until at least this elementary fact is known.

Cell Surfaces and Neoplastic Transformation

A SET of observations which may be as relevant to an understanding of cancer as the discovery of RNA dependent DNA polymerase is reported on page 512 of this issue of *Nature*. Burger and Noonan report that the pattern of growth of transformed mouse fibroblasts in culture is restored to that of untransformed fibroblasts when certain binding sites on the surface of the transformed cells are covered by fragments of concanavalin A. Furthermore, this change is reversible.

Concanavalin A is one of about a dozen phytagglutinins or lectins, glycoproteins isolated from various plant seeds, which bind to and agglutinate animal cells. Various phytagglutinins have been available for several years, but only recently, as a result of the work of Burger and his co-workers at Princeton and of Sachs's group at Rehovoth, have they assumed great importance to cancer researchers. The key observation is that the extent to which a cell binds phytagglutinins is correlated with its sensitivity to contact inhibition of growth in cultures. Burger has shown that cells transformed *in vitro* by chemical carcinogens or tumour viruses, and spontaneous transformants selected by growing cultures of cells at high densities, always bind concanavalin A and the other plant agglutinins, while untransformed cells only bind these substances when they are actually in mitosis or just recovering from it. (There are exceptions to this rule; a phytagglutinin extracted from soya beans, for example, binds to untransformed cells but not to transformed cells; the pattern of differential binding is reversed.) Because of this phenomenon, cultures of untransformed cells which have reached their saturation density, with the cells in contact with each other, and have stopped or almost stopped increasing in density, do not bind phytagglutinins. On the other hand, cultures of transformed cells, which are not subject to contact inhibition of growth, always bind phytagglutinins.

This difference provides another criterion for judging whether or not a cell is transformed, but Burger and his colleagues believed that it had more significance than this. They suggested, and now seem to have proved, that the change in the cell surface on transformation, which results in the exposure of sites which bind concanavalin A and its relatives, is directly related to the fact that transformed cells are not subject to contact inhibition of cell division. First, Burger discovered that if monolayers of untransformed cells, which have stopped dividing because the cells are in contact and have also stopped binding concanavalin A, are briefly exposed to dilute solutions of trypsin, the cells undergo at least one more round of division. And parallel cultures similarly treated were found to become capable of transiently binding concanavalin A.

Burger and Noonan (page 512) now report the reciprocal experiment. They transformed with polyoma virus some 3T3 mouse fibroblasts, which before transformation stop dividing when they form a monolayer culture. The transformed cells, Py-3T3, however, continue to multiply even when the culture becomes very crowded and there are sufficient cells to form several monolayers. Furthermore, the transformed cells always bind concanavalin A. Burger and Noonan asked what happens if the sites which bind concanavalin A, and which are permanently exposed on Py-3T3 cells, are covered by a fragment of concanavalin A. This fragment, produced by digesting concanavalin A with trypsin, retains the property of binding to cells but cannot agglutinate or kill them. Burger and Noonan reasoned that if the exposure of the surface binding site reflects a change in the cell surface which renders the cells unsusceptible to contact inhibition, recovering these sites with a fragment of concanavalin A might restore contact inhibition. This was indeed what they found.

Transformed Py-3T3 cells plated at a low density and grown in the presence of sufficient fragmented concanavalin A stop multiplying at about the same density as that reached by untransformed 3T3 cells. This indicates that in the presence of fragmented concanavalin A, transformed cells behave in culture as do untransformed cells. Contact of cell with cell is crucial in inhibiting the multiplication of transformed cells when concanavalin A is present to bind to and form a covering layer on the surface of the cells. The significance of contact inhibition of cell division, as opposed to contact inhibition of cell movement, has been questioned, but at least as far as mouse 3T3 cells are concerned Burger and Noonan's experiments show the former as well as the latter to be real.

The restoration of growth control by fragmented concanavalin A is reversible as expected. When the agglutinin is removed from resting transformed cells they start to divide once more. And from the kinetics of the inhibition of cell division achieved by adding

fragmented concanavalin A, Burger and Noonan suggest that all Py-3T3 cells in a dense culture are potentially susceptible to inhibition within one cell cycle of exposure, but that inhibition can only occur if the concanavalin A is bound at a particular stage in the cell cycle. Cells in the G1 phase are immediately prevented from dividing, while cells which bind concanavalin A at other stages complete the cycle and stop dividing when they reach the G1 phase of the succeeding cycle.

Burger and Noonan propose a simple model to explain these results. They suggest that the phytagglutinin receptors are part of a whole subsurface layer of cells. In untransformed cells this layer is covered by some, so far uncharacterized, substance or architecture, whereas in transformed cells or untransformed cells treated with trypsin the subsurface layer is, respectively, permanently or transiently exposed. Fragments of concanavalin A bind to receptors in this exposed layer and, at least in a functional sense, cover it, rendering it cryptic. Burger and Noonan further suggest that a cell can only propagate and/or respond to short range chemical messages which lead to a cessation of cell division when this subsurface layer containing the phytagglutinin receptors is cryptic. Moreover, a cell's permeability or lack of it to specific ions, nutrients or specific growth controlling factors may vary according to whether this layer is exposed or covered. They suggest that when the agglutinin receptor layer is exposed, cells may not be able to recognize and respond to the near presence of other cells. On the other hand, when the layer is covered the social behaviour of the cells is restored.

PROTEINS

Natural History of the Ribosome

from our Molecular Biology Correspondent

THE state of ribosomes in bacterial cells, and the operational relation between polysomes, 70S monomers and their subunits, remains a controversial and confusing topic. In mammalian systems the fog has not yet gathered, and a good clear account of the situation in mouse liver by Falvey and Staehelin offers the hope that it may be averted altogether. In their first article (*J. Mol. Biol.*, **53**, 1; 1970) they observe that the polysomes cannot be broken down to the ribosomal subunits, without irreversible inactivation, which is caused by removal of magnesium. This, of course, is not new, and other workers, notably Wool and his colleagues, also found ways of preparing subunits which could be made to reassociate to functional 80S ribosomes. The work of Falvey and Staehelin enlarges on these observations.

The dissociation of the complex of ribosomes with messenger and peptidyl-tRNA can best be achieved by allowing the ribosomes to complete synthesis of their polypeptide chains in a cell-free system, and run off the messenger. These resulting monomers can be made to dissociate with some ease, simply by making the solution 0·5 M in potassium chloride. The 60S and 40S subunits are then separated by centrifugation and collected by precipitation with ethanol (a useful technical trick that had previously escaped practitioners in the field). When the subunits are redissolved in a solvent containing magnesium, they forthwith associate to 80S ribosomes, which perform satisfactorily in a poly U-stimulated cell-free system.

In their second article (*ibid.*, 21) Falvey and Staehelin consider how this property of reversible dissociation of the messenger-free ribosome relates to the protein synthesis cycle. Labelled 40S subunits were added to the cell-free system. Examination of the 80S ribosomes accumulating on release showed the incorporation of counts. The 80S ribosomes alone, on the other hand, showed only very slow assimilation of labelled subunits. Neither is there appreciable exchange at initiation, for when the components of a cell-free system were added to a mixture of 80S and labelled 40S particles, and after an appropriate interval potassium chloride was introduced, the ribosome–messenger-tRNA complex, which alone remains undissociated, contained no label. It follows that 80S ribosomes, and not their subunits, attach to the messenger at initiation (at least in the unphysiological poly U system, which requires no initiation factors). The principal message of this work then is that dissociation of subunits occurs at the end of each traverse of the messenger. The small fraction of undissociable 80S particles found in the cell lysates is thought to represent a quite different state of unknown significance.

A different approach to the same problem is that of Okubo and Raskas (*Biochemistry*, **9**, 3458; 1970), who have studied the incorporation of newly synthesized ribosomes into polysomes *in vivo*, using KB cells. The label identifying new RNA appears first in the small, then in the large, subunit. Thereafter the labelled subunits turn up in the polysomes, and only later in monosomes, with a lag of six hours before the label is uniformly distributed among all the ribosomal particles in the cell. This is in excellent accord with the findings of Falvey and Staehelin, except that six hours is much longer than the matter of minutes required for completion of one protein chain. It is too short, on the other hand, to be explicable simply in terms of the utilization of the new ribosomes only in new cells. Several explanations are possible—that dissociation of the 80S particles does not occur at each cycle or, more satisfactorily, that equilibration depends on the rate of distribution of new ribosomes through the cytoplasm.

HYBRID CELLS

Suppressing Malignancy

from our Cell Biology Correspondent

PROFESSOR HENRY HARRIS entertained his audience for an hour when he gave the sixth Michael Cross memorial lecture at the Royal College of Surgeons on October 27. He summarized the work which he and his colleagues at Oxford and his collaborators in Dr George Klein's laboratory in Stockholm have done during the past few years. He believes that this work indicates that malignancy behaves in Mendelian terms as a recessive trait, and in Darwinian terms as a property selected from a population of cells in which the chief source of genetic variation is the loss of chromosomes.

Professor Harris's approach to the genetic questions posed by malignancy was centred on the exploitation of the technique of cell fusion mediated by Sendai virus. He asks: is the hybrid cell produced by fusing a malig-

nant to a non-malignant cell malignant or non-malignant, and what can the properties and chromosomal composition of such hybrids and variants derived from them tell about the chromosome(s) which carry the determinants for malignancy ? It is well known that he and his colleagues found that when several sorts of highly malignant mouse cells were fused with non-malignant A9 mouse cells the hybrids were by several orders of magnitude less malignant than the parental cells, as judged by their capacity to induce tumours in irradiated newborn mice. Moreover, the cells of the few tumours which did arise had far fewer chromosomes than the hybrid cells inoculated.

Professor Harris interprets these results as showing that malignancy is recessive and that the non-malignant hybrids must lose chromosomes to regain malignancy. But chromosome loss *per se* does not generate malignancy; hybrids cultured *in vitro* until they have lost many chromosomes are not malignant.

But A9 cells, the non-malignant partner in the hybrids, are an established cell line and Barski, Defendi, Ephrussi and others have reported that hybrids formed by spontaneous fusion between malignant cells and normal mouse fibroblasts are malignant. Professor Harris and his colleagues therefore repeated their experiments using normal diploid fibroblasts freshly put in culture, instead of A9 cells. When Ehrlich cells were the malignant partner such hybrids were malignant, but they also lost chromosomes extremely rapidly, which, according to Professor Harris, is the way to generate malignant cells. He and his colleagues therefore used SEWA cells, which are malignant but have a karyotype closer to that of normal cells than Ehrlich cells. Three clones of hybrids between SEWA cells and normal fibroblasts were much less malignant than SEWA cells alone, and moreover these three hybrids lost chromosomes more slowly than clones of similar hybrids which were malignant. Professor Harris interprets these results as showing that normal fibroblasts can suppress malignancy in the same way as A9 cells, but that this suppression only occurs if the hybrids have a stable chromosome composition. If, on the other hand, the hybrids are unstable and readily shed chromosomes, they form an ideal population from which to select *in vivo* cells which have lost the particular chromosomes which suppress malignancy. Professor Harris commented that he was now certain that antigenic differences between the hybrid cells and the recipient mice were not responsible for the results he reported. Unfortunately he did not present in detail the evidence from which his conviction stems, and it is the cornerstone of his interpretations, for the results could be interpreted in terms of the selection for the loss of a chromosome which determines foreign histocompatibility antigens rather than the suppression of malignancy.

ENZYMES

Recombining Nucleases

from a Correspondent

INTEREST in the processes of recombination has recently focused on a new class of deoxyribonucleases which seems to be widely distributed in bacteria (*J. Biol. Chem.*, **245**, 767; 1970). These ATP-dependent nucleases are interesting from both mechanistic and biological viewpoints. Their absence in certain mutants (*rec*) of *Escherichia coli* which are deficient in recombination suggests that these enzymes play an important part in this process.

A new enzymatic property reported by Goldmark and Linn (*Proc. US Nat. Acad. Sci.*, **67**, 434; 1970) may provide a clue to the function they fulfil. Goldmark and Linn purified from *E. coli* an ATP-dependent activity that cleaves single stranded circular phage fd DNA. This endonucleolytic activity is lacking in *recB* and *recC* recombination mutants, as is the previously described ATP-dependent exonucleolytic activity.

Apart from its ability to make a first endonucleolytic cleavage of circular single stranded fd DNA, Goldmark and Linn's preparation of enzyme has properties similar to those described for ATP-dependent deoxyribonucleases from *Micrococcus lysodeikticus* and *Escherischia coli*. Double stranded circular DNA is not attacked, but linear single stranded and linear double stranded DNAs are degraded in a sequential fashion: any molecule of DNA attacked is completely broken down into oligonucleotides before the enzyme relinquishes it to attack another molecule of DNA. This is shown by the lack of accumulation of products of intermediate size and a rate of change of viscosity relative to the appearance of acid soluble products that is characteristic of exonucleolytic activity. At the *p*H optimum for the exonucleolytic activity (above 9·5) native DNA is a better substrate, but at neutral *p*H denatured DNA may be better.

It remains to be shown whether a single enzyme in *E. coli* embodies both the endonucleolytic and the exonucleolytic activities, but these properties are evocative of a nuclease that does not require a free end, but rather might act best at a site in DNA where both single stranded and double stranded DNA are present, namely a branch point. The action of the ATP-dependent DNase might in one way be analogous to the 5′ to 3′ exonucleolytic activity of *E. coli* DNA polymerase (*Nature*, **224**, 495; 1969) which cleaves oligonucleotides from non-homologous single stranded branches. In genetic recombination, on the other hand, structures with homologous single stranded branches are plausible intermediates that would require trimming by one or another mechanism.

A prominent difference between the experiments of Goldmark and Linn and earlier ones is the presence of an inhibitor of the ATP-dependent endonucleolytic action. Extracts of *recB* and *recC* mutants have an inhibitor, which is not present in *recA* mutants or *rec*$^+$ wild type that specifically inhibits the endonucleolytic action on fd DNA but does not seem to inhibit the exonucleolytic action on native DNA. The existence of this inhibitor raises some questions about the exact relationship of *recB* and *recC* genes to the ATP-dependent activities, because no evidence has yet been produced that these genes code for the ATP-dependent DNase.

CYCLIC AMP

Transmission and Development

from our Neurophysiology Correspondent

CYCLIC AMP is in vogue as an important intermediary in cellular control processes. It is now plain that this

nucleotide has an important function in the nervous system; indeed, Rasmussen (*Science*, **170**, 404; 1970) suggests that it has a role whenever there is intercellular communication. He points out that elements common to both neural and humoral transmission are: calcium ions, cyclic AMP, intracellular microtubules, microfilaments, secretory vesicles and protein kinases. He elaborates this suggestion in order to postulate a common origin, followed by divergent evolution, of neural and humoral signalling systems.

In the light of this it is interesting that Goodwin and Cohen (*J. Theoret. Biol.*, **25**, 49; 1969) suggested that the nervous system itself might have evolved from a developmental control system needed for the precise ordering of cellular events in embryogenesis. This suggestion gains weight with the growing evidence for the involvement of cyclic AMP in neural processes (H. Mellwain, *Nature*, **226**, 803; 1970; *Neurosciences Research Program Bulletin*, **8**, 221; 1970). In the only developmental control system that has so far been analysed in any detail, cyclic AMP also acts as an intermediary. In this case it is the chemotactic attractant controlling the aggregation of some cellular slime moulds (Konijn, Barkley *et al.*, *Amer. Naturalist*, **102**, 225; 1968). All the elements Rasmussen mentions, with the exception of vesicles, have been observed in this developmental control system, and so the suggestion of a common origin for developmental control systems and neural and humoral systems may not be too far fetched. In any case, a general theory of biological signalling processes in which excitation occurs through membranes is clearly developing.

In Rasmussen's model there are two fundamental intermediary signalling agents, cyclic AMP and calcium ions. Both are increased by membrane excitation of the enzyme adenyl cyclase which converts ATP to cyclic AMP. An increase in cyclic AMP activates phosphorylating enzymes which make products that are sensitive to calcium ions. The structure of these products can be changed by calcium ions, leading, for example, to contraction, or to a change in their interactions with other cellular components, allowing, for example, release of vesicle-containing transmitters. Calcium ions can also affect their own rate of transport through the cell membrane. In a similar way it has been postulated that the reception of cyclic AMP by aggregating slime mould amoebae can initiate amoeboid movement, the release of a further cyclic AMP signal and, eventually, differentiation.

ENVIRONMENT

Conservation in the Tropics

from a Correspondent

THE trials which beset ecologists working in the tropics were aired on October 21, when the Tropical Ecology Group of the British Ecological Society met in London. The complexities of tropical ecosystems were well described by Dr J. S. Weir (University of Leicester), discussing elephants, termite mounds, diversity and stability in savanna ecosystems. Some *Macrotermes* mounds contain concentrations of biogenic resources (such as minerals and water) and have ecological attributes (such as thermal stability, well aerated soil and refuge from fire and flood) which maintain a diverse and specialized flora and fauna. Stability is theoretically associated with species diversity in tropical ecosystems. These mounds, which may be very old (more than 700 years in one dated case), form mosaics of high species diversity, and their destruction is likely to have far reaching effects on the savanna ecosystem. Recent studies have shown that *Macrotermes* has deserted its mounds in areas of National Parks where there are large numbers of elephants; the reasons for this are not yet clear, but seem to be related to the damage which the elephants cause to the habitat.

Dr M. E. D. Poore (Nature Conservancy) pointed out that sustained use of a natural resource may be compatible with the conservation of an ecosystem, but that when land use is intensified these aims often cease to be compatible and very careful planning is essential. The best balance must be struck between human needs and the resources available; imbalances should not be introduced into the system until it is known how to control them, and it is important to maintain freedom of choice.

In Western Malaysia, Dr A. G. Marshall (University of Aberdeen) said, the virgin rain forest is being increasingly exploited and destroyed as a consequence of the rapid growth of human population and economic development. Conservation is no new idea there, but there is no organization to coordinate efforts. One delegate pointed out that air photographs suggest that one-third of the rain forest of Borneo has been destroyed since the Second World War. In Brazil, although the number of National Parks and designated protected areas has been increased recently, as Mr I. R. Bishop (University of Leicester) showed, it is extremely difficult to enforce conservation laws in remote areas. A large scale road building programme is leading to the destruction of Amazonian forest at an alarming pace, and farmers are moving in, often ahead of the road, to clear the very diversified forest with the intention of replacing it with a monoculture of grass for cattle. The extreme poverty of nutrient salts in the soils means that the land is likely to deteriorate rapidly after the removal of forest cover.

The priorities, problems and practice of the conservation of natural resources in tropical Africa were discussed by Drs M. J. Coe (Animal Ecology Research Group, Oxford) and M. J. Delany (University of Southampton), who found that there has been a great increase in the number of field biologists there in the past ten to fifteen years. But efforts badly need to be coordinated, both between workers with similar interests in different territories and between people traditionally thought to have conflicting interests. Even within one country departments may have conflicting policies, for example with respect to the conservation of game or its control for medical or veterinary reasons. In the francophone territories, recently visited by Dr C. H. Fry (University of Aberdeen), much has depended on the enthusiasm of a few individuals. Certain schemes call for regional consideration, such as a suggested river diversion for hydroelectric power which might lead to desiccation of much of Lake Chad. Clearly the need for more ecological work in tropical areas is urgent, and a meeting is being planned to consider ways of encouraging and helping with this. Not only are scientific studies and the coordination of accumulated data required, but there is still often a need for the authorities to be made aware of the importance of sound ecological advice.

NUCLEAR PHYSICS

Direct Proton Decay

by our Solid State Physics Correspondent

A FORCEFUL reminder of how important scientific results can be collected by the exploitation of chance situations is contained in the discovery by J. Cerny *et al.* of the process of direct proton decay in an excited nucleus of cobalt (*Phys. Lett.*, **33**; 1970).

The extraordinary feature of this experiment is that direct proton decay, long predicted to occur in highly unstable nuclei, has been observed in a relatively stable isomer of cobalt-53 with a half life of about a quarter of a second.

About sixty years ago Lord Rutherford first identified alpha decay as a means by which a nucleus could be transmuted by radioactive decay. Beta decay was also recognized at about the same time and about 1940 a third decay process was reported, in which the nucleus breaks up of its own accord in spontaneous fission. The possibility of observing direct proton decay has recently excited a lot of interest particularly among Soviet physicists, but calculations showed that this fourth process of decay could only occur in very unstable nuclei beyond the reach of present day accelerators.

Cerny and his team were first alerted to the possibility of observing proton decay at the Atomic Energy Research Establishment at Harwell. They were studying the interaction of calcium nuclei with a beam of oxygen-16 on the heavy ion cyclotron when they found, unexpectedly, that protons were being produced in the reaction. Although the intended product was nickel-53, the most obvious explanation was that an unbound isomer of cobalt-53 had been synthesized and was then decaying to give iron-52 with the emission of a proton and two neutrons.

But the experiments at Harwell were inconclusive. Although the energies involved were such as to eliminate nickel-53 as a source of protons, the interaction of nuclei of oxygen and calcium is complicated and it proved difficult to identify all the particles accurately. Moreover, it was not feasible to rule out the possibility of delayed proton decay, in which the fundamental mode is beta decay with the proton ejected from the excited nucleus only as a secondary process.

On returning to California, Cerny and colleagues at Berkeley carried out some experiments designed to measure direct proton decay. They bombarded iron-52 nuclei with protons from the Berkeley cyclotron. In this reaction two neutrons were again emitted yielding the same excited nucleus of cobalt-53. They succeeded in identifying the decay of the nucleus by opening and shutting the beam about once a second. Those protons detected in the dead time must then have come from the short lived cobalt nucleus.

Several questions are naturally thrown up by these experiments. Why was proton decay observed in a nucleus with a deficiency of only six neutrons (from the stable state) when theory had demanded a deficit of ten neutrons ? And by what process did the protons escape ? Cerny *et al.* suggest that the escaping proton is not energetically bound to the nucleus but is held in by barriers from other physical effects. They anticipate that proton decay will be observed widely among exotic nuclei, but not among more stable ones. In any event, they foresee the need for rethinking some of the basic ideas about nuclear structure.

X-RAY STARS

Iron Lines from Sco X-1

by our Cosmology Correspondent

RECENT confirmation of emission lines of iron in the X-ray spectrum of Sco X-1 (L. W. Acton *et al.*, *Astrophys. J.*, **161**, L 175; 1970) shows that the model of this source being built up by theoreticians is essentially correct. Sco X-1 is of particular interest to both theoretical and observational astronomers because it has been the strongest single emitter of X-rays in our galaxy ever since its discovery more than ten years ago. Most X-ray stars are very short lived as emitters of X-rays, flaring up and dying away in months rather than years. This makes their study very difficult, particularly when detectors have to be flown above the atmosphere in order to measure their X-ray emission at all and balloon or rocket borne experiments are planned months in advance.

Sco X-1 has therefore been seen by astronomers as the key to unlock the secrets of X-ray stars—once its properties are explained the properties of all other X-ray stars might, with luck, be explained by similar mechanisms operating on a smaller scale. It is equally likely that Sco X-1 will turn out to be completely different from "normal" X-ray stars, but astronomers have become used to gleaning a wealth of information from small quantities of data, and there are few pessimists left in a field where so few data are available.

One feature of the optical spectrum of Sco X-1 has been the presence of many lines associated with heavy elements; taken with the necessity for very high temperature before X-rays can be produced, this has encouraged speculation that the line radiation of heavily ionized iron (Fe^{+24}, Fe^{+25}) might be seen in the X-ray emission band of the source. Several attempts have been made to detect these lines; one proved successful in finding features in the expected part of the spectrum (S. Holt, E. Boldt, and P. Serlemitsos, *Astrophys. J.*, **158**, L155; 1969), but the features had too low a significance to be conclusive on the basis of a single experiment.

Now, however, Acton *et al.* have independently carried out a similar experiment, using a large area proportional counter carried above the atmosphere in an Aerobee rocket. They too have found a feature which may arise from the radiation of highly ionized iron. The feature occurs at 6·6 keV, and has a significance of about six standard deviations relative to the background noise. Taken together, these two experiments show conclusively that Sco X-1 contains at least the amount of iron expected from averaging the quantities of all the elements in our galaxy. Probably there is rather more iron than this actually present, for the high densities and pressures in Sco X-1 ensure that much of the radiation is scattered by electrons to produce broad lines which seem less important than they would if the emission were coming from a less dense source.

This addition to the information which has been gained about Sco X-1 in recent months fits in well with the ideas of the theoreticians, suggesting that the eventual union of all this information into a reasonably complete model of the source may not be too far away.

100 m Radio Telescope in Germany

by
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The world's largest steerable radio telescope is coming into operation at Effelsberg. In this article Dr Wielebinski describes the 100 m dish.

ADVANCES in experimental sciences are often connected with the completion of new instruments. In radio astronomy the development of antennae has made an important contribution to the accumulation of observational data. In one very familiar form of radio telescope, the paraboloidal reflecting antenna, size and surface accuracy are the two important criteria. Size is limited, among other factors, by rapidly increasing building costs, and the improvement of surface accuracy depends on improvements in structural design. Apart from its imposing size, the most outstanding feature of the 100 m radio telescope nearing completion at Effelsberg, about 40 km south-west of Bonn, is its high surface accuracy.

Usually improved surface accuracy is achieved by providing stiffer structural members. With a large structure, such as a 100 m fully steerable paraboloidal reflector, however, increased stiffness requires, apart from enormous expenditure, so much additional steel that only marginal improvement in surface accuracy can be obtained. For some time there has been talk of dynamic and static methods of surface correction and in the case of the 100 m telescope[1] the latter have been applied rigorously. In the static method, often called homology design[2], a light steel structure is designed in a way that under all loads, such as movement in elevation and varying loads such as wind, the surface remains a paraboloid. Absolute deflexions of up to 10 cm are allowed, but the r.m.s. surface accuracy is expected to remain within 1 mm in all conditions of operation. Movement of the focus which ensues requires continuous correction, an easy task for a computer.

Two companies, Krupp and Man, submitted designs to the set specifications. In each case elaborate computations were required to meet a converging homology design. In the end the Krupp design was preferred and the two companies were asked to form a joint organization —Arge-Star—which was given the task of constructing the telescopes. The finance for the project, some DM32,000,000, was principally guaranteed by the Volkswagen-Stiftung. Construction began in 1968 at Effelsberg. The choice of site was dictated by the desire to remain within the boundaries of North Rhine–Westphalia, while being protected from the many sources of electrical interference. The site is a deep valley running north–south and the telescope is just visible above the neighbouring ridges when in the zenith position. The dimensions and other features of the telescope are given in Table 1.

Table 1. THE 100 M TELESCOPE

Reflector diameter	100 m
Focal length	30 m
Gregory reflector diameter	6·5 m
Gregory focal length	~360 m
Azimuth track diameter	64 m
Height of elevation axis above azimuth track	50 m
Elevation axis length	45 m
Elevation gear radius	28 m
Azimuth movement	±360°
Elevation movement	+5 to 94°
Maximum azimuth drive rate	40°/min
Maximum elevation drive rate	20°/min
Expected positional accuracy	6″
Total moving weight	~3,200 tons

The first stage of construction was preparation of the site and foundations. About 140 piles, 7–10 m long, were required to form the support for a ring foundation for the azimuth track. This ring foundation is 4 m high and rests on a 4 m wide base connected to the piles. At the centre of the ring a pivot-cone was constructed as the central turning axis of the telescope. A forged steel track carries the four bogies, each with eight wheels, which form the azimuth drive system for the telescope. The elevation drive is made through two double gear-motor systems which are carried on the 56 m diameter elevation gear wheel, and connected through two pinions to the elevation platform. The elevation platform is supported on a tetrahedron connected to the azimuth base structure.

The elevation axis is made up of a cross-like structure to which are attached, in one direction, the four supports for the primary focus cabin and, in the other direction, four members which form an octahedron supported at two points. The 100 m shell which supports the reflecting panels loads the octahedron basically at two points—just above the elevation platform at the bottom of the octahedron and at the centre of the elevation cross. In designing the telescope, care was taken to ensure that the

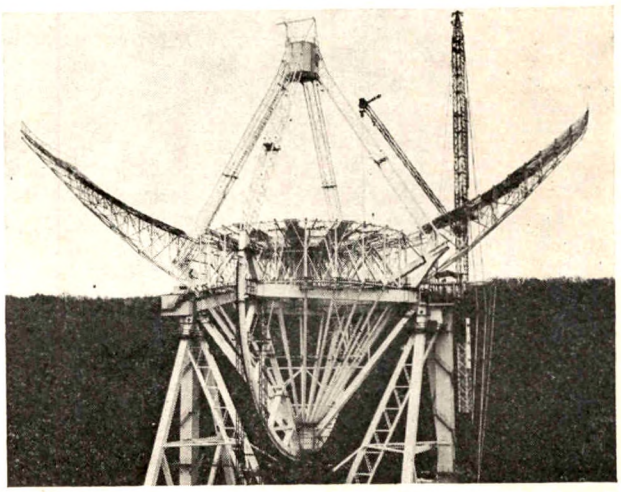


Fig. 1. An earlier construction stage showing the octahedron support and the partly assembled 100 m shell.

deflexions at the upper point of suspension, the elevation cross and paraboloid apex were identical. This umbrella-like construction is obvious in Fig. 1. The result is that the 100 m reflecting structure is virtually supported only at one point, the lowest apex of the octahedron.

The reflecting panels are constructed in three ways. The inner 60 m of the diameter has an aluminium sandwich construction in which two formed aluminium sheets are glued to a honeycomb inner sheet. Measurements have shown that by this method of construction r.m.s. surface accuracy of 0·22 mm has been achieved. The panels covering the next 20 m of the diameter are of aluminium "cassette" construction. Aluminium sections support a formed aluminium sheet. The r.m.s. accuracy of this type of panel is 0·27 mm. The outermost ring of cassette panels was made using perforated sheet to join the surface aerodynamically to the outer panels. The outer ring of panels from 85 to 100 m was made using 6 mm square stainless steel mesh. The measured r.m.s. surface deviations are 0·55 mm for the mesh panels. Each of the panels is supported on four adjusting screws. It is expected to operate with full efficiency down to 5 cm wavelength. Operation down to 8 mm wavelength is hoped for, using the 60 m central section of the telescope. The fully assembled telescope is shown in Fig. 2.

The telescope has a focus to diameter ratio (f/D) of 0·3. This is intermediate between those of the deep dishes, such as Jodrell Bank MkI (f/D = 0·25), and shallow dishes such as Parkes (f/D = 0·41). The 100 m telescope is a Gregorian reflector system which allows both prime focus operation and secondary focus work. A "collar" has been added around the aperture to reduce local interference to a minimum. For prime focus operation, a tube, about 3 m long and 1 m in diameter, will be projected through the centre of the secondary sub-reflector. A receiver box will be inserted from the prime focus cabin into this tube, which is rotatable for polarization measurements. In the secondary focus cabin, provision has been made for at least nine independent feeds. The feed horns of the hybrid-mode type will be able to operate at various frequencies. The prime focus will be used for all short wavelengths (5 cm and below) and for all wavelengths of 18 cm and above. Initially the secondary focus will provide feed horns for 6 cm, 11 cm and 13 cm (space) receivers. Multibeam operation is intended in this mode of observation.

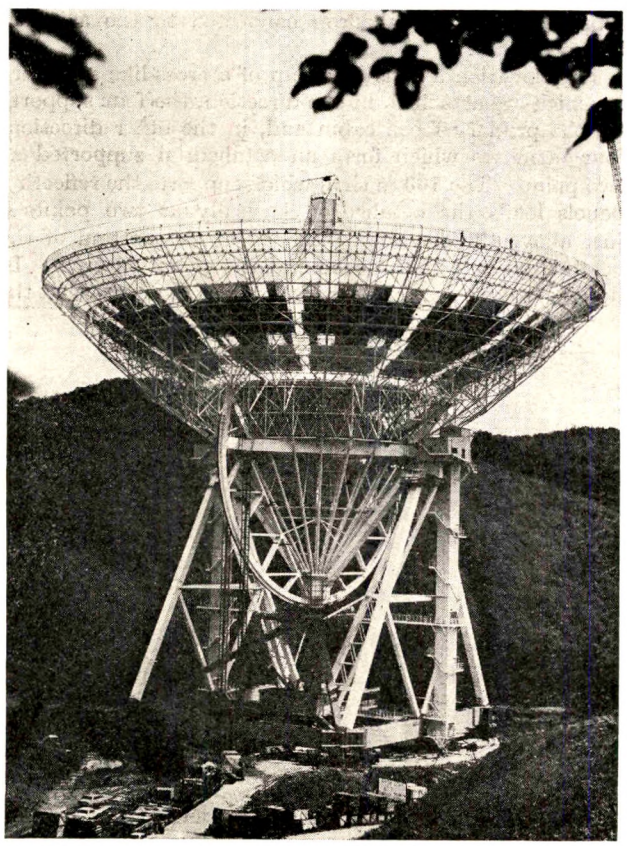


Fig. 2. Completed steelwork of the 100 m telescope.

A radio telescope of this size requires an elaborate receiving installation. Cryogenically cooled parametric amplifiers are being provided for wavelengths of 21 cm, 18 cm, 11 cm, 6 cm and 3 cm. Receivers for decimetre wavelengths will also be available. For centimetre wavelengths low noise front ends are being developed. A rubidium clock will be used for the basic timing reference for the universal oscillator in line measurements, for pulsar timing and for VLB observations. Radiometers are available for continuum measurements. Multi-channel filter receivers with bandwidths of 100, 16, 8 and 4 kHz are being constructed. An elaborate data processing system is being provided for the observers.

The basic data processor and steering computer is a Ferranti ARGUS 500. The purpose of this digital computer is to coordinate transformations, to control the telescope drive and the receiver and to process real time data.

From the control desk the ARGUS 500 computer allows the observer to choose a system of astronomical coordinates and to determine the method of scanning. The computer carries out the coordinate transformations and instructs the telescope drives of necessary drive velocities in azimuth and elevation. Because only a portion of the computer cycle is necessary for these functions, the computer is able to store data on magnetic tape, operate a fast line printer and a Calcomp plotter, as well as carrying out some on-line data processing. Further special purpose digital data processors to be provided are a 1,024 channel memory for pulse height analysis and a 384 channel autocorrelation spectrograph for line observations. At the institute in Bonn a Control Data 3300 digital computer system is available for further analysis of data.

The construction of a large instrument of this sort required the formation of a research organization and, from the Radio Astronomy Institute of the University of Bonn, a Max-Planck-Institut für Radioastronomie was formed. Three directors (Professor O. Hachenberg, Dr P. G. Mezger and myself) head an institute of about sixty scientists, forty of them astronomers. Three "service" divisions are entrusted with the operation of the telescope, data processing and electronics. An astronomical division will be responsible for the observational programmes, planned to embrace galactic and extragalactic radio astronomy. With supporting technical and administrative personnel the staff of the Max-Planck Institute is expected to grow to 150, and research students will also be able to carry out supervised research. Provision is being made for guest observers, both from Germany and from other countries. An international board of visitors meets annually to discuss and assess the performance of the institute.

[1] Hachenberg, O., *Beiträge zur Radioastronomie*, **1**, No. 2, 31 (1968).
[2] von Hoerner, S., *Astron. J.*, **72**, 35 (1967).

Nomenclature of Immunoglobulins

by
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As the problem of biological nomenclature grows, the effective system worked out for the immunoglobulins can provide a model for the clarification of a confused situation.

THE gentle art of good name calling does not come easily to most scientists, at least in regard to the objects of their study. None the less, clear and unambiguous names are needed for described phenomena and if possible the name and the phenomenon should be logically associated. There are special problems of nomenclature in immunology which arise partly from the complexity of the immune response itself, and also from the great rate at which knowledge is acquired. The great variability of immunoglobulins has presented a major problem of nomenclature. This article is concerned chiefly with the nomenclature of immunoglobulins in man, and is intended as an introduction for non-specialists. It also indicates one way in which problems of nomenclature have been tackled on an international basis.

Although the phenomenon of antibody activity in plasma has been recognized since 1890 from the work of von Behring and Kitasato on tetanus antitoxin, the finding that antibody molecules belong to a distinguishable group of plasma proteins had to wait for the development of adequate techniques of protein analysis. In 1938 Tiselius and Kabat laid the foundation for subsequent work by showing that antibody to diphtheria toxoid in man was found in the γ-globulin electrophoretic fraction. γ-Globulin preparations, although now made by methods such as Cohn's ethanol fractionation procedure rather than electrophoresis, contain most of the antibody present in human serum. They are used therapeutically in man when the passive transfer of antibodies is required.

The idea that all antibodies were to be found in a homogeneous group of proteins did not, however, survive for long. Analysis in the ultracentrifuge, for example, showed that some antibodies were present in two molecular forms, sedimenting at different rates. The γ-globulin concept finally became outmoded by Grabar and Williams's simple and elegant technique of immunoelectrophoresis, which revealed several immunochemically distinct proteins within the γ-globulin region. These proteins were electrophoretically heterogeneous, and overlapped into the β-globulin regions.

What then was the relationship between these various proteins in the β and γ-globulin regions and antibodies? Three of the proteins which could readily be distinguished by immunoelectrophoresis were soon discovered to include molecules with antibody activity. These, according to their immunoelectrophoretic designation, were γ-globulin, β_{2A} or γ_{1A}-globulin and β_{2M} or γ_{1M}-globulin. Diversity of nomenclature had already appeared. The major protein, γ-globulin, was further designated by names such as γss (γ-sensu strictu) by a classically trained school of immunologists; and 7S γ-globulin where the orientation was perhaps more towards physicochemical concepts than towards dead languages. Moreover, β_{2M}-globulin turned out to be none other than a protein designated 19S γ-globulin on the basis of ultracentrifugal analysis.

This confusing situation was not a novel one in science. It reflected the rapid progress of the protein chemistry of γ-globulins. None the less, the diversity of names was confusing and could seriously have retarded progress in research and its application to medicine. A novel way was available to solve this problem. In 1963 the World Health Organization (WHO) established its immunology unit, charged with the responsibility of ensuring the effective development of immunology for human welfare on an international scale. The first WHO meeting on the nomenclature of human immunoglobulins took place in Prague in 1964, and WHO has subsequently sponsored a series of meetings and publications on this subject (refs. 1–5). In the publications some of the leading scientists in the field have made proposals for solving problems of nomenclature. Many of the proposals have found general acceptance, and have resulted in great simplification; an exception is the proposal concerning the nomenclature of genetic markers of immunoglobulins which has appeared less appropriate in the light of subsequent research, and is not generally accepted.

In 1964, the term "immunoglobulin" was proposed as the generic name for all types of proteins with antibody activities, and for structurally related proteins. "Immunoglobulin" thus superseded "γ-globulin". Two symbols were proposed to indicate immunoglobulin; Ig was regarded as the logical abbreviation, but the symbol "γ" was also retained in recognition of its historical association with immunoglobulins. As will be seen, although the γ nomenclature suffers from a degree of ambiguity, both Ig and γ are now well established symbols, and immunologists remain typically faithful to the symbol of their choice.

Classes and Types of Immunoglobulins

Names were also proposed in 1964 for the different classes of immunoglobulins. Clear distinction between classes is essential, for these proteins differ widely in their biological properties. The major protein in the γ-globulins was designated IgG; the macroglobulin characteristic of 19S γ-globulin (γ_{1M} etc.) was recognized in the symbol IgM, while for the previous β_{2A} or γ_{1A}-globulin, the highest common factor was indicated in IgA. In this way rational-

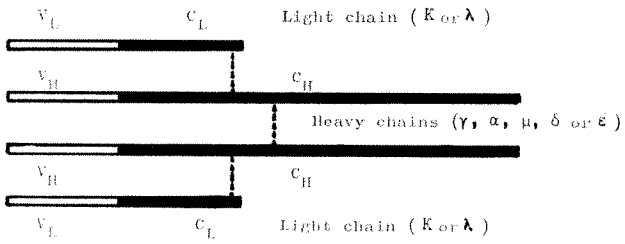


Fig. 1. A diagram of the structural unit of immunoglobulins and its constituent polypeptide chains and regions. Immunoglobulin molecules may consist of one or more of such units, for example, IgG molecules comprise one unit, IgM molecules five units. See text for a description of the symbols used for the regions.

ity was introduced while preserving a form of continuity with previous terms. The terminology provided for the introduction of names for new classes of immunoglobulins; IgD and IgE have since been added. The names of these proteins, together with their salient characteristics, are shown in Table 1.

These terms IgG, IgA and so on served to define the major classes of immunoglobulins. At the same time a terminology was established for the constituent polypeptide chains of immunoglobulin molecules. The fundamental unit of structure of immunoglobulins irrespective of their class, consists of two pairs of identical chains as shown in Fig. 1. The larger chains were designated heavy chains and the smaller chains light chains. Because differences between classes of immunoglobulins are due entirely to differences between their heavy chains, these were designated by the Greek letter corresponding to their class, for example, the heavy chains of IgG as γ-chains, the heavy chains of IgA as α-chains and so on. The light chains are common to all immunoglobulin classes but occur in two forms or types. These were designated type K or type L and the chains as κ or λ. At the time when this nomenclature was established the evidence of heterogeneity of chains was derived almost entirely from immunochemical analyses. Subsequent studies of the primary structure of immunoglobulins have shown that the differences between chains are due to major differences in primary structure.

Table 1. CLASSES OF HUMAN IMMUNOGLOBULINS

Name	Median serum concentration (mg/ml.)	Molecular weight	Biological properties
IgG (γG)	1·2	150,000	The major immunoglobulin in serum
IgA (γA)	0·4	180,000	The major immunoglobulin in secretions. Does not activate complement
IgM (γM)	0·12	900,000	"Early" antibodies often occur in this class
IgD (γD)	0·003	186,000	Function unknown
IgE (γE)	0·00025	200,000	Responsible for immediate type sensitivity

Sub-classes and Genetic Markers

Further sub-divisions of immunoglobulins are now recognized. For example, sub-classes occur in IgG, IgA and IgM. Human IgG can be divided into four sub-classes on the basis of antigenic differences between γ-chains. These are designated IgG1, IgG2, IgG3 and IgG4 in the order of their relative serum concentrations. Differences between sub-classes are due to a series of differences of amino-acid residues within heavy chains. Sub-classes are functionally distinct, for example, IgG1 and IgG3 sub-classes readily activate complement, whereas IgG_2 activates weakly and the IgG4 sub-class not at all.

Whereas all immunoglobulin sub-classes are present in all (normal) members of the species, genetic markers or allotypic specificities of immunoglobulins are present in some, but not all, individuals. In man, genetic markers have been found in γ and α-chains and in λ-chains, and allotypic specificities have also been recognized in rabbits and other animal species. Two principal series of factors have been recognized in man. Grubb, working with antiglobulins present in the serum of patients with rheumatoid arthritis, was the first to recognize one series of factors which he designated Gm. This indicated that the factor was present in gamma-globulin. More than twenty Gm factors are now recognized, usually designated by symbols such as Gm a, Gm f, although a numerical notation has been proposed[2]. Individual Gm factors are associated with individual sub-classes of IgG and Gm factors in the different sub-classes are found to be genetically closely linked. A second unrelated genetic system present in man is designated by the symbol Inv, described initially by Ropartz to be present in the serum of patient V. The factor is a property of λ chains. Inv a+ differ from Inv b+ light chains by the substitution of leucine for valine at residue 191 in the constant region of the chain (discussed later). Another variability, termed Oz, has been demonstrated at a similar position in the constant region of κ chains. Oz, however, differs from InV in that both Oz+ and Oz − κ-chains can occur in the same individual,

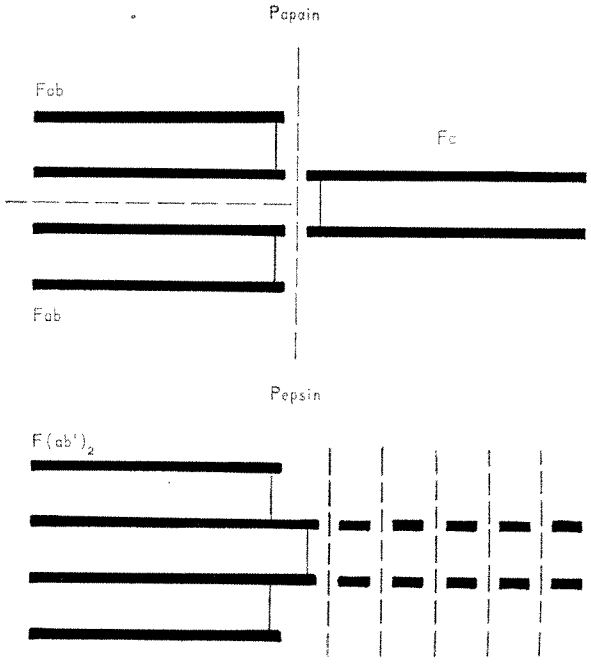


Fig. 2. The actions of papain and pepsin on IgG. Papain yields two identical Fab fragments and one Fc fragment. Pepsin yields one $F(ab')_2$ fragment, the carboxy terminal halves of the heavy chains are split into small peptides.

Enzymatic Fragments

Names have also been given to the fragments of immunoglobulins obtained by enzymatic digestion (Fig. 2). The system which has been most extensively studied is the action of papain on IgG. Three major fragments are produced and the study of these has been valuable in

elucidating the molecular structure of IgG and in assigning different functional properties to different regions of the molecule. The fragment which is composed of the carboxy terminal halves of the heavy chains is sometimes crystallizable and is designated Fc (fraction crystallizable) on this account. The two remaining fragments are identical and each comprises the amino terminal half of a heavy chain and one light chain. When obtained from antibody molecules these fragments can bind antigen, and so have been designated Fab (fragment antigen binding). That part of the heavy chain included in Fab has been designated Fd. Pepsin cleaves the heavy chains of IgG at a site slightly closer to the amino terminus, yielding one $F(ab')_2$ fragment, the remaining part of the heavy chain being further split into several smaller fragments. Most of the biological properties of IgG antibody molecules other than antibody binding are present in the Fc fragment, for example, the ability to activate complement, to attach to cells as cytophilic antibody and to be transferred across the placenta.

Constant and Variable Regions

Studies of primary structure have shown that light chains may be divided into two approximately equal regions. These are the carboxy terminal half of the chain where within a given type the sequence is invariable (except for Inv and Oz differences I have noted), and the amino terminal half of the chain where numerous differences in sequence are found, even among light chains of the same type. These two regions are therefore referred to as constant regions and variable regions. Light chain type is determined by the sequence of the constant region. Variable regions do not consist of an infinite variety of amino-acid sequences; rather a distinct group of variable regions has been found to be associated with a constant region of given type, that is, one group is found in κ-chains, and a different group in λ-chains. Further patterns of variability have been recognized within groups. On the basis of existing sequence data, three sub-groups of κ variable regions and five sub-groups of λ variable regions can be distinguished in man.

Heavy chains also have constant and variable regions. The variable regions of the heavy chains are at the amino terminal and and are of approximately the same length as the variable regions of light chains. The variable regions of heavy chains are also comparable with those of light chains in the occurrence of groups of distinctive sequence patterns. Contrary to the light chains, however, there is evidence that the groups of individual patterns may not be restricted to heavy chains of a given class, that is, the same individual pattern may occur in γ, α and μ chains. There is much indirect evidence that the antigen binding activities of immunoglobulins are determined by the primary structure of the variable regions of heavy and light chains.

The constant region of heavy chains which has been most extensively studied is that of IgG. Three linearly arranged adjacent regions have been found which have sequence similarities both to each other and to the constant region of the light chains. These three regions have been called homology regions. In addition, that region of the heavy chains close to the site of the action of papain is notably rich in proline residues and evidence derived from electron microscopy suggests that this is the most flexible region of the immunoglobulin molecule. This general region is therefore sometimes referred to as the "hinge region".

Symbols have been proposed for these different regions of immunoglobulin molecules. Constant regions of heavy and light chains are indicated by CH and CL respectively. Variable regions are similarly indicated as VH and VL. To specify a particular class or sub-class of heavy chain the symbol H could be replaced by the symbol of the chain. Thus the constant region of α-chains would be indicated by the symbol Cα. Similarly, to indicate an individual type of light chain the symbol L could be replaced by the symbol for the chain, for example, the variable region of κ chains would be indicated by V_κ. A more detailed account of proposals for the nomenclature of the regions, of half-cystinyl residues, and for formulae for immunoglobulin molecules will be found in ref. 5.

Immunoglobulins in Species other than Man

The nomenclature I have described has been developed primarily with regard to the immunoglobulins of man. Nomenclature in other species is more confused; diverse systems have grown up partly based on evidence from within a species, and partly on comparisons with other species. It would be desirable that the nomenclature which has been successfully applied to the classes and types of human immunoglobulins also be used for other species. The logical basis of such nomenclature would be the demonstration of evolutionary homology between the immunoglobulins of different species. Thus names for immunoglobulins in species close to man would be relatively easy to assign, whereas nomenclature in more distant species could prove to be more difficult.

The firmest ground for the demonstration of evolutionary homology is the finding of similarities of primary structure. Although rapid progress has been made it is hardly to be expected that this information will soon be available for many species, especially for those immunoglobulins which are difficult to isolate in purified form. In these circumstances antigenic cross reactivity forms a valuable tool for the investigation of similarities of structure, and it seems reasonable to designate immunoglobulins on this basis. Other types of comparison are on less sure ground. Criteria such as molecular weight and carbohydrate content must carry less weight than those based on primary structure.

Functional criteria could be seriously misleading. For example, it was generally considered that IgA was the major immunoglobulin of colostrum and other secretions, but in the bovine species a protein more closely related to IgG seems to be the major immunoglobulin of colostrum, whereas the protein more comparable with IgA is only a minor component.

In summary, the nomenclature of human immunoglobulins, although necessarily complex, has now been placed on a rational and generally agreed basis which is sufficiently flexible to accommodate new information. Studies of immunoglobulins in other species have important applications to the understanding of the activities of comparable proteins in man, and the establishment of homology is therefore of broader significance than its applications to questions of nomenclature. None the less, a satisfactory nomenclature for the immunoglobulins of various species can hardly be established before adequate evidence for homology is obtained.

[1] *Bull. WHO*, **30**, 447 (1964).
[2] *Bull. WHO*, **33**, 721 (1965).
[3] *Bull. WHO*, **35**, 953 (1966).
[4] *Bull. WHO*, **38**, 151 (1968).
[5] *Bull. WHO*, **41**, 975 (1969).

Restoration of Normal Growth by Covering of Agglutinin Sites on Tumour Cell Surface

by
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By covering sites on transformed cells with monovalent concanavalin A, the growth pattern of transformed fibroblasts is restored to that of normal cells. The process can, moreover, be reversed.

NORMAL fibroblasts in tissue culture stop growing when they have reached a confluent monolayer; that is, they are sensitive to growth control, the mechanism of which is not known. Virally transformed cells grow past the monolayer stage and therefore may be considered to have lost this type of growth control. Even though this phenomenon, called contact inhibition of growth or density-dependent inhibition of growth[1], is dependent on factors in the growth medium, normal cells do show a clearly different sensitivity to the growth factors compared with that of tumour cells. A correlation between tumourgenicity and loss of contact inhibition of growth has been shown earlier[2].

We have previously described a change in the surface membrane of tumour cells which seems to be correlated with the loss of contact inhibition of growth in several tissue culture lines[3,4]. This change consists in the appearance of certain agglutinin receptor sites on the tumour cell surface which were not available on the surface of normal cells, but which could be exposed by a brief and light proteolytic treatment[5]. On the basis of these findings we postulated that among the changes brought about by such a proteolytic treatment, some may be directly related to the loss of contact inhibition of growth.

Two entirely different types of experiments could lend support to such a working hypothesis and both should be able to shed some light on the mechanism of contact inhibition of growth. First, an attempt may be made to bring about a transient escape from growth control by treating the cells in a monolayer with proteases, thereby temporarily producing the type of surface change seen in transformed cells. Second, an attempt may be made to recover growth control in virally transformed cells by covering up the exposed layer of surface receptors and thereby mimicking the surface structure of normal cells. We have recently substantiated the first prediction by demonstrating a transient escape from growth control after a brief proteolytic treatment which lasts approximately as long as the surface change persists, that is, until the surface is repaired[6].

We describe here an attempt at the complementary experiment where it is proposed that covering the agglutinin sites on the tumour cell surface should bring about a reversion of the growth pattern of transformed cells to that of normal cells. We demonstrate that if one covers the agglutinin sites on tumour cell surfaces such cells will phenotypically revert to normal growth behaviour and continue such behaviour until the cover layer is removed again, at which point the same cells again begin to grow like tumour cells.

Monovalent Agglutinin for Blocking Surface Sites

Concanavalin A (Con A), a phytagglutinin or lectin, isolated[7] from Jack Bean meal (Calbiochem) was used in these studies, and was shown to react specifically with carbohydrates and preferentially to agglutinate tumour cells[8]. We decided to use Con A rather than wheat germ agglutinin because agglutination brought about by wheat germ agglutinin[9] could be overcome by serum glycoproteins and serum[10], thereby prohibiting its use in growth medium that requires serum.

To cover the agglutinin site and at the same time to avoid the criticism that any growth control which we observed was simply the result of the immobilization of two neighbouring cells by the normal agglutination process, we tried to split or degrade the agglutinin molecule into pieces which had only one binding site. First we tried papain which was earlier used to split γ-globulin, then pronase, both without success. Then we found that chymotrypsin treatment resulted in such a split agglutinin which stopped tumour cell growth at confluency, and data similar to those in Fig. 1 were obtained with chymotrypsin. A better procedure involving trypsin was independently developed by M. S. Steinberg and I. A. Gepner (in preparation). A modification of that method was used for most of the experiments described here. 1 mg Con A was dissolved in 1 ml. phosphate buffer (0·2 M, *p*H 7·0) and treated with 0·1 per cent trypsin (Difco) for 5 h at 37° C. Enzymatic digestion of Con A was stopped by the addition of 0·5–1·0 per cent soybean inhibitor (Sigma). Two pieces of evidence suggest that the trypsin did break Con A and that the monovalent pieces were attached to the agglutinin receptor site. Table 1 shows the agglutination with polyoma virus of transformed mouse fibroblasts (Py3T3) before and after preincubation with trypsinized Con A. The procedure used for the agglutination assay was as follows. Mouse fibroblasts (3T3) and Py3T3 cells were harvested by rinsing the culture dishes twice with saline at 37° C and then suspending the cells with an EDTA solution at 37° C as described earlier[3]. The agglutination tests were performed in the well of a porcelain spot plate and standardized to 10 min of mixing on a rocking platform with a constant temperature plate at 22° C. The agglutinations were scored in terms of half-maximal agglutination concentrations, that is, that concentration of agglutinin necessary to agglutinate 75 per cent of the cells. It can be seen that preincubation of the Py3T3

Table 1. AGGLUTINATION OF 3T3 AND Py3T3 CELLS BEFORE AND AFTER PREINCUBATION WITH TRYPSINIZED CON A

Cell type	Additions	Intact Con A necessary for half-maximal agglutination (μg/ml.)	Cell type	Additions	Intact Con A necessary for half-maximal agglutination (μg/ml.)
3T3	—	>1,500	Py3T3	250 μg/ml. trypsinized Con A preincubated with 5×10^{-3} M α-methyl-mannoside; added to cells and incubated for 20 min, washed and tested	250
Py3T3	—	250			
Py3T3	250 μg/ml. trypsinized Con A preincubated with cells for 20 min	>1,500	Py3T3	250 μg/ml. trypsinized Con A preincubated with cells for 20 min, washed and tested	>1,500
Py3T3	Trypsin + soybean inhibitor	250			
Py3T3	Bovine serum albumin (250 μg/ml.)	250	Py3T3	Cells preincubated with 5×10^{-3} M α-methyl-mannoside, washed and tested	250

All agglutinations were carried out according to the procedure described in the paper and are scored as concentration of Con A necessary for half-maximal agglutination.

cells with trypsinized Con A radically increases the amount of agglutinin necessary to bring about half-maximal agglutination. The remainder of the table suggests specificity of the agglutinin for a chemically defined site on the cell surface. Analytical ultracentrifugation with trypsinized Con A resolves only one peak that is moving slightly slower than the untrypsinized Con A, suggesting that the Con A was reduced in size by trypsinization.

Using this "split" agglutinin to cover the receptor sites on the cell surface, we have been able to bring about contact inhibition of growth in cultures of Py3T3 cells which normally do not exhibit contact inhibition of growth or low saturation density. Whether other properties of these transformed cells have also returned to normal after this treatment is being investigated.

We used the cell lines 3T3 and Py3T3, obtained from Dr H. Green. All experimental cell lines were grown in 60 mm Falcon tissue culture dishes with Dulbecco's modified Eagle medium with 10 per cent calf serum or 15 per cent foetal calf serum. All cultures were seeded at low densities and then allowed to grow to the required density, at which time the desired concentration of trypsinized Con A was added. This was done by removing the old medium and replacing it with fresh medium or conditioned medium in which the desired concentration of trypsinized Con A was dissolved. By seeding the cells at one density and allowing them to grow to the desired density we hoped to exclude the possibility that the effect of trypsinized Con A was on the attachment of the cells to the plastic surface and had nothing to do with contact inhibition of growth. Cell counts were made every 24 h for 6 days with a haemocytometer. New medium with trypsinized Con A was added every 2 days to prevent the accumulation of catabolites and to supply the necessary metabolites. The *p*H was kept stable in an incubator with constant pCO_2.

Fig. 1 demonstrates the graded response of cell growth observed when varying quantities of trypsinized Con A were added to the culture medium, and indicates that the higher the concentration of monovalent agglutinin in the medium the more the growth behaviour of the transformed cells approaches that of the normal fibroblasts. This suggests a quantitative relationship between the covering of the agglutinin receptor layer and the degree to which contact inhibition of growth is expressed. The more surface sites that are closed with monovalent agglutinin the lower the resultant saturation density. Such a relationship has suggested itself from earlier work in which we demonstrated that more easily agglutinable cell lines showed a higher saturation density than lines which agglutinated less at the same concentration of agglutinin[3]. Thus there seems to be a reasonable quantitative relationship between the number of sites opened and the saturation density of the particular cell line. The ultimate proof for such a correlation will require a measurement of the exact number of sites per unit area that are opened with respect to those that are covered.

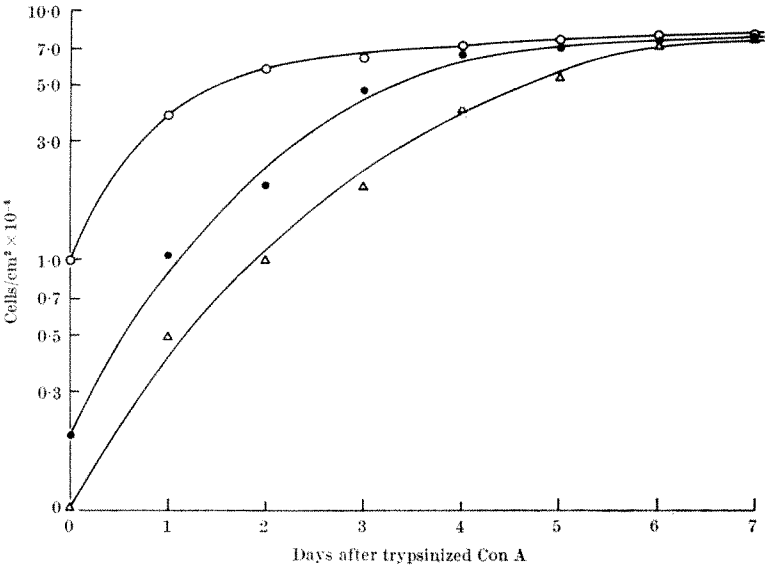


Fig. 2. Effect of 50 μg trypsinized Con A on the growth of Py3T3 cells seeded at different densities. ○—○, Py3T3 cells seeded at 1×10^4 cells per cm² + 50 μg trypsinized Con A; ●—●, Py3T3 cells seeded at 2×10^4 cells per cm² + 50 μg trypsinized Con A; △—△, Py3T3 cells seeded at 1×10^3 cells per cm² + 50 μg trypsinized Con A.

The addition of monovalent agglutinin to Py3T3 cells does not interfere with growth *per se*, for Py3T3 cells grow normally for 24–48 h before reaching confluency (Fig. 1). Further, monovalent agglutinin does not interfere with the growth of 3T3 cells at the concentration where Py3T3 overgrowth is clearly inhibited, suggesting that we are not observing merely a toxic effect of trypsinized Con A on the growth of Py3T3 cells. The addition of soybean inhibitor or of soybean inhibitor and trypsin had no effect on the growth of 3T3 or Py3T3 cells in a control experiment.

Although no toxic effects could be found with monovalent agglutinin, native, bivalent agglutinin led to the death of both 3T3 and Py3T3 cells. This toxicity might be the result of two different effects. Adjacent cells could be irreversibly bound to each other, or single cells without neighbour cells could be immobilized by bivalent molecules folding back on themselves, thereby connecting adjacent sites of the same cell surface and reducing the membrane mobility and flexibility.

Fig. 2 demonstrates that trypsinized Con A does not simply decrease or prevent growth of transformed cells *per se* but rather plays a part in a density dependent phenomenon, similar to the contact inhibition of growth observed in normal cells. In this experiment, monovalent agglutinin was added to cells previously seeded at

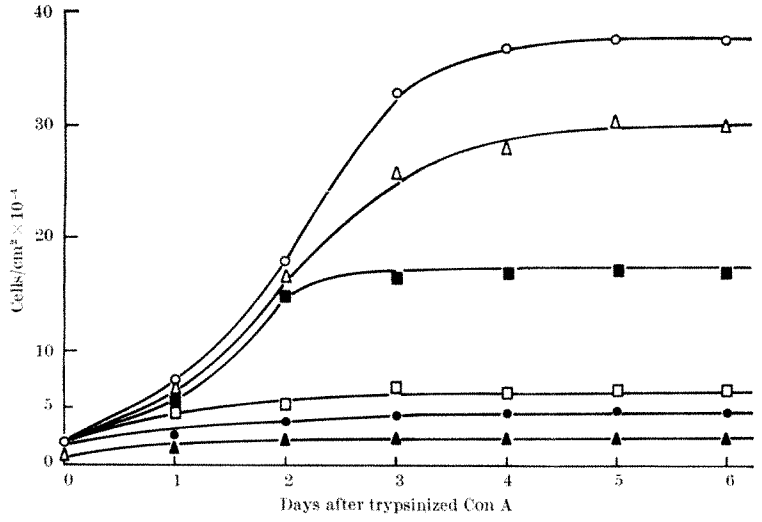


Fig. 1. Growth response of Py3T3 cells to varying concentrations of trypsinized Con A. ○—○, Py3T3, control; △—△, Py3T3 + 10 μg trypsinized Con A; ■—■, Py3T3 + 25 μg trypsinized Con A; □—□, Py3T3 + 50 μg trypsinized Con A; ●—●, Py3T3 + 74 μg trypsinized Con A; ▲—▲, 3T3, control.

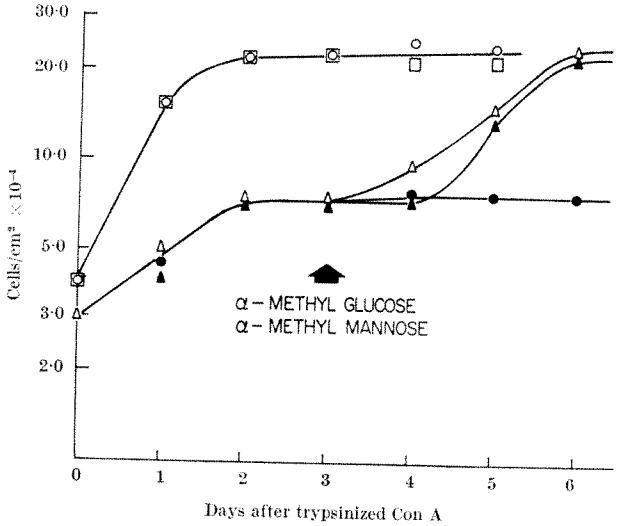


Fig. 3. Reversal of the effect of trypsinized Con A on the growth of Py3T3 cells. ○—○, Py3T3, control; □—□, Py3T3 cells + 50 μg trypsinized Con A incubated with 10^{-2} M α-methyl-glucoside for 12 h; △—△, Py3T3 cells + 50 μg trypsinized Con A + 10^{-2} M α-methyl-glucoside or 10^{-2} M α-methyl-mannoside added to the contact inhibited culture at day 3; ▲—▲, Py3T3 cells + 50 μg trypsinized Con A + 10^{-3} M α-methyl-glucoside of 10^{-2} M α-methyl-mannoside added to the contact inhibited culture at day 3; ●—●, Py3T3 cells + 50 μg trypsinized Con A.

different densities and the cells were allowed to grow. Cells seeded at the highest density stopped growing first, followed by the cells seeded at lower densities, suggesting that cell density and therefore cell to cell contact are significant in the restrictive growth control observed after covering the surface with monovalent agglutinin.

If the medium containing monovalent agglutinin is removed and replaced with fresh medium before the cultures reach confluency, the cells will grow past confluency and no growth control will be observed. If, however, new medium is added only after the cells have reached confluency, such cells will not overgrow the monolayer and the growth control exerted by the monovalent agglutinin will remain effective. This again suggests that although the attachment of the monovalent agglutinin to the cell surface is necessary to produce the growth control reported here, it is not sufficient to bring about growth inhibition but must be coupled with a particular cell density to be effective. This finding is again analogous to observations made for contact inhibition of growth in normal 3T3 cells.

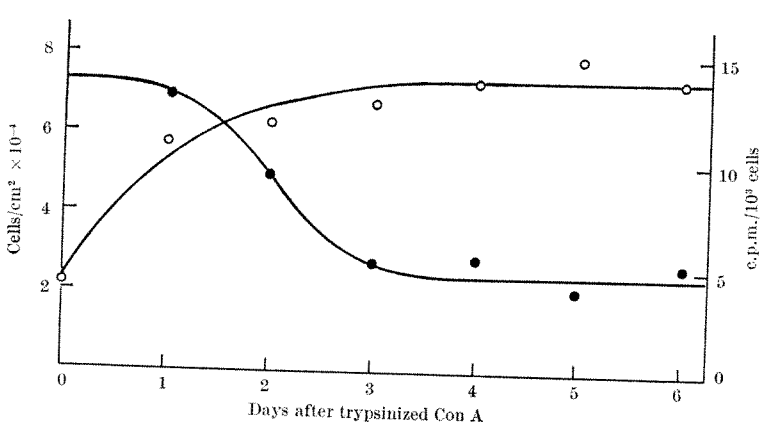


Fig. 4. ^{3}H-Thymidine uptake by Py3T3 cells grown with 50 μg trypsinized Con A. Cells were seeded at the desired density and each day a 2 h pulse of 1 μCi/ml. was added to the plate to be used the next day. After the pulse, the medium was replaced with conditioned medium + 50 μg trypsinized Con A. The next day the cell number and the radioactivity of ^{3}H-thymidine incorporated into DNA was determined. ○—○, Py3T3 cells + 50 μg trypsinized Con A; ●—●, ^{3}H-thymidine, c.p.m./10^3 cells per ml.

Evidence for Reversibility and Non-toxicity

To exclude the possibility that cessation of growth at confluency may just have been brought about by damage to the cell by the attachment of monovalent agglutinin to the cell surface, and to demonstrate that the attachment was site specific and that this site specificity accounted for the inhibition of growth, the experiment in Fig. 3 was performed. Transformed cells to which monovalent agglutinin was added together with either α-methyl-glucopyranoside or α-methyl-mannopyranoside (the hapten inhibitors of Con A) grew to the expected saturation density of untreated transformed cells. Moreover, if the cells were allowed to come to the density at which their growth was inhibited, and a hapten was then added, contact inhibition of growth was overcome, the cells resumed growth and eventually reached the cell density of the control culture. These results confirm the quantitative nature of the inhibition. The cell number of the culture released with 10^{-3} M hapten always lags behind the cell number of the culture released with 10^{-2} M hapten (Fig. 3) indicating that, the more sites that are reopened, the more rapid the release from contact inhibition of growth. Incomplete reversion with still lower concentrations of hapten is likely but this remains to be proved.

Time Dependence of the Effect of Concanavalin A

To determine the time dependence of the trypsinized Con A effect on growth, cells were allowed to grow to a density of 8×10^4 cells/cm², which exceeds the density to which trypsinized Con A treated cells normally grow. Trypsinized Con A (50 μg) was added and cell numbers were determined for the next 6 days. Somewhat less than one complete cell division could still be observed before the onset of complete inhibition of growth, suggesting that at such high cell densities all the cells are potentially sensitive to contact inhibition by monovalent agglutinin within one cell cycle but actual inhibition can only occur at a defined part of the cycle. By analogy with contact inhibition of growth in normal fibroblasts the stage in the cell cycle at which they stop is probably G_1. According to this model, any cell in G_1 will stop growth immediately while all other cells will do so only when they have reached G_1. Thus at this density the cell number of the culture should almost, but not quite, double. A slight discrepancy from exact doubling would be predicted because of all those cells that were in the sensitive part of G_1 when trypsinized Con A interrupted their further progress in the cell cycle. This prediction is substantiated in Fig. 4, and we hope to verify it in synchronous culture.

Release from growth inhibition seems to occur in much less time than the acquisition of growth inhibition. Such a short lag suggests that once the growth control induced by trypsinized Con A is released, the cells need considerably less than a full cycle to reinitiate growth.

Fig. 4 illustrates the pattern of ^{3}H-thymidine uptake into DNA by cells which have been exposed to monovalent agglutinin, and demonstrates that within 24 h after the onset of inhibition by trypsinized Con A the rate of DNA synthesis decreased rapidly, reaching a stable minimum at about the time that the cell number was maximum. This again suggests that certainly not more than one round of replication is necessary to induce growth inhibition once the cells have reached the proper density and that less than one round occurs before cessation of growth. Further, ^{3}H-thymidine uptake is still normal at least 24 h after addition of trypsinized Con A. If trypsinized Con A had blocked trans-

port in general by interfering with binding sites, pores or other mechanisms of membrane translocation, such an effect would have been expected to occur immediately or at least within 24 h. General transport[10,11] is therefore not the most likely basis for an explanation of the type of growth control involved in our experiments, but it cannot be ruled out as a secondary effect if only the transport of very specific components were altered.

A number of possible models for the role of the exposed agglutinin receptor layer in contact inhibition may be suggested. The components which cover the agglutinin receptor layer may affect the following important processes: its presence or absence may directly or indirectly change physicochemical surface properties like adhesiveness or membrane flexibility important for cell mobility and the division process; it may affect membrane turnover; the surface components may influence attachment or permeability for specific ions, general nutrients[13] or specific growth factors[14]; and they may be crucial for the propagation of messages relating to the control of cell division. Such messages could act at high cell density and close range between cells or could be exchanged intracellularly between the cell nucleus and the membrane as a means of coordinating events in the cell cycle and cell division. The validity of each of these hypotheses will be tested.

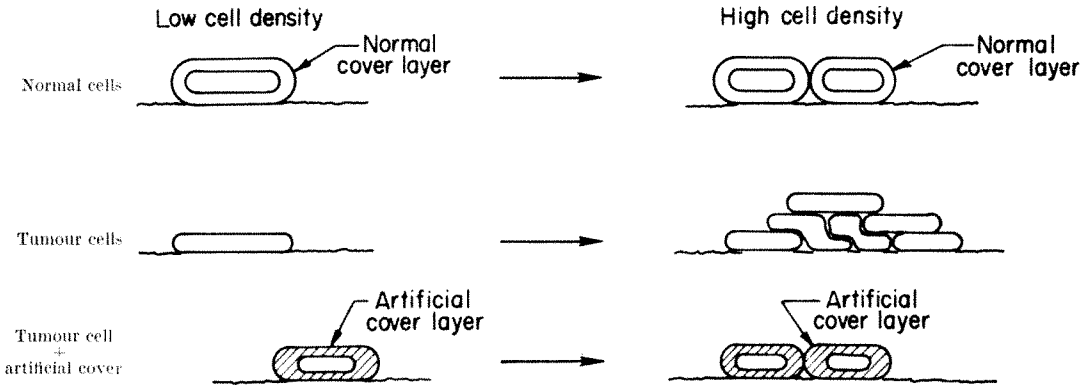


Fig. 5. Possible model for contact inhibition of growth.

Model for Contact Inhibition of Growth

Taken together with our earlier findings that exposure of the agglutinin receptor layer with trypsin can overcome contact inhibition of growth, these results demonstrate the importance of certain glycoproteins on the cell surface in the maintenance of growth control in the normal cell. The results reported here have the additional advantage of not producing the type of unspecific damage to the cell surface which trypsin must produce. Of course, we do not think that the concanavalin site is the only site that has to be covered to bring about contact inhibition of growth. Nor do we think that the wheat germ agglutinin site is the site which, if uncovered, brings about loss of contact inhibition even though we previously used it to monitor for escape from contact inhibition of growth[6]. With the evidence we now have on nine different agglutinins, most of which react with different sites exposed on tumour cells, we have suggested[6,13] and now reiterate that a whole subsurface layer is exposed in the course of transformation or brief proteolytic treatment. We propose that trypsinized Con A restores, in a functional way, a cover layer which is present in the normal cell.

Fig. 5 is an attempt to depict our conclusions in a very simplistic way; it should in no way be construed as a demonstration of a specific mechanism for contact inhibition of growth. The upper part shows a normal cell with a normal cover layer which is subject to contact inhibition of growth. On the surface of the transformed cell in the middle of the figure the agglutinin receptor sites are uncovered and growth control is lost. In the lower part the transformed surface is repaired with a substitute cover layer in the form of a monovalent agglutinin and in these circumstances the transformed cell has regained the type of growth control seen in normal cells.

Our findings allow two interpretations: loss of contact inhibition can be ascribed to the absence of the cover layer or exposure of the agglutinin receptor layer. Because the contact inhibited state can be restored by substituting the cover layer with a plant protein which is probably chemically different from the natural exterior components of mouse fibroblasts, we envisage more significance in the agglutinin receptor layer, although more work is necessary to decide between the two possibilities.

This work was supported by US Public Health Service grants, a training grant, and a grant from the American Cancer Society and the Anita G. Mestres Fund.

Note added in proof. Shoham *et al.* (*Nature*, **227**, 1244; 1970) have also come to the conclusion that native Con A kills transformed cells, a result not unexpected in view of the possible explanations offered in the text above. It should be stressed, however, that in order to avoid that killing effect and nevertheless cover the surface receptor sites we decided to split the molecule into monovalent pieces. Only this procedure allowed us to bring the transformed cells back into a state of normal growth control.

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Three-dimensional Fourier Synthesis of Human Deoxyhaemoglobin at 3·5 Å Resolution

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Electron density maps of human deoxyhaemoglobin at a resolution of 3·5 Å reveal new conformations of the C-terminal residues of the β chain and confirm their contribution to the alkaline Bohr effect. A preliminary comparison of this structure with that of horse oxyhaemoglobin shows that the main differences occur at the chain termini and at certain side chains in the $\alpha_1\beta_2$ interface.

MAMMALIAN haemoglobins undergo a reversible transition between two alternative structures. One of these is taken up when a ligand occupies the sixth coordination position at the iron atoms; the other when this position is empty. X-ray analysis of human deoxyhaemoglobin and horse deoxyhaemoglobin at 5·5 Å resolution had revealed large differences in quaternary structure between oxy and deoxyhaemoglobins[1,2]. It was hoped that the X-ray analysis at 3·5 Å resolution would identify the much smaller changes in tertiary structure which must precede the alteration in quaternary structure and also show whether the residues responsible for the alkaline Bohr effect had been correctly identified[3,4].

Much information about the mechanism of haem-haem interaction, oxygen affinity and the Bohr effect could be obtained from X-ray studies of abnormal human haemoglobins where these functions are impaired[5,6]. Some of these abnormal haemoglobins crystallize with the same space group and unit cell dimensions as normal human haemoglobin. One of us (J. G.) prepared difference Fourier maps[7] of several of these mutants at 5·5 Å resolution but found them difficult to interpret. This work was undertaken partly to determine phase angles to a resolution sufficiently high to allow the stereochemical differences between normal and mutant human haemoglobins to be clearly distinguished. One such comparison is described in a forthcoming article.

Table 1. HEAVY ATOM DERIVATIVES USED FOR ISOMORPHOUS REPLACEMENT

Derivative No.	Hg No.	Abbreviation	Substituent on S of cysteine No. 93β	112β	104α
1	1+2	PMB	$-HgC_6H_4COO^-$	—H	—H
2	1 to 6	MM	$-HgCH_3$	$-HgCH_3$	$-HgCH_3$
3	3+4	$HgAc_2$	$-CH_2-CONH_2$	$-Hg-OOC-CH_3$	—H

Experimental Procedures

Crystals of human deoxyhaemoglobin were grown in ammonium sulphate solution, buffered to *p*H 6·5 as described by Muirhead *et al.*[1]. The crystals belong to the monoclinic space group $P2_1$ with unit cell dimensions $a = 63{\cdot}2$ Å, $b = 83{\cdot}4$ Å, $c = 53{\cdot}8$ Å and $\beta = 99° 15'$. The unit cell contains two molecules so that the crystallographic asymmetric unit contains one complete molecule of molecular weight 64,000. The limiting sphere of radius 3·5 $Å^{-1}$ contains 6,900 independent reflexions. Human haemoglobin contains three pairs of sulphydryl groups which differ in reactivity[8,9]. Three heavy atom derivatives were used to determine phase angles by the multiple isomorphous replacement technique[10]: in one cysteines 93β were substituted with *p*-mercuribenzoate (PMB), in the second cysteines 93β, 112β and 104α with methyl mercury (MM), and in the third cysteines 112β with mercuric acetate ($HgAc_2$) (Table 1). The unit cell dimensions of crystals of all three derivatives were the same as those of the parent compound within experimental error.

A complete set of three-dimensional data between 6·0 and 3·5 Å resolution was measured for the native protein and for each of the three derivatives. The phases of all the reflexions to a resolution of 5·5 Å had already been determined from photographic measurements[1]. 11,600 X-ray diffraction intensities comprising 5,800 independent reflexions were measured for each data set on a Hilger and Watts four-circle diffractometer, which was fitted with helium-filled input and output collimators and connected on-line to a time-shared Ferranti Argus 312 computer, using CuKα radiation from a Philips sealed fine-focus X-ray tube run at 40 kV and 24 mA. Each reflexion was measured by an ordinate analysis procedure as described by Watson *et al.*[11]. The measured intensities were corrected for Lorentz, polarization and absorption factors[12] using a program written by T. H. Gossling.

Because of radiation damage only about 1,000 Bijvoet pairs of reflexions could be measured from a single crystal. Each data set contained measurements from five to eight different crystals and these measurements were scaled together using a least squares procedure. For a single crystal the agreement residual for the structure amplitudes of Bijvoet pairs of reflexions

$$\left(2{\cdot}0\frac{\Sigma(\|F_{hkl}-|F|_{h\bar{k}\bar{l}}|)}{\Sigma(|F|_{hkl}+|F|_{h\bar{k}\bar{l}})}\right)$$

varied between 0·03 and 0·07, the largest values being obtained for the crystals with six molecules of methyl mercury per molecule of haemoglobin, while when the different crystals in each set were scaled together the percentage standard deviation in F varied between 2·4 and 3·2. It is impossible to determine accurately the overall temperature factor of the crystals using data to a resolution of only 3·5 Å, but it seems to be less than the temperature factor for crystals of horse oxyhaemoglobin.

Approximate heavy atom coordinates were known already[1] but three-dimensional difference Patterson syntheses were calculated for all three derivatives to check their quality. The heavy atom parameters were refined using alternate cycles of phase determination and least squares refinement[1,13,14] on 1,800 selected reflexions, anomalous scattering being ignored. These reflexions were chosen from the medium-to-strong intensities so that there were equal numbers in equal ranges of $\sin^2\theta/\lambda^2$. The parameters derived from the last cycle of the refinement process are given in Table 2.

The centroid[15] phase angles of all 5,800 independent reflexions were calculated from the final heavy atom parameters. Fig. 1 shows the variations of the mean parent and calculated heavy atom structure amplitudes for the complete data sets, and of the accuracy of the phase determination as a function of resolution. The mean figure of merit, averaged over all reflexions, is 0·81 corresponding to a mean error in phase angle of 36°. For all three derivatives the root mean square lack of closure of the phase triangles, E, is significantly less than the root mean square calculated heavy atom structure amplitude, f_H, indicating that all three derivatives are providing accurate phase information at 3·5 Å resolution

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The diffractometer data and the 5·5 Å resolution photographic data were scaled together using overlapping reflexions, and weighted averages of the phase angles were used in the Fourier calculations.

Electron Density Map

The "best"[15] Fourier synthesis was calculated in sections perpendicular to the a^* axis at intervals of $a/80$, $b/120$ and $c/80$ using a Fortran program written by R. A. Crowther. Sections of the Fourier perpendicular to the molecular dyad axis were calculated on a Cartesian coordinate system[1] at 1 Å intervals, using a skew planes program written by J. M. Baldwin. These sections were displayed in contour form on a computer controlled oscilloscope and recorded on 35 mm film[16]. The zero contour was at 0·15 e/Å^3 and the contour interval was 0·15 e/Å^3. The maximum density was 2·30 e/Å^3. They were enlarged by projection and copied on to 'Perspex' sheets to the scale 2 cm = 1 Å for subsequent interpretation and model building. The electron density map was sufficiently clear to construct a skeletal model of human deoxyhaemoglobin using a half silvered mirror to superimpose the model and the electron density map[17].

The absence of any ligand on the haem group in deoxyhaemoglobin as in deoxymyoglobin[18] is clearly seen by comparing the electron density on the distal side of the haem group in Fig. 2*a* with that of the β haem group in horse oxyhaemoglobin (Fig. 5 of ref. 19): a difference of one or two oxygen atoms can be detected. The only large differences in tertiary structure between oxy and deoxyhaemoglobins are those involving the termini of the polypeptide chains affecting the environment of the residues thought to be responsible for the alkaline Bohr effect[3].

Fig. 2*a* (sections −14 to −8 of the map) shows the carboxyl terminus and the surroundings of the haem group of the β-chain. Note that Tyr HC2(145)β is very clearly defined in contrast to the low density found for the phenolic group in the horse oxyhaemoglobin high resolution map[20]. The carboxyl group (labelled COO^-) and the terminal histidine side chain, His HC3(146)β, are also clearly visible; the latter bonding to the γ-carboxyl of Asp FG1(94)β in the same chain. The formation of this bond in deoxy as opposed to oxyhaemoglobin[20] was predicted and must be responsible for the contribution of the β-chains to the alkaline Bohr effect[3]. Unexpectedly, a salt bridge from the terminal carboxyl group to the ε-amino group of Lys C5(40)α stabilizes the conformation of the terminus and forms an $\alpha_1\beta_2$ (ref. 21) link which is absent in oxyhaemoglobin.

Fig. 2*b* (sections 8 to 15) shows salt bridges between the two α chains: one between the guanidinium group of the terminal Arg HC3(141)α_2 and Asp H9(126)α_1; the other bridges form part of a system of four polar groups involving Asp A4(6)α_1, Lys H10(127)α_1, the alpha amino group of Val NA1(1)α_1 and the alpha carboxyl group of Arg HC3(141)α_2. The array is such that Asp A4(6)α_1 can bond only to Lys H10(127)α_1. Lys H10(127)α_1 could bond to both Asp A4(6)α_1 and to the α-COO^- of Arg HC3(141)α_2. The α-NH_3^+ of Val NA1(1)α_1 is too far away to bond to Asp A4(6)α_1 but may be within the correct distance to form a salt bridge with the α-COO^- of Arg HC3(141)α_2. The system thus contains one salt bridge linking two polar groups within the same α-chain: Asp A4(6)–Lys H10(127); this is common to both oxy and deoxy structures. In addition there are salt bridges between the two α-chains which exist only in deoxyhaemoglobin: Asp H9(126)α_1–Arg HC3(141)α_2 and possibly NH_3^+ of Val NA1(1)α_1–COO^- Arg HC3(141)α_2. The tetramers will, of course, contain twice the number of bridges listed here, because of their dyad axis. These bridges would have to break if the tetramer were to dissociate into αβ dimers. As in the β-chain (Fig. 2*a*) the penultimate Tyr HC2(140)α is clearly defined in the deoxy structure.

Table 2. FINAL HEAVY ATOM PARAMETERS AND *R*-FACTORS

	Z	*z*	*y*	*x*	*B*	*R*
PMB						
Site 1	81	0·234	0·050	1·112	29	0·44
2	80	0·240	0·0	0·420	28	
MM						
Site 1	88	0·242	0·059	1·103	71	0·43
2	89	0·246	0·002	0·422	75	
3	66	0·023	0·181	0·744	11	
4	79	0·507	0·125	0·753	20	
5	86	0·086	0·167	0·844	25	
6	73	0·439	0·110	0·655	13	
$HgAc_2$						
Site 3	47	0·024	0·185	0·739	16	0·60
4	65	0·507	0·128	0·763	32	

Z is the occupancy; *x*, *y* and *z* fractional coordinates and *B* the isotropic temperature factor. All six sites lie in a single molecule. Sites 1 and 2, 3 and 4 and 5 and 6, respectively, are related by the non-crystallographic dyad axis.

$$R = \frac{\Sigma(\| F_H \|_{obs} - \| F_H \|_{calc} \|)}{\Sigma(\| F_H \|_{obs} - \| F_P \|_{obs} \|)}$$

The heavy atom parameters were refined using 1,800 selected reflexions. The *R* factors are based on all the measured reflexions.

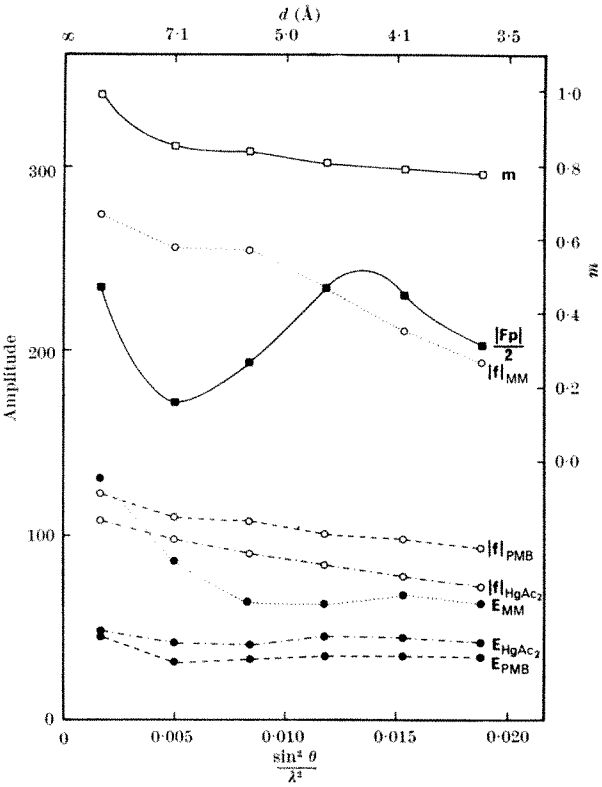
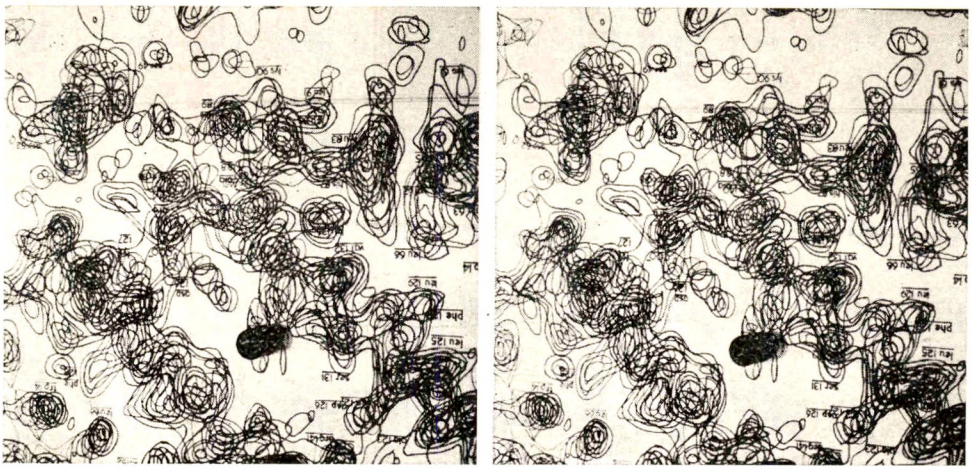


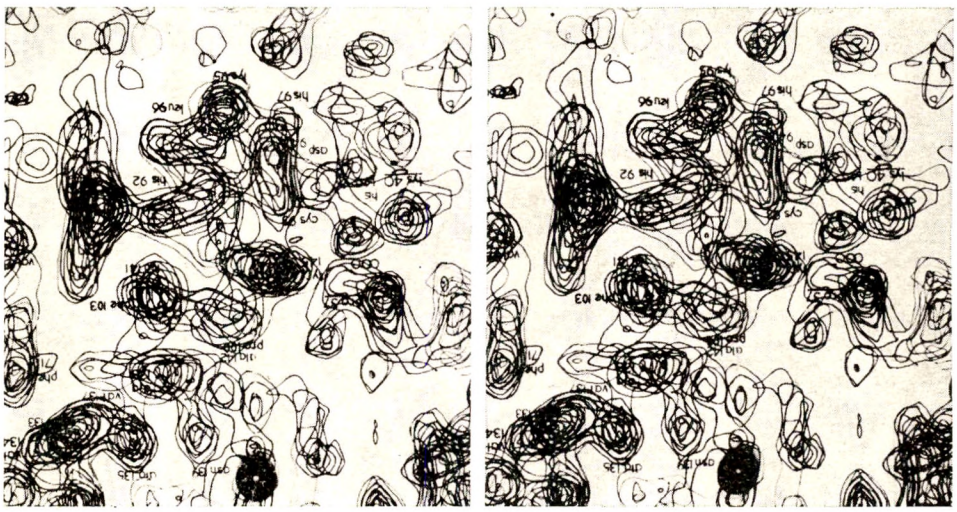
Fig. 1. Mean structure amplitudes of the native protein and of the heavy atoms, and accuracy of phase determination as a function of $\sin^2\theta/\lambda^2$. The meaning of the curves from the top downwards is as follows: *m* (ordinate at top right) is the average figure of merit of the phase angles[14]. $|F_P|$ is the average structure amplitude of the native protein. The three curves for the $|f|$ values (○) of the heavy atom derivatives refer to the average root mean squares of $|f_H|$. The *E* values (●) represent the root mean square lack of closure errors of the phase triangles for the three derivatives. – – –, PMB; · · ·, MM; — · — · —, $HgAc_2$.

The amino terminal group of each α-chain is involved in the alkaline Bohr effect[4]. The formation of a salt linkage between the amino and carboxyl termini of opposite α-chains in the deoxy form only has been predicted in order to explain this contribution to the Bohr effect[3]. It is not yet possible to confirm the existence of this salt linkage without refinement of the atomic coordinates. All other changes in tertiary structure are small and those which have been interpreted occur in the contacts between subunits.

The $\alpha_1\beta_1$ contact is almost identical in the oxy and deoxy molecules as expected from the comparison at 5·5 Å resolution[1,2]. The only significant change is linked to the stabilized conformation in deoxy of the carboxyl termini of the α-chains. In horse oxyhaemoglobin there is probably a hydrogen bond between Asp H9(126)α_1 and



a



b

Fig. 2. Stereoscopic figures of the 3·5 Å deoxyhaemoglobin electron density map. The sections are perpendicular to the molecular dyad axis and are spaced at 1 Å intervals. The centre of the black ring (top centre in both figures) marks the dyad axis. The zero contour is at 0·15 e/Å^3. The contour interval is 0·15 e/Å^3. Underlined residues form part of the α chains; plain ones part of the β chains. *a*, A superposition of sections −14 to −8 of the electron density map of human deoxyhaemoglobin. The scale is 2 mm to the Å. Part of the β haem group is seen edge on at the centre right attached to the proximal histidine (his 92). Tyr HC2(145)β (*Tyr* 145) is at the centre. Below and to the left is the salt linkage between His HC3(146)β and Asp FG1(94)β. The β-chain carboxyl terminus (COO^-) is bonded to Lys C5(40)α (*Lys* 40) as is shown at the centre left. *b*, Sections 8 to 15 of the electron density map showing one carboxyl terminus and part of the H helices of the α-chains. The scale is 1·5 mm to the Å. Part of the α haem group is seen edge on at the lower left attached to its proximal histidine (*His* 87). The two salt linkages are to the right of and below the molecular dyad; one between Lys H10(127)α_1 (*Lys* 127) and the terminal carboxyl of α_2 (COO^-), and the other between Asp H9(126)α_1 (*Asp* 126) and Arg HC3(141)α_2 (*Arg* 141). The phenolic group of Tyr HC2(140)α (*Tyr* 140) lies 12 Å below the dyad and half way between the labels *His* 87 and COO^-.

Tyr C1(35)β_1[20]. This aspartate is bonded to Arg HC3(141)α_2 in deoxyhaemoglobin; the tyrosine is lying in the $\alpha_1\beta_1$ interface and does not seem to form a hydrogen bond.

The largest relative movement between subunits occurs at the $\alpha_1\beta_2$ interface[1,2]. Remarkably, this large change in quaternary structure is accompanied by only very small changes in tertiary structure at the interface. Small adjustments in the Gα_1 helix and the Cβ_2 helix allow Pro G2(95)α_1 to remain in van der Waals contact with Trp C3(37)β_2 in spite of the relative movement of the two chains.

The flexibility of the side chain of Arg C6(40)β_2 allows it to maintain contact with Tyr C7(42)α_1 in both structures, although the detailed interaction may be different. The γ-hydroxyl of Thr C6(41)α forms a hydrogen bond to the main chain carbonyl of Thr C3(38)α in the same chain in deoxyhaemoglobin. In oxyhaemoglobin the proximity of Thr C6(41)α_1 to His FG4(97)β_2 suggested the possibility of a hydrogen bond between these residues[20]. A comparison of the electron density maps of horse oxy and human deoxyhaemoglobins indicates, however, that the threonine bonds back into its own α chain in the oxy structure as well.

As a result of these changes in tertiary and quaternary structures, there are four extra salt bridges between α_1 and α_2 in the deoxy structure. It therefore seems that the deoxyhaemoglobin tetramer would be less likely to dissociate into dimers than that of oxyhaemoglobin, as has been observed by Kellett, Midgarden and Schachman (private communication). There are also differences in the van der Waals contacts at the subunit interfaces, which are very small at the contacts $\alpha_1\beta_1$ and large at the contacts $\alpha_1\beta_2$. Without accurate atomic coordinates, however, it is impossible to predict whether the van der Waals contacts increase or decrease in stability.

The relationship between ligand bonding and the changes in tertiary and quaternary structures is still

obscure and these results do not suggest any mechanism for cooperativity. The identification of all the changes in tertiary structure as well as a complete description of the quaternary structure change will require a more detailed comparison of the oxy and deoxy structures. This may be possible on the basis of a Fourier synthesis of horse deoxyhaemoglobin at 2·8 Å resolution determined by Bolton (to be published).

Copies of the structure amplitudes and electron density maps are available on request.

We thank Dr M. F. Perutz for discussion, Miss Valerie Coulson for technical assistance and Mrs J. M. Baldwin, Dr R. A. Crowther and Mr T. H. Gossling for making computer programs available to us. For computing facilities we thank Professor F. Hoyle and the computer staff of the Institute of Theoretical Astronomy, Cambridge, and the manager and staff of the Computer Unit at the Rutherford High Energy Laboratory, Harwell. One of us (J. G.) thanks the US National Science Foundation for a fellowship.

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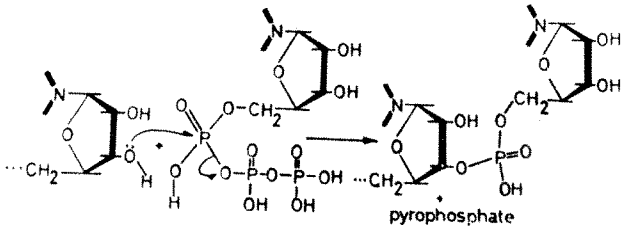
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RNA Polymerase: Two-step Mechanism with Overlapping Steps

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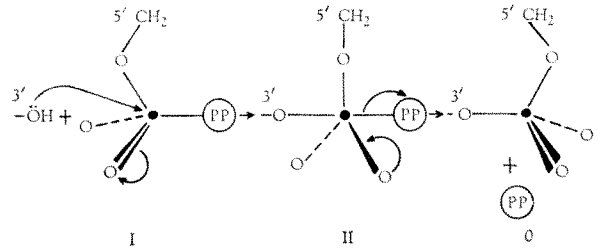
RNA transcription could take place by Watson–Crick base pairing between the growing RNA chain and the DNA template if the DNA unwinds locally after changing from its usual B form to the less stable A form.

RNA polymerase (EC 2.7.7.6) synthesizes a polyribonucleotide with the aid of a DNA template according to the following scheme.



The chain lengthening proceeds by linkage of the ribose 3′-hydroxyl to the 5′-phosphate group of an attacking nucleotide, the base sequence of the new polymer being determined by the base sequence of the DNA[1].

Findings concerning the general mechanism of hydrolysis of phosphate esters indicate that the splitting of the ester bond proceeds by way of an intermediate in which the five covalent bonds of the phosphorus atom are in a trigonal bipyramidal configuration[2]. The overall reaction of nucleotide addition may therefore be depicted by the following two-step scheme.



In this reaction, intermediate II is thermodynamically unstable, and its formation requires energy. But the next step, pyrophosphate liberation, proceeds with release of considerable free energy. It is tempting to suggest that RNA polymerase operates in such a way that these two stages are concerted, so that in the course of addition of a new nucleotide, formation on the thermodynamically unfavoured "pyramidal" triphosphate intermediate is coupled with the pyrophosphate release.

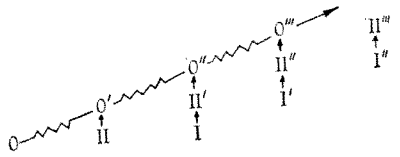
Here, we suggest a model for the molecular mechanism of RNA polymerase action based on the above hypothesis, assuming an overlap of steps in the process of sequential addition of two nucleotides (see ref. 3 for a more detailed review of the data). We will deal only with the polymerization mechanism proper (chain elongation) and not with other known functions of the enzyme, such as the recognition of an operon promoter and the breaking of the polynucleotide chain at the operon termination region.

The two types of atomic models that have been used in this study are spacefilling models ("Eugon", Hungary) and structural models of the Dreiding type. Models of the A form of DNA and of nucleotides conforming to the "Eugon" were manufactured for us by Yu. N. Symakin according to atomic coordinates from ref. 4.

Model for Operation of RNA Polymerase

By "state I" we designate the state of an adding nucleoside triphosphate on attachment to the active site of the RNA polymerase system before covalent binding to the 3′-hydroxyl of ribose. The state of a nucleotide in pyramidal configuration at the α-phosphate group will be designated "state II". A nucleotide completely incorporated into the RNA chain will be indicated by the symbol "0". Then, according to the above "overlap hypothesis",

the nucleotide pattern at the growing end of RNA would be



Conformation of the Template at the Growing Point

We shall assume that DNA transcription involves the Watson–Crick base pairing mechanism. This assumption seems to be the most reasonable one at present, although alternative schemes have been proposed in which a triple complex with non-uncoiled DNA is formed[5].

For the unwinding of DNA with the concomitant formation of a new hybrid A-like structure to be "economical", we think that the A form of template is more suitable than the B form. Indeed, the base pairs in the glycosidic groove (that which is faced by glycosidic bonds) of the A form are located near the periphery of the molecule, so that only a small displacement is required for a base to be found outside the parental helix. We can therefore also assume that the normally unstable A form of DNA helix is stabilized in a section of the template adjoining the active site of the system. The idea that RNA synthesis is connected with the B→A transition was emphasized in the studies of Hamilton[6] and Arnott *et al.*[7]. Thus RNA polymerase from *M. lysodeikticus* can operate with two-stranded polyribonucleotides which can exist only in the A conformation[8]. That one and the same enzyme may operate with such different template structures as the B form and A form is contradictory to the concept of enzyme specificity. Because it is impossible to induce the A→B transition in RNA double helices we are faced with the necessity to induce the B→A transition in DNA. Such a local B→A transition in the course of polymerase operation might be induced by exclusion of some water molecules from the DNA section bound to the polymerase protein. Removal of water provides, at the same time, appropriate conditions for the reaction of nucleotide polycondensation to occur.

Examination of three dimensional molecular models shows that there is no substantial steric hindrance to the unfolding of a base in one DNA strand from the glycosidic groove of A form DNA after disruption of hydrogen bonds linking it to the complementary base of the other strand (Fig. 1). This unfolding is caused by rotations in the sugar phosphate backbone. It can be demonstrated with atomic models that, when DNA bases are "extracted" in this way, the conformational change is restricted to one DNA strand only, while the conformation of the other strand remains practically unaltered. If the "extracted" part of the chain consists of two or three bases, a small disruption will be produced in the regular A structure (Fig. 1).

Structure of the Hybrid at the Growing Point

If our basic assumption of reaction step overlap is correct, two nucleotides are involved at a time. The adding nucleotide is in state I and the preceding nucleotide is in state II; both are base paired to the DNA. The minimum number of bases at the unfolded region of the template would be two.

A few experimental data are available which testify to the existence of "stacking" interaction between base pairs in the hybrid region[9,10]. Inspection of molecular models shows that a couple of the "extracted" bases, together with the complementary counterparts of the ascent RNA, can be fitted into a right handed helix similar to that of A form (Fig. 1). The spatial arrangement of the helices of DNA and of the hybrid portion seems to be far from random: the approximate axis of the hybrid helix is inclined to the DNA axis at an angle, the minimum magnitude of which is restricted by the necessity to provide room for the last two ribonucleotides; this angle is about 40°. It can be measured in space-filling atomic models as the angle of inclination between the average planes of the pentose rings (Fig. 1). If one postulates a requirement for the conservation of template regularity the regular hybrid must be short because of the divergence of the axes of the hybrid and the template. (If no such postulate is made, the hybridized portion may be long. Note that the term "helical axis" is not applicable in a strict sense to a hybrid of two base pairs, but the general conformation of such a hybrid is similar to that of a short piece of A structure.)

Thus at each step in the RNA polymerase operation a new base is "extracted" from the DNA template, and the deoxynucleotide, the complementary ribonucleotide of which has lost pyrophosphate, folds back into the DNA with restitution of the DNA hydrogen bonds. The helix axis of the hybrid moves in a helical way along the DNA, thereby implying a simultaneous shift of the enzyme.

To clarify our model for the molecular mechanism of the enzyme and the functions of the protein moiety of RNA polymerase, it is necessary to consider the question of binding points between the enzyme and the template and the detailed stereochemistry of the RNA chain elongation.

Binding between RNA Polymerase and Template

In the absence of the substrates, RNA polymerase is known to be bound with DNA rather weakly, so that an increase in ionic strength will lead to dissociation of the enzyme from the DNA[1]. During the synthesis of RNA, however, the bonding of the enzyme with DNA becomes sufficiently strong to form a non-dissociating complex[1]. In the model that we propose, this specific strong attachment, arising in the course of RNA synthesis, results from interaction between the negatively charged pyrophosphate groups of the two interacting ribonucleoside triphosphates (II and I) and the cationic sites of the polymerase subunits. The base moieties of these triphosphates from Watson-Crick pair with the DNA bases. Nucleotides I and II, together with nucleotide "0", form the transient hybrid region (the A form part of the hybrid

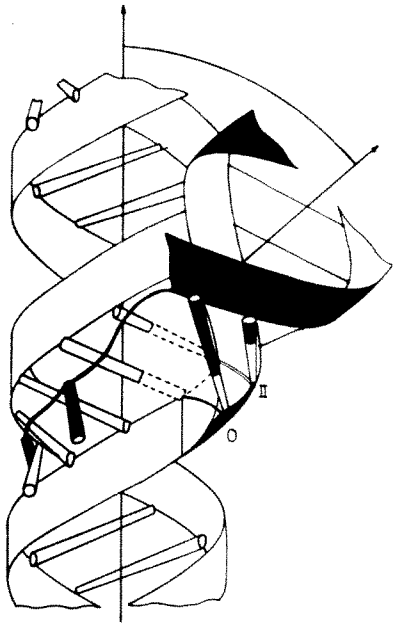


Fig. 1. Conformation of the template and structure of the hybrid at the growing point of the RNA chain. In the A form, the approximate planes of the sugar rings are represented by the ribbons. The A form hybrid really consists of two pairs but is extrapolated in order to demonstrate the interrelationship of the helices.

is formed by the 0 and II pairs). Because two ribonucleoside triphosphates function at any one time, our model explains the strong binding of the enzyme; besides its non-specific interaction with DNA (which is probably electrostatic), there are additional specific bonds during the synthesis. This proposition is attractive because, on release of pyrophosphate, firm attachment of the given binding site to DNA is abolished. It is also important to the consideration of a molecular mechanism for the polymerase movement.

Conformations of the Nucleotides and Enzyme Movement

By assuming an A type conformation for the hybrid region (0, II) the sugar-phosphate backbone can be depicted as in Fig. 2. One can see that the presence of a pyramidal phosphate does not lead to significant distortion of the A form backbone. The conformation of the pyrophosphate part in nucleotide II will be considered later. The deviation of the backbone structure from the A form will be smallest in the case of the apical bond cleaved in the pyrophosphate. Disposition on one line of the ribose-α-phosphate and α,β-phosphate bonds also follows from the stereochemistry of phosphate ester hydrolysis[2]. We have therefore defined the conformation of the sugar-monophosphate part of nucleotides 0 and II.

The non-covalently bound nucleoside triphosphate I has a very different position from that of a nucleotide in the A form. The phosphorus atom attacking the ribose 3′-hydroxyl should be no less than ~3·5 Å from the 3′-oxygen (the sum of their van der Waals radii). In the next step (I→II), the covalent bond formation, this distance will be reduced to 1·98 Å (this being the length of an apical bond in pyramidal phosphate[11]. Moreover, because the resulting (0′II′) situation is connected with the preceding (0 II) situation by helical symmetry operation (with the translation value of A form), the nucleotide I should be located above nucleotide II (with respect to the DNA axis) (Fig. 2). The restrictions stipulated by these requirements permit fairly precise localization in space of the atoms of nucleotide I (Fig. 2).

Essentially, the only conformations that remain unknown are those of the pyrophosphate groups of nucleotides I and II. With space-filling atomic models one can see that the pyrophosphate of the pyramidal intermediate (II) can be arranged in such a way that its terminal oxygen will be at a distance of a hydrogen bond from a ribose 3′-hydroxyl of this nucleotide (Fig. 3). If such a conformation is possible in nucleotide II, the attractive

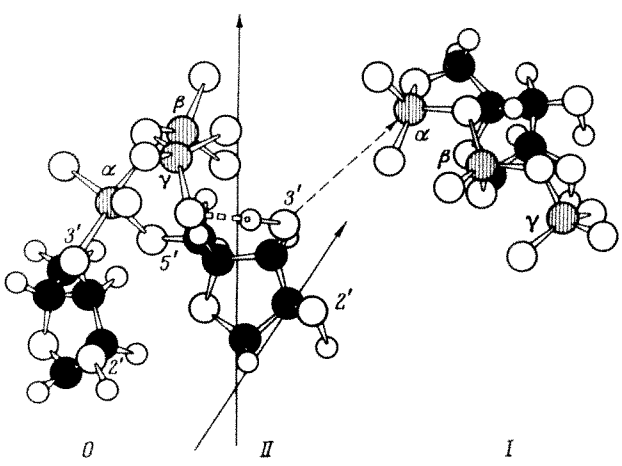


Fig. 2. Stereochemistry of the sugar-phosphate backbone at the growing end of RNA. Solid arrows show the direction of the axes of the DNA template and of the hybrid. Dotted arrow indicates the direction of nucleophilic attack. The binding points for the enzyme are the terminal pyrophosphate groups of nucleotides I and II. Note the pyramidal α-phosphate group and the hydrogen bond between the γ-phosphate and the 3′-hydroxy groups of nucleotide II.

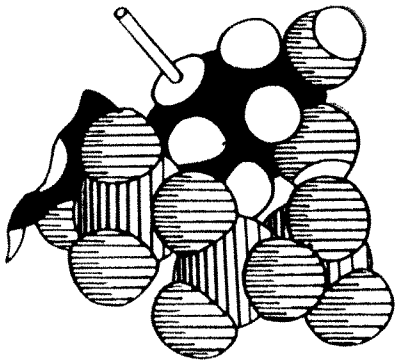


Fig. 3.

possibility arises that the pyrophosphate group acts as a catalyst for nucleophilic addition of the nucleotide I. Moreover, the basicity of the pyrophosphate would steadily increase in the course of formation of the transition state, thereby promoting proton removal from 3′-hydroxy group. This is the condition for the reaction to occur effectively. The mechanism also provides for synchronization of the steps I→II′ and II→0′, and suggests a molecular basis for the coupling of the exergonic step of pyrophosphate release with the endergonic step of pyramidal phosphate formation.

The last structural aspect to be examined is the conformation of the pyrophosphate group in nucleotide I. If we assume that the binding sites of the enzyme for successive nucleotides are on one level with respect to the DNA axis, then, because nucleotide I is situated above nucleotide II in the helical system, its pyrophosphate group must be pulled down relative to the ribose ring (Fig. 2, right). Only at the next step (I→II′) will this pyrophosphate group attain the hydrogen bonded conformation characteristic of state II. We now have all the prerequisites for a description of the movement of RNA polymerase along the DNA molecule.

At the moment when the next deoxynucleotide folds out from the DNA template, the thermal energy of the movement would probably be sufficient for a neighbouring nucleotide to assume the required position. (It is known that several terminal base pairs of a two-stranded DNA molecule are unwound at physiological temperatures[12].) If an appropriately situated binding site on the polymerase holds a complementary ribonucleoside triphosphate, the DNA base extracted will be fixed in the new position to form a hybrid pair (state I). We thus obtain an A type conformation constituted by the nucleotide pairs 0 and II, and the hybridized nucleotide I, which at that step does not form part of the A structure. To achieve a new A type conformation (II′ 0′) from the pairs (II and I), pair II must be displaced to the right and upwards (Fig. 4). When this shift reaches the extent when a ribose 3′-hydroxyl is at a distance equal to the sum of van der Waals radii from the P atom of the α-phosphate group in the ribonucleoside triphosphate I, nucleophilic addition will occur with the formation of the intermediary pyramidal phosphate. This is coupled with pyrophosphate release from the preceding nucleotide: I→II′; II→0′. In the course of pyramidal phosphate formation and of the preceding displacement of a nucleotide II, the pyrophosphate group of the latter will also be shifted; this shift, as can be seen from Figs. 2 and 4, must have a component along the DNA axis. Because the pyrophosphate group is attached to an enzyme subunit, it is shifted upwards by the translation value for the A form (2·56 Å). Inspection of atomic models shows that the sugar-phosphate backbone has the mobility required to make such a displacement possible. Precisely the required amount of displacement is achieved automatically, because the resulting conformation follows from the previous one by a helical

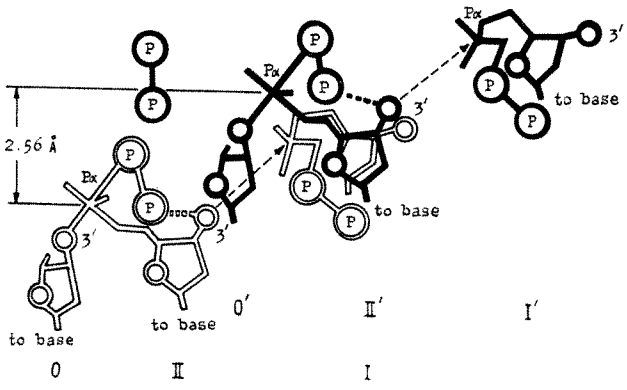


Fig. 4. Repetitive steps during the RNA synthesis. "White" (0, II, I) and "black" (0′, II′, I′) are the situations at the successive stages of the RNA polymerase operation. I⟶II′, II⟶0′ are the simultaneous reaction stages. The ribose 2′-hydroxyls are omitted.

symmetry operation. Displacement of the polymerase subunit entrains the adjacent one and thereby the whole enzyme macromolecule. Because the pyrophosphate conformation at the right hand binding site (Fig. 2, state I) is stretched downwards, the displacement of the enzyme will lift the pyrophosphate group to the position where the γ-phosphate group will be "locked" by the hydrogen bond to a ribose 3′-OH group (I→II′) (Fig. 4). It is important that the pair I itself remains motionless in the course of the transition I→II′. While a nucleotide II moves and is converted to state 0′, its pyrophosphate group is being released; its strong binding to the respective enzyme subunit is abolished, and the DNA base is folded back into the template with liberation of a new RNA link from the hybrid.

So far, the Mg^{2+} (or Mn^{2+}) ions, which are essential components of the system, have not been involved in the model. In reactions involving phosphates these ions are regarded as quenchers of the negative charges at the oxygen atoms in ionized phosphate groups. This neutralization is necessary for the reactions to occur[13]. In our model, the negative charges of the terminal pyrophosphate group of a nucleoside triphosphate are neutralized by attachment to protein. But the α-phosphate group is not bound to the protein, and the divalent metal ions might be involved in its neutralization.

Quaternary Structure of RNA Polymerase

RNA polymerase is known to have a complex structure, being composed of different types of polypeptides (σ, α, β) which perform different functions[14]. We consider only those subunits which are involved in propagation of synthesis (β ?). One of the features of our model is the simultaneous operation of two protomers (or binding sites) connected with the nucleotides in states I and II. It was not possible to devise a simple mechanism for RNA synthesis using only the two "acting" protomers. This difficulty is abolished, however, if the "acting" part of the polymerase consists of several identical protomers (binding sites) in a circular arrangement around the DNA. Then these protomers may replace each other sequentially at each state of the enzyme operation. Clearly the number of binding sites involved will determine the degree of rotation of the polymerase ring around the DNA.

Conclusion

In an earlier report[15] concerning a molecular model for the action of a transaminase, we concluded that the main distinction between enzymatic reactions and reactions in the corresponding model systems lies in the enzyme's capacity to provide appropriate conditions for each stage of a multistep process. These conditions, which are usually alternating ones, are provided by structural rearrangements at a preceding stage of the catalytic process. The model considered here shows that repetitive sequential reactions of one and the same type can be achieved very effectively by coupling the exergonic and endergonic steps between two successive reaction cycles. One may speculate that some "non-template" enzymes composed of identical protomers may also have "overlap mechanisms".

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A Suggested Mechanism for DNA Transcription

by

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RNA transcription could take place without unwinding the DNA double helix if the RNA chain grows in the wide groove of the DNA by a specific stereochemical interaction between the ribonucleotides and the base pairs of the DNA.

THERE is good evidence that DNA replication is semi-conservative and involves separation of the two strands which then act as templates for the synthesis of new complementary strands[1–4]. Alternatively DNA could act as a template by remaining in its native double stranded state with the paired bases acting as the template for an RNA base inserted in the major groove. This would result in asymmetric transcription from a double stranded DNA template—a condition which is known to exist[5–8]. The possible involvement of a triple stranded intermediate in transcription has been suggested in a general way by Watson[9] and in detail by Stent[9] and Zubay[10,11]. Stent's model[9] involves the growth of a third polynucleotide chain within the major groove of the DNA double helix, leading to a three chain helical structure, but the base triplets involved are unsatisfactory because the hydrogen bonds are strained, an improbable tautomeric shift in the position of the hydrogen from N1 to the oxygen on C6 of guanine is

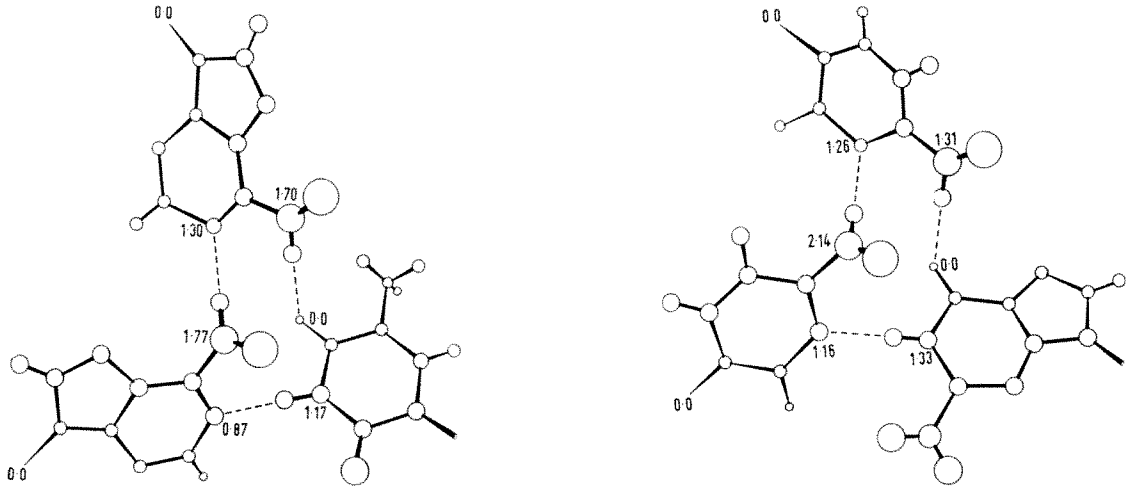


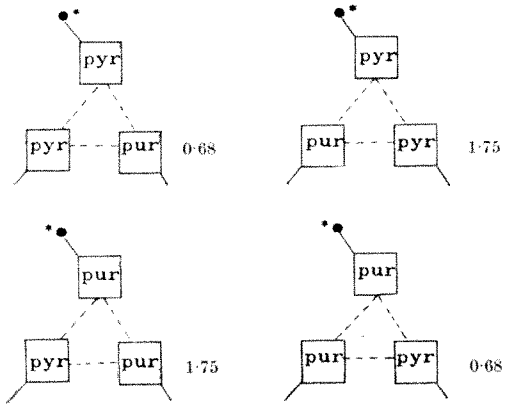
Fig. 1. Schematic view of base triplets for purine and pyrimidine copying with the inserted N_{base}–C_{sugar} bond facing left into the major groove. The triplets shown are (*a*) adenine–thymine–adenine, and (*b*) cytosine–guanine–cytosine. The displacements out of the plane of the paper marked in Å are those measured on the model and not necessarily optimal. The amino groups are rotated by about 40° to the plane of the ring. Hydrogen bonds are shown by dotted lines.

involved, and there is evidence which implicates base complementarity involving hydrogen bonding groups of the Watson–Crick type[12–15]. Base triplets of this kind have been suggested by Zubay but the model lacks template specificity[10,11].

The Watson–Crick model of DNA[16] provides a molecular basis for genetic continuity by virtue of a complementary template, the specificity of which is implicit in two fundamental parameters. The first of these is strain-free hydrogen bonding between the purine and pyrimidine bases, and the second is a limitation of the overall size of the base pairs. Here we suggest a model of transcription based on nucleotide triplets with Watson–Crick hydrogen bond donor and acceptor groups and optimum hydrogen bonding between the bases.

If a third base is to be inserted into the major groove of DNA, the hydrogen bond donor and acceptor groups which will be involved in forming the base triplet will be those attached to C4 of pyrimidine and C6 of purine. The position of these groups differs according to the disposition of the purine and pyrimidine combining in the molecule. As a result of this there is a size limitation on the inserted base. In planar terms this is shown by the distances between the average position and the actual position of the C′1 of the inserted nucleotide when hydrogen bond lengths of 3 Å are assumed (Table 1). If the base triplets are limited to those which deviate by less than 1 Å from the average position there are two possible dispositions of the bases—those shown in Fig. 1 and their mirror image But a planar arrangement of the bases is unsatisfactory because there is a large deviation from linearity by the hydrogen bonds.

Table 1. DEVIATION FROM AVERAGE POSITION IN VARIOUS TRIPLETS (Å)



The average position is indicated by the asterisk.

Table 2. TEMPLATE SPECIFICITY OF BASE TRIPLETS

DNA base pairs	RNA base: N_{base}–C_{sugar} bond facing left	RNA base: N_{base}–C_{sugar} bond facing right
A–T	A	U
G–C	G	C
T–A	U	A
C–G	C	G

The arrows show the direction of sugar-phosphate strand (C′3⟶C′5).

To satisfy the conditions for strong hydrogen bonding the base pairs of the DNA have to be rotated about the symmetrical hydrogen bond between the N3 of the pyrimidine and N1 of the corresponding purine on an axis perpendicular to the helix axis to an angle of approximately 40° to each other. This allows a third base to be inserted from the major groove side and to form strong hydrogen bonds through the carboxyl and amino groups on the DNA base pair. If the hydrogen bond donor and acceptor group limitations are applied to these base triplet configurations the template specificity is such that the inserted base is a copy of the base in the strand of the DNA in which the sugar phosphate chain runs in the same direction as that in the strand which is being synthesized (Table 2). This is ensured by the bonding angles which are such that the tilt of the inserted base is the same as the tilt of the base in the strand being copied and which requires the sugar to be in the C′3-endo conformation. This is the observed conformation of the sugar ring in all forms of RNA studied[17].

The distortion of the DNA which is envisaged involves alteration in the conformation of the sugar–phosphate backbone including a change from the C′2-endo position of the native B-DNA in which the bases are coplanar, to the C′3-endo position. This type of conformational distortion can be reversibly obtained *in vitro* by alterations of the relative humidity[18–21]. This alteration in the conformation of the sugar results in a tilting of the nucleotide base. Because the sugar-phosphate backbones run antiparallel, the effect could be in the opposite direction for the paired base (Fig. 2). This would cause rotation of the bases. Some increase in the distance between base pairs would

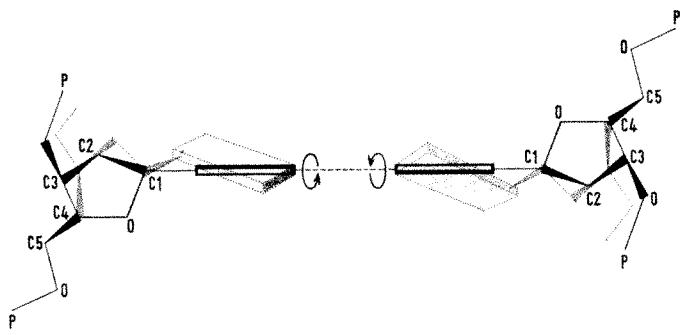


Fig. 2. Diagram illustrating the relative rotation of the DNA bases resulting from the distortion of the two sugar-phosphate strands.

accompany such a distortion, together with alterations in the bond rotations in the phosphate moiety of the backbone. This type of conformational distortion could be produced by RNA polymerase. A consideration of the atomic displacements involved shows that the effect would be localized and the base triplet transient, probably existing at one point only at any time. The RNA chain would be displaced from the major groove by the recovery of the DNA from the distortion as the enzyme proceeds.

Examination of models shows that an abrupt transition from coplanar to rotated base conformation is not possible and therefore a wavelike perturbation of DNA is likely to occur whereby progressive increments of base tilting occur from the front of the wave to the fully distorted maximum and then diminishing in its wake (Fig. 3). This conception of polymerase activity may offer an explanation for the inhibitory effects of certain dyes and drugs which by interaction with DNA prevent the transcription of RNA. For example, because the principal feature of the proposed model is the rotation of the DNA bases to an angle of about 40° relative to each other during transcription, it follows that agents which restrict or interfere with this rotation will be inhibitory. Thus the mechanism of action of intercalating agents, such as acridines[22], is readily explained: their interposition between the DNA bases precludes rotation and prevents the transcription of a segment of the genome. That the inhibitory effect of actinomycin D is a result of its binding to DNA was proposed by Hamilton *et al.*[23]. In our model, it would impede the rotation of the guanine residue relative to the sugar-phosphate backbone. Moreover, it is a feature of this proposed transcriptional mechanism that gene expression can be restricted by attaching material through covalent bonds to the backbone of each of the DNA strands. By preventing the distortion of the deoxyribose sugar a segment of DNA may be prevented from being transcribed without affecting the ability of the strands of the molecule to act as templates for replication. There is evidence that in physiological conditions RNA synthesis begins at certain specific initiation sites on the DNA[24,25] and therefore by preventing initiation at these sites certain portions of the genome could be made inaccessible to transcription.

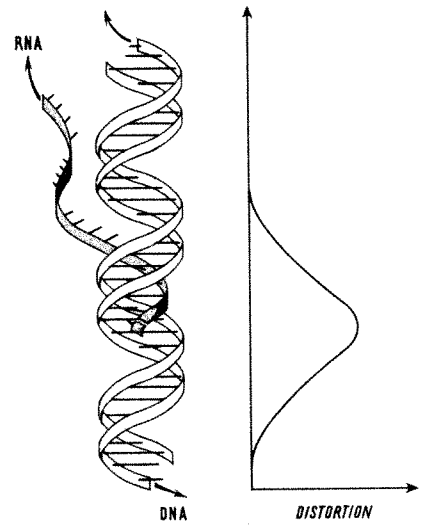


Fig. 3. Synthesis of a strand of RNA on a DNA template as a transient triplet helical structure. In the diagram the distortion of the DNA double helix represents a 5 per cent rotation per base pair.

The process of initiation requires the attachment of sigma factor[26]. This does not seem to be an essential component of the polymerizing enzyme, but it is instrumental in priming the DNA at the initiating site[27]. In our model, the sigma factor could produce the necessary initial distortion in the DNA for it to act as a template for RNA synthesis, and it may be that a section of the initiation site is therefore not transcribed. The initial distortion would be an energy requiring reaction consistent with the kinetic data of Walter *et al.*[28]. The requirements for base rotation are less rigorous for a purine template than for a pyrimidine template and it would be anticipated that purines would predominate at the 5′ terminus of the transcribed RNA. This has been shown to be the case both *in vivo* and *in vitro*[29–31]. It follows that the initiation of RNA transcription would be favoured by a purine rich region in one strand. This would also determine which strand is copied and thus the direction in which RNA synthesis takes place. This is in agreement with the finding of deoxycytosine clusters asymmetrically distributed between the two strands of DNA isolated from a variety of organisms and which have been proposed as the initiation sites[32].

It is difficult, using the triplet model of transcription, to provide a satisfactory mechanism for RNA synthesis on a single stranded DNA template[33]. Because no distortion of the DNA is involved, it is possible that the descending strand could fit into the enzyme site normally occupied by the distorted ascending strand and thus act as a template for the synthesis (in the opposite direction) of RNA with the complementary base sequence. It follows that there would be loss of selectivity in initiation for there is no limitation of attachment sites for the polymerase.

A feature of the transcriptional mechanism proposed in this report is the sensitivity of the polymerase to small regions of denaturation of the DNA duplex, for if RNA synthesis is to proceed, it is an indispensable requirement that the DNA is in the base-paired state. It would therefore be expected that RNA synthesis can be reversibly inhibited by lowering the ionic strength, whereas the reverse might be predicted for the alternative local melting model. The results of Fuchs *et al.*[34] agree with the prediction of the triplet model although they interpret their findings in terms of an effect on "strand exchange" in a model of transcription by local unwinding. Unfortunately, other relevant evidence, such as the resistance of the nascent RNA to ribonuclease digestion[35,36], can as easily be explained in terms of either model. Possibly X-ray diffraction studies of the enzyme–DNA complex are the only satisfactory means of distinguishing between them.

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Subunit Structure of Qβ Replicase

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Qβ replicase comprises four different polypeptides, only one of which is coded for by the phage genome.

THERE is currently considerable interest in the structure of the RNA of phage Qβ and its relationship to the virus-specified proteins[1–3]. The Qβ phage particle comprises two minor proteins (A_1 and A_2 (ref. 4; unpublished results of Farron and C. W.)), besides the coat protein. Labelling experiments on actinomycin-inhibited *Escherichia coli* infected with Qβ revealed a fourth phage specified protein, tentatively identified as a Qβ replicase subunit[4]. We have examined highly purified Qβ replicase preparations and have found them to contain four polypeptides of different electrophoretic mobility, only one of which, with a molecular weight of about 69,000, is Qβ specific. The functional and structural relationship of the three host-specific polypeptides to the Qβ replicase molecule is unclear. Because the molecular weight of Qβ replicase is 130,000 or greater, the enzyme must consist of more than one subunit. We estimate that the four polypeptides thought to be specified by the Qβ genome, A_1, A_2, coat and replicase β subunits, comprise about 1,500 amino-acids; on the other hand, the number of nucleotides available for coding in Qβ RNA corresponds to not more than 1,150 codons[2].

Qβ replicase was purified by a slightly modified version of the method of Eoyang and August[5] (see Table 1) to a specific activity of 7,000 units/mg, the highest value reported so far. It was dependent on host-specific factor for activity with Qβ RNA as template[6]. The sedimentation coefficient of the enzyme was determined by preparative zonal sedimentation through a sucrose gradient using human haemoglobin ($s_{20,w} = 4{\cdot}31$)[7], rabbit muscle aldolase ($s_{20,w} = 7{\cdot}35$)[8,9] and beef liver catalase as reference proteins ($s_{20,w} = 11{\cdot}1$)[10]. The $s_{20,w}$ value of Qβ replicase was interpolated to be 6·7 from the calibration curve shown in Fig. 1. Assuming the relationship between sedimentation coefficient and molecular weight to be the same for replicase as for an average globular protein, an approximate molecular weight of 130,000 can be estimated. There was no significant effect on the relative sedimentation rate by varying the ammonium sulphate concentration between 0·02 and 0·8 M although, at low ionic strength (0·01 M Tris, 0·005 M Mg^{2+}), aggregation occurs[11].

Highly purified Qβ replicase was dissociated with sodium dodecyl sulphate (SDS) and mercaptoethanol and analysed by polyacrylamide gel electrophoresis in the presence of 0·1 per cent SDS. Four bands labelled α, β, γ and δ were found in amounts that varied from one preparation to another. The β band was usually the strongest component (Figs. 2 and 3). Similar results were obtained at higher concentrations of SDS and in the presence of 0·5 M urea. To determine which of the polypeptides was specified by the virus we did the following experiment. Two batches of *E. coli* labelled with ^{35}S

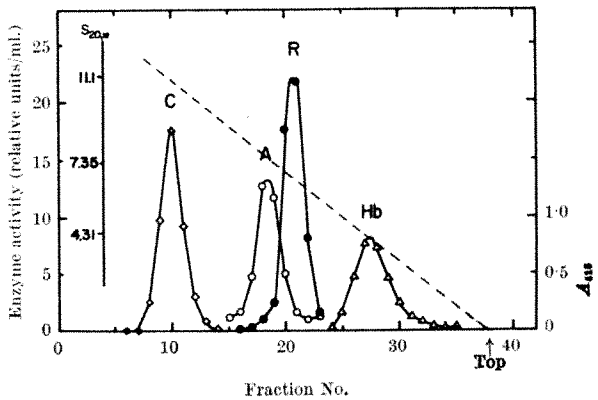


Fig. 1. Determination of the sedimentation coefficient of Qβ replicase. Qβ replicase (specific activity, 4,800 units/mg, about 150 units), rabbit muscle aldolase (about 200 μg, Boehringer) and beef liver catalase (about 300 μg, Boehringer) were mixed and dialysed against a buffer containing 50 mM Tris (*p*H 7·8), 5 mM $MgCl_2$, 1 mM EDTA, 5 mM β-mercaptoethanol and 0·1 M ammonium sulphate for 2 h. Human haemoglobin (about 200 μg) was added to the mixture which was centrifuged through a 5–23 per cent sucrose gradient in the same buffer as that used for dialysis, at 40,000 r.p.m. and 4° C for 24 h in the Spinco 'SW 41' rotor. Qβ replicase (R, ●) activity was assayed as usual[5]; the value 10 on the ordinate equals 5 replicase units. Catalase (C, ◇) was assayed according to the method of Beers and Sizer[15]; the value 10 on the ordinate corresponds to 50 catalase units. Aldolase (A, ○) was assayed according to Jagannathan *et al.*[19]; the value 10 on the ordinate corresponds to 5 aldolase units. Haemoglobin (Hb, △) was measured spectrophotometrically at 415 nm.

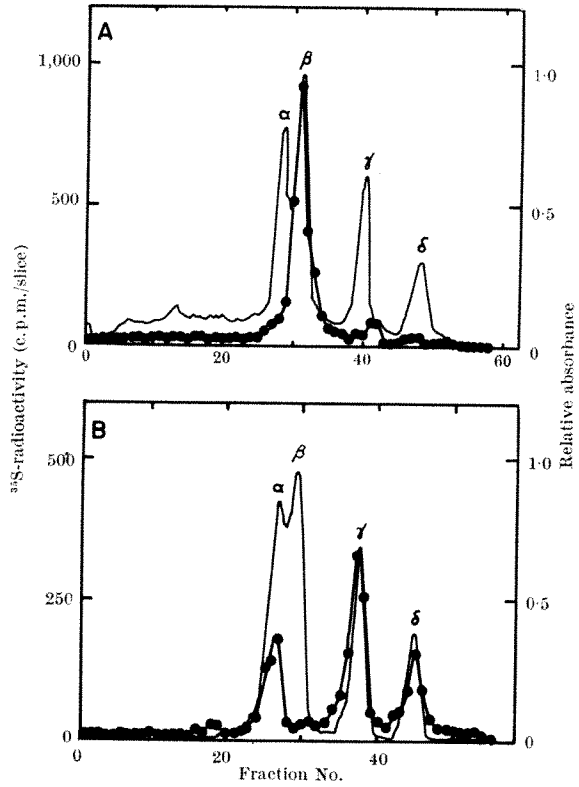


Fig. 2. Analysis of ^{35}S-labelled polypeptides of Qβ replicase preparations by gel electrophoresis. ^{35}S-labelled Qβ replicase (step 5, about 500 units) was incubated for 2 h at 37° C in a buffer containing 50 mM Tris (pH 7·8), 5 mM $MgCl_2$, 1 mM EDTA, 2·0 per cent β-mercaptoethanol, 0·22 per cent SDS and 3·3 per cent sucrose. The samples (about 75 μl.) were mixed with 5 μl. of bromphenol blue (0·25 per cent) in 30 per cent sucrose, layered on 10 per cent polyacrylamide gels[13,17] (7 × 100 mm) and run at 8 mA per tube for 6 h. The gels were stained with 0·25 per cent Coomassie brilliant blue in 50 per cent methanol–7 per cent acetic acid overnight, destained with the same solvent and scanned with the Joyce–Loebl 'Chromoscan' at 620 nm. After soaking in 0·1 per cent SDS for several hours, the gels were frozen and cut into 1 mm slices. The slices were digested with H_2O_2 and alcoholic hyamine hydroxide and counted in Bray's solution[20]. The general methods and buffers have been described[13,17]. *A*, Qβ replicase purified from a mixture of ^{35}S-labelled Qβ-infected cells (1 g) and unlabelled Qβ-infected cells (100 g); 6·6 μg (9,600 c.p.m. of ^{35}S) of the step 5 preparation was applied to the gel. *B*, Qβ replicase purified from a mixture of ^{35}S-labelled uninfected cells (1 g) and unlabelled Qβ infected cells (100 g); 88 μg (4,800 c.p.m. of ^{35}S of the step 5 preparation was used.

were prepared, one uninfected and labelled with ^{35}S throughout log phase growth, the other labelled with ^{35}S after infection with Qβ. About 1 g of each preparation was mixed with 100 g of Qβ-infected *E. coli* and Qβ replicase was purified as usual. Table 1 shows that after the final purification step (zonal centrifugation) the ratio of ^{35}S to enzymatic activity was ten times lower when the radioactive proteins were from non-infected rather than from infected cells. After dissociation with SDS and mercaptoethanol both preparations were subjected to polyacrylamide gel electrophoresis. The gels were stained and scanned to locate the four bands; the gels were then sectioned and the radioactivities were determined. The distribution of (unlabelled) protein among the four bands was similar in both preparations (Fig. 2), but in the sample containing ^{35}S-labelled protein from infected cells virtually all radioactivity was in band β, whereas in the sample containing ^{35}S-labelled proteins from uninfected cells bands α, γ and δ but not β were labelled. Thus β corresponds to the virus-specified polypeptide. The same conclusion has been reached independently by R. Kamen (personal communication and following article[12]).

The molecular weight of the virus-specified replicase subunit was determined in an SDS-containing polyacrylamide gel system[13,14], by comparing its mobility with that of aldolase, catalase, bovine serum albumin and phosphorylase a (all dissociated in mercaptoethanol and sodium dodecyl sulphate) side by side in split gels or gel slabs as well as by co-electrophoresis. Fig. 3 shows that the mobility of the β band is slightly less than that of bovine serum albumin (68,000)[15]. The mobility of the reference proteins was plotted against log molecular weight[13,14] and the molecular weight of the β band was interpolated to be 69,000. The values for the α, γ and δ

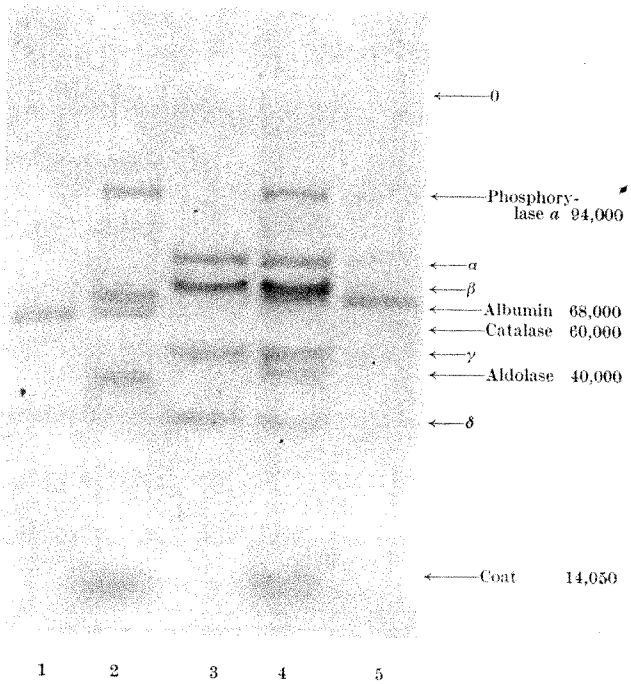


Fig. 3. Polyacrylamide gel electrophoresis of Qβ replicase and reference proteins. The conditions for electrophoresis are described in the legend to Table 2. 1, Catalase; 2, phosphorylase a, bovine serum albumin, catalase, aldolase, Qβ coat protein; 3, Qβ replicase; 4, Qβ replicase, phosphorylase a, bovine serum albumin, aldolase, Qβ coat protein. 15 μg of Qβ replicase, 8 μg Qβ coat protein and 4 μg each of the other reference proteins were run in one lane.

Table 1. PURIFICATION OF Qβ REPLICASE FROM ^{35}S-LABELLED *E. coli*

Step	Replicase activity (units) A	Replicase activity (units) B	^{35}S Radioactivity (c.p.m.) A	^{35}S Radioactivity (c.p.m.) B	Specific activity (units/mg) A	Specific activity (units/mg) B	^{35}S Radioactivity/replicase activity (c.p.m./units) A	^{35}S Radioactivity/replicase activity (c.p.m./units) B
1 $(NH_4)_2SO_4$	6,700	5,300	$2·8 \times 10^7$	$9·2 \times 10$	9·1	6·4	4,180 (1,000)*	17,360 (1,000)
2 DEAE-cellulose	8,200	9,400	$2·4 \times 10^6$	$3·6 \times 10^6$	119	162	293 (70)	383 (22)
3 Hypatite	5,400	5,400	$1·1 \times 10^6$	$2·5 \times 10^6$	—	—	204 (49)	463 (27)
4 'Biogel'	12,000	5,000	$3·0 \times 10^5$	$1·3 \times 10^5$	4,690	3,270	25 (6)	26 (1·5)
5 Sucrose gradient	6,200	4,600	$1·2 \times 10^5$	$3·2 \times 10^4$	7,210	6,770	19 (45)	7 (0·4)

* Normalized to an initial value of 1,000 c.p.m./unit.

(*A*) Starting material was a mixture of 100 g of unlabelled, Qβ-infected *E. coli* Q13 and 1 g of Qβ-infected *E. coli* Q13 labelled with ^{35}S (7×10^8 c.p.m.) after infection. For the labelled preparation, cells were grown in 1 l. of medium containing 0·5 g NaCl, 3·0 g KCl, 1·1 g NH_4Cl, 0·2 g $MgCl_2 \cdot 6H_2O$, 12·1 g Tris, 0·023 g KH_2PO_4, 0·294 g $CaCl_2 \cdot 2H_2O$, 50 μg $FeSO_4 \cdot 7H_2O$, 0·01 g sodium citrate, 1·6 g casamino-acids, 10 g glycerin, 10 mg thiamine, 160 μmoles $(NH_4)_2SO_4$. When the cell number reached $1·6 \times 10^8$ cells/ml., the bacteria were harvested, suspended in 1 l. of the above medium lacking sulphate and glycerin. After 20 min at 37° C 10 g of glycerin and 20 mCi of ^{35}S-SO_4^{-2} were added to give a final SO_4^{2-} concentration of 5 μM. After 10 min, Qβ was added at a multiplicity of infection of 10. The infected cells were harvested after 40 min at 37° C. (*B*) Starting material was a mixture of 100 g Qβ-infected *E. coli* Q13 and 1 g of uninfected *E. coli* Q13 labelled with ^{35}S (10^9 c.p.m.). The cells were grown in the medium described above except that ^{35}S-SO_4^{2-} (80 μM, 0·25 mCi/μmole) was present throughout growth; the cells were harvested in log phase (3×10^8 cells/ml.). Qβ replicase was purified as described by Eoyang and August[5], except that (*a*) elution from the DEAE-cellulose column was with an NaCl gradient (0·1 to 0·3 M), (*b*) chromatography on 0·5 M 'Biogel' was substituted for that on 'Sephadex G-200', and (*c*) zonal centrifugation was carried out as described by Feix *et al.*[11].

Table 2. MOLECULAR WEIGHTS OF POLYPEPTIDE FROM Qβ REPLICASE PREPARATIONS AND Qβ PARTICLES

(1) Polypeptides from Qβ replicase preparations	Mol. wt.	(2) Polypeptides from Qβ particles	Mol. wt.
α	74,000	A_1	38,000
β	69,000	A_2	44,000
γ	47,000	Qβ coat	14,050*
δ	33,000		

* From the amino-acid composition given by Konigsberg *et al.*[16]. Molecular weights were estimated by the general procedure of Shapiro *et al.*[13] and Weber and Osborn[14]. All proteins were incubated for 2 h at 37° C with 0·1 per cent mercaptoethanol and 0·1 per cent SDS before electrophoresis. Samples were either run side by side with a mixture of reference proteins in split gels (0·7×12 cm tubes filled with 3 ml. of gel for 6 h at 10 mA/gel) or in adjacent slots in gel slabs (6×13 cm, 4 mm thickness, 12 h at 30–35 mA), or co-electrophoresis from the same slot of a gel slab flanked by unmixed samples (see Fig. 3). Buffers and gels were prepared according to Viñuela *et al.*[17]. All gels contained 10 per cent acrylamide and 0·27 per cent *bis*-acrylamide; gel slabs contained 0·5 M urea in addition. The same relative mobilities were found whether or not urea was present. The molecular weights were obtained from a plot of log (molecular weight of the reference proteins) against distance travelled.

The values in column 1 were obtained from eleven independent experiments; those in column 2 from two. The standard error of the mean was less than 5 per cent. The reference proteins[14] were phosphorylase *a* (94,000), bovine serum albumin (68,000)[18], beef liver catalase (60,000), rabbit muscle aldolase (40,000) and Qβ coat protein (14,050)[16].

bands are given in Table 2. None of the components occurring in Qβ replicase preparations has the same mobility as any of the subunits of DNA-dependent RNA polymerase. It is not immediately evident which, if any, of the subunits besides β constitute the native enzyme. Two observations are of interest in this connexion. First, a Qβ replicase preparation derived from a mixture of labelled uninfected and unlabelled infected cells contains radioactivity in the α, γ and δ bands. This means that the labelled polypeptides either exchange with the putative subunits of replicase, or that they are present in the preparation as independent protein molecules. Because Qβ replicase prepared from a mixture of a small amount of *E. coli* labelled with ^{35}S after infection and a large excess of infected unlabelled cells contains more than ten times more radioactivity in the β than in all the other bands, it seems that synthesis of α, γ and δ is strongly depressed after infection. Second, if a Qβ replicase preparation is incubated with Qβ RNA and the resulting 30S binding complex[11] is isolated, only the α and β polypeptides are recovered. This may mean either that α and β are associated as subunits of one protein, or that they constitute two different proteins both of which have the capacity to bind to Qβ RNA. If all four polypeptides are part of a structural unit, it is necessary to account for the fact that their added molecular weights (223,000) greatly exceed the value of 130,000 estimated from the sedimentation properties of the enzyme. Such an apparent discrepancy could arise if the enzyme were highly asymmetric or if it were in an association–dissociation equilibrium with its subunits. The latter possibility would also account for the finding that the four polypeptides occur in non-stoichiometric and variable amounts within the sharply sedimenting enzyme band.

Assuming an average molecular weight of 110 for an amino-acid, the added molecular weights of the polypeptides thought to be specified by the Qβ genome correspond to 1,500 amino-acids (Table 2). Qβ RNA comprises 3,500 nucleotides of which not more than about 3,400 are used for coding[2,3]; it can therefore specify only 1,150 amino-acids. Beyond assuming errors in the molecular weight determinations of the RNA or the polypeptides the discrepancy could be explained in several ways. First, only one of the A proteins is virus-specified while the other is derived from the host. Neither the data of Garwes *et al.*[4] nor labelling experiments done in our laboratory encourage this view, but it cannot be ruled out. Second, the virus preparations consist of a mixture of two kinds of particles, containing different A proteins. Third, the smaller of the A proteins is derived from the larger by proteolytic cleavage. Comparison of the fingerprints of A_1 and A_2 may provide the answer.

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Characterization of the Subunits of Qβ Replicase

by
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The RNA replicating enzyme induced by RNA phage Qβ consists of a single phage specific and three host specific polypeptide chains. At least one of the host subunits is shown to be essential for enzymatic activity, and none are known subunits of *E. coli* DNA-dependent RNA polymerase.

In RNA bacteriophages a common RNA molecule is both the replicating genetic material and the messenger for protein synthesis. Because no RNA-dependent RNA polymerase is known to exist in bacteria, the RNA phage must induce a new enzyme for replication and, indeed, such a RNA polymerizing activity appears in cells shortly after infection[1–3]. The induction of the enzyme requires the protein produced by only one of the cistrons of the RNA phage[4–8].

Purified[9–13] RNA dependent RNA polymerase induced by phage Qβ (replicase) directs the replication of Qβ RNA *in vitro*. New infectious RNA molecules[14] are produced by copying of minus strands synthesized on the original phage RNA plus strand[15]. The phage enzyme is able to replicate its homologous RNA in a host cell full of

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potentially competitive RNA species because of its extreme template specificity: the only natural RNA molecules it will use as templates are Qβ plus strands[16], Qβ minus strands[11,17,18] or a 6S RNA found in Qβ infected cells[19]. Because all RNA molecules initiated by Qβ replicase start with 5′-pppGpG . . .[17,18,20], it is not surprising that the enzyme will also synthesize RNA complementary to synthetic ribopolymers containing cytidylate[12,21] as in the polymerization of poly G on poly C templates. It will not, however, replicate the synthetic RNAs to generate copies of the original template. Extensively purified replicase requires two host factor proteins[17,21,22] called F_I and F_{II}, to copy Qβ plus strands, but these factors are not required for activity with the other templates. Although it has often been assumed that the replicase itself—defined as the enzyme which copies minus strands or poly C (but which requires F_I and F_{II} to copy plus strands)—is an oligomer of a single phage specific polypeptide chain, its subunit structure has not been elucidated. The fact that the enzyme sediments at about 7S (refs. 13, 21, 24), while there is room on the phage RNA for information specifying a protein molecular weight of only about 60,000 devoted to RNA replication, suggests that subunits are indeed present. Here I report that pure Qβ replicase contains four different polypeptide chains, only one of which is Qβ specific. The others pre-exist in the host cell.

I have developed a new isolation procedure (to be published) which yields enzyme more than 95 per cent pure. It involves use of the host factor independent synthesis of poly G on a poly C template as a routine assay. Total specificity for replicase was ensured by adding the antibiotic rifampicin[25] to inhibit the synthetic polymer activity of the host RNA polymerase. Rifampicin does not inhibit replicase[26,27] and I found no rifampicin resistant GMP incorporation in extracts of uninfected cells (unpublished results). Enzyme overproduction was obtained by using a Qβ coat amber mutant (amber 12, generously provided by Dr K. Horuichi) which makes three to five times the wild type level of replicase at late times after infection.

The procedure used is a modified combination of the Babinet[28] and Burgess[29] methods originally developed for the isolation of host DNA-dependent RNA polymerase. Infected cells are broken with glass beads; the resulting extract is mildly digested with deoxyribonuclease and fractionated by multi-stage liquid polymer phase partitioning, DEAE-cellulose and phosphocellulose column chromatography with a final zonal sedimentation in high salt glycerol gradients. The last step resolves host polymerase (12·3S) and replicase (7S). A small scale version of the procedure is described in the legend to Fig. 2. The final protein fraction sediments as a single 7S peak in high salt glycerol gradients (see Fig. 9), and has a specific activity (measured by the poly C assay) of approximately 500 nmoles GMP incorporated/mg protein/min at 30° C. No RNA polymerase, poly A synthetase, polynucleotide phosphorylase or nuclease activity can be detected. Although the poly C dependent activity is used throughout the purification, the enzyme obtained is active with Qβ RNA templates, provided host factors are added, and is active without host factors on denatured double stranded Qβ RNA as a source of minus strands.

Sodium dodecyl sulphate (SDS)–polyacrylamide gels[30,31] were used throughout the purification procedure to follow the size and number of individual polypeptides. The purest enzyme still contained four polypeptide chains (Fig. 1): their molecular weights, calculated by the method of Weber and Osborn[31], and by co-electrophoresis with close marker proteins (R. K., to be published) are 70,000, 65,000, 45,000 and 35,000 for bands "I–IV", respectively. None has the same molecular weight as any known subunit of host RNA polymerase[32]; the bands are distinct from the RNA polymerase β, β′, σ or α bands.

Efforts to develop alternative purification schemes or to fractionate further the final product just described have failed to produce active enzyme with fewer bands on an SDS gel. I therefore proceeded with experiment to test the hypothesis that all four polypeptides are subunits of Qβ replicase. (While this manuscript was in preparation I learned that Weissmann, Kondo and Gallerani had obtained the same four polypeptide chains using a slight modification of the method of Eoyang and August[13]; personal communication and preceding manuscript[24].)

One Phage and Three Host Polypeptide Chains

The RNA of phage Qβ can specify protein of molecular weight not exceeding about 150,000. The total molecular weight of the three structural proteins found in mature virions, and also synthesized in antibiotic inhibited infected cells (see Fig. 5), is approximately 90,000, leaving only about 60,000 for any protein(s) involved in RNA replication. Thus it is unlikely that more than one of the four replicase polypeptides could be phage specific. To determine the origin of the polypeptides, tracer amounts of ^{35}S-labelled uninfected cells were mixed with unlabelled, infected cells; an extract was made, and the replicase purified to determine whether any of the host radioactivity was associated with the purified enzyme. As a control, the same labelled cells were mixed with uninfected cells to verify that, in this case, no protein purified as did the replicase.

The phosphocellulose (PC) column elution profiles of the parallel infected and uninfected carrier cell purifications are shown in Fig. 2. Most of the protein from the previous DEAE-cellulose step flowed through the PC column, for the two oppositely charged resins were run at the same *p*H. The main peak of protein eluted by the NaCl gradient is slightly broader in the purification using infected carrier cells (Fig. 2*B*) and the Qβ replicase activity lies in the leading half of this peak. No rifamycin resistant GMP incorporation was detected in the protein eluted in

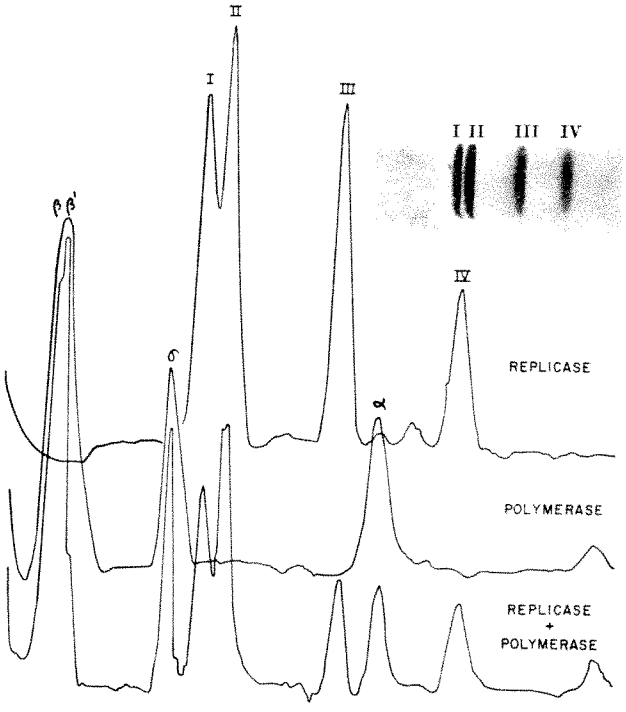


Fig. 1. SDS-polyacrylamide gels of Qβ replicase and *E. coli* RNA polymerase. The gels (10 per cent acrylamide, 0·27 per cent *bis*-acrylamide, 0·1 per cent SDS, 0·1 M sodium phosphate; *p*H 7·2) were prepared, run and stained with Coomassie brilliant blue, as described by Weber and Osborn[31]. The photograph shows highly purified Qβ replicase. The gel scans (done at 600 nm in a Gilford scanner) compare replicase and *E. coli* RNA polymerase, and show that the two enzymes share no common subunits.

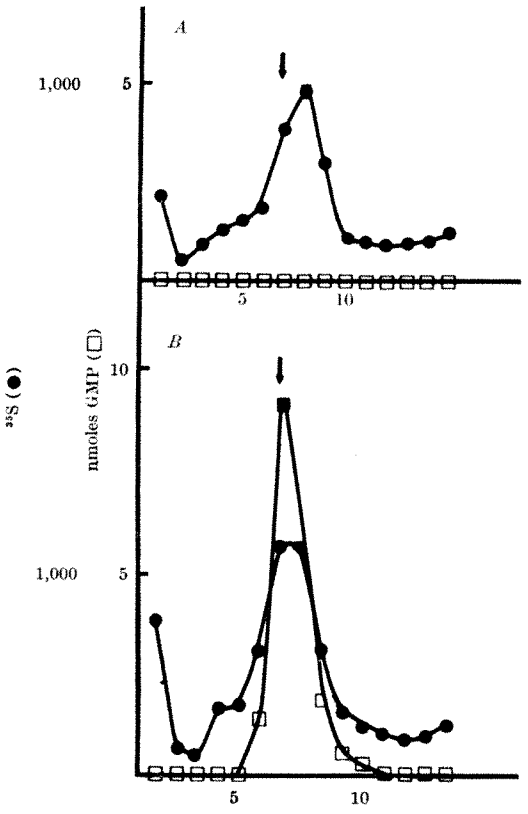


Fig. 2. Phosphocellulose elution profiles of protein from uninfected (*A*) and Qβ amber 12 infected (*B*) cells mixed with tracer amounts of uninfected ^{35}S-labelled cells. The steps used before the phosphocellulose chromatography were the following: a female strain, PR-13 (ref. 41) (RNase$_3^-$, PNPase$_3^-$) was labelled by growth in $^{35}SO_4^{2-}$ medium (TA medium—see Fig. 5—modified by decreasing the $MgSO_4$ to 2×10^{-4} M, adding carrier-free $^{35}SO_4$ at 10 mCi/100 ml., and omitting cysteine and methionine) and 0·8 mCi mixed with either 5 g of unlabelled Qβ amber 12 infected cells or with 5 g of unlabelled PR-13. The two cell mixtures were purified for replicase in the same way although activity was only detected in the infected cell mixture. All steps were at 0–4° C. The cells were ground in alumina, and the resulting cell pastes were taken up in 10 ml. of standard buffer plus Mg^{2+}: 0·05 M Tris-HCl (*p*H 7·5 at 25° C), 0·001 M EDTA, 0·1 mM dithiothreitol, 20 per cent glycerol, 0·005 M magnesium acetate, and clarified by low speed centrifugation. To minimize protease action, 0·5 ml. of a saturated ethanolic solution of phenylmethylsulphonyl fluoride was added to the extracts. The extracts were then fractionated by multi-stage liquid polymer phase partitioning. One-third weight of a standard buffer plus Mg^{2+} solution containing 6·4 per cent Dextran 'T500' (Pharmacia) and 25·6 per cent polyethylene glycol 6,000 ('Carbowax 6,000', Union Carbide) was added, and the phase system produced was stirred for 30 min. The phases were then separated by brief low speed centrifugation, and the top phases discarded. To the bottom phases, an equal volume of 6·9 per cent 'Carbowax 6,000' in 2·0 M NaCl standard buffer plus Mg^{2+} was added, and the phases were separated after stirring for 30 min. The top phases were removed and saved, and the bottom phases extracted twice more as just described. The pooled 2·0 M NaCl phases were then dialysed against standard buffer (no Mg^{2+}) overnight. The resulting dialysates (about 65 ml.) had conductivities of 2·5 mΩ^{-1}. Absorption on DEAE–cellulose was achieved batchwise without removal of the polyethylene glycol or concentration, by the direct addition of a slurry of Whatman 'DE-52' (equilibrated with standard buffer). About 10 ml. of resin was added to each dialysate, and after one hour of slow mixing, the 'DE-52' was removed by slow speed sedimentation. It was washed twice with 25 ml. of standard buffer, twice with 25 ml. of 0·12 M NaCl in standard buffer, and finally three times with 0·2 M NaCl in standard buffer. The pooled 0·2 M washes were diluted two-fold with standard buffer, and applied slowly to 2 ml. Whatman 'P-11' phosphocellulose columns which had been extensively equilibrated with standard buffer. To ensure that the two parallel purifications were as identical as possible, the PC columns were eluted from a common multi-outlet gradient maker (Buchler) using the same peristaltic pump as well as a single timed fraction collector. The profile of protein eluted by an NaCl gradient (0·12–0·5 M NaCl, total volume 40 ml.) is illustrated. ●, ^{35}S protein; □, replicase activity. Activity was measured in the standard poly C dependent assay, which contained 0·05 M Tris-HCl (*p*H 7·5) at 25° C, 0·010 M magnesium acetate, 0·001 M EDTA, 0·1 mM DTT, 0·001 M phosphoenolpyruvate, 5 μg/ml. pyruvate kinase, 2·5 mg/ml. rifampicin, 0·1 mM polycytidylic acid, and 0·2 mM GTP (specific activity 1–2,000 ^{14}C c.p.m. per nmole), in 10 per cent glycerol, at a final volume of 0·2 ml. Reactions were incubated for 10 min at 30° C, stopped with the addition of 3 ml. 5 per cent trichloroacetic acid (TCA), 1·0 M NaCl, and 0·01 M sodium pyrophosphate; the RNA precipitates were collected on 0·65 μm membrane filters, washed six times with 3 ml. of the TCA–salt solution, and once with 95 per cent ethanol. After drying, the filters were counted in 3 ml. of toluene–'Omnifluor' scintillation fluid.

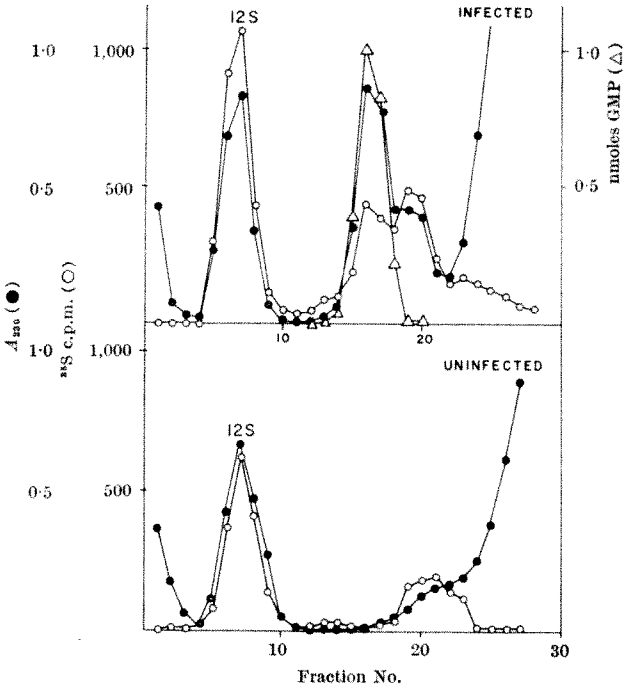


Fig. 3. High salt glycerol gradients of the phosphocellulose fractions purified from Qβ amber 12 infected cells or uninfected cells mixed with ^{35}S-labelled female cells. The labelled protein fractions indicated by the arrows in Fig. 2 were mixed with egg white lysozyme carrier and concentrated by dialysis against standard buffer containing saturated $(NH_4)_2SO_4$. The precipitates were dissolved in standard buffer plus 5 mM magnesium acetate and 0·2 M $(NH_4)_2SO_4$, dialysed to lower the glycerol concentration to 5 per cent, and loaded on 11·5 ml. glycerol gradients (10–30 per cent glycerol in standard buffer plus Mg^{2+} and 0·2 M $(NH_4)_2SO_4$). The gradients were centrifuged at 41,000 r.p.m. in an International 'SB283' swinging bucket rotor at 12° C for 23 h. Replicase activity (△) was measured with the standard poly C assay, but ^{3}H-GTP (7,500 c.p.m./nmole) replaced ^{14}C radioactivity so that the ^{35}S-protein (○) could be determined in the same TCA precipitates. The absorbance (●) at the top of the gradients is the carrier lysozyme. No replicase activity was found in the gradient from uninfected cells. Sedimentation was from right to left.

the uninfected carrier cell purification (Fig. 2*A*), nor at earlier stages in the purification. This confirms that the activity is totally phage specific.

The PC fractions indicated by the arrows in Fig. 2, corresponding to the leading half of the protein peaks eluted from the two columns, were concentrated separately (after adding lysozyme as carrier) and then run on high salt glycerol gradients. The two resulting gradients (Fig. 3) are similar, except for a 7S peak of absorbance and ^{35}S only present on the gradient with the infected carrier cell. Qβ replicase activity is coincident with this 7S peak. The other principal peak, at about 12S, common to both gradients, is host DNA-dependent RNA polymerase. The co-sedimentation of ^{35}S-labelled (and therefore host specified) protein with the 7S replicase activity, plus the absence of ^{35}S or absorbance in the 7S region of the control "blank" purification, leads to two important conclusions. First, the 7S replicase is about 95 per cent pure, because little or no protein is found in this position in the "blank" purification, and second, the pure replicase must contain at least one host polypeptide because it is ^{35}S labelled. The ratio of absorbance (derived almost entirely from the carrier cells) to ^{35}S is higher in the 7S replicase peak than it is in the 12S peak on the same gradients. Thus one or more of the replicase subunits is not radioactive and must be phage specific.

The ^{35}S labelled replicase, obtained from the gradients shown in Fig. 3, was analysed by SDS–acrylamide gel electrophoresis to resolve its constituent polypeptide chains. The gel was stained for protein with Coomassie brilliant blue, and then examined for ^{35}S by autoradiography using the procedure described by Fairbanks, Levinthal and Reeder[33]. Fig. 4 shows the stained gel

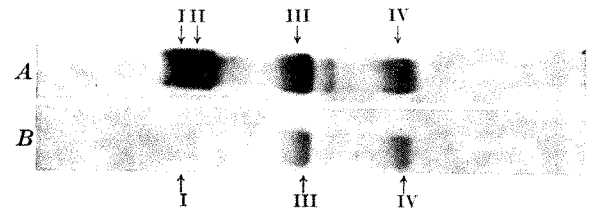


Fig. 4. SDS gels of ^{35}S-labelled Qβ replicase. The radioactive enzyme obtained from the gradient shown in Fig. 3 was analysed by electrophoresis on a 10 per cent SDS–polyacrylamide gel. After running and staining for protein in the usual manner[31], the gel was sliced longitudinally and the slices dried down on a sheet of filter paper under vacuum[34]. A photograph of a dried gel slice is shown in *A*. There are four stained bands, corresponding to subunits I–IV in Fig. 1. The other minor bands are only detectable here because the gel has been very heavily loaded and the photograph overexposed during printing to maximize contrast. They constitute less than 5 per cent of the total protein. The autoradiogram of the dried gel (*B*) shows that subunits I, III and IV are radioactive, and therefore host specific. Subunit II contains no detectable ^{35}S.

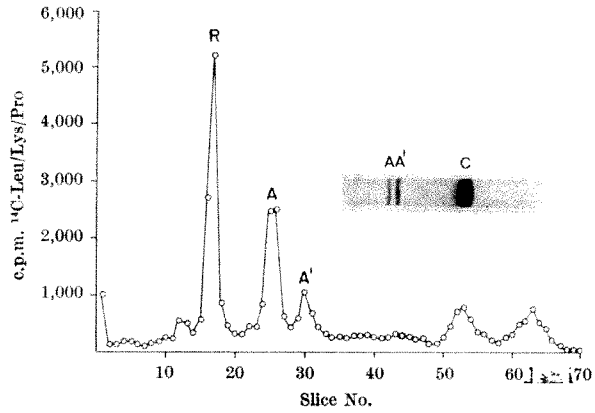


Fig. 5. SDS-acrylamide gel of rifampicin inhibited cells infected with Qβ coat amber 12. *E. coli* F11 (ref. 42, an actinomycin and rifampicin sensitive derivative of Q13) grown in TA medium* minus leucine, lysine and proline was used (1 ml.) to infect 75 ml. of fresh medium in a 500 ml. flask. The cells were grown at 37° C, to a density of 1×10^8 cells/ml., and then infected with **Qβ** amber 12 (at a multiplicity of infection of 10 with 0·06 per cent revertants). 25 min later, rifampicin was added to 33 μg/ml. After allowing 20 min for complete inhibition of host protein synthesis (verified in preliminary control experiments), 2 μCi each of ^{14}C-leucine, ^{14}C-lysine and ^{14}C-proline (specific activities 280, 309 and 205 μCi/μmole) were added and the culture was incubated for a further 45 min. This labelling period corresponds to the time of maximal replicase synthesis in amber 12 infected cells. At 90 min after infection, the cells were chilled, harvested and resuspended in 0·5 ml. of 0·1 M sodium phosphate buffer (*p*H 7·2). After heating to 100° C, SDS and mercaptoethanol were added to 0·5 per cent and 1 per cent respectively, and the cells were disrupted by boiling for a further 20 min. The sample was then dialysed against 0·1 M sodium phosphate–0·1 per cent SDS–1 per cent mercaptoethanol and 10 per cent sucrose at room temperature overnight. The dialysate, which contained 73,000 c.p.m./5 μl. (counted in NEN 'Aquasol') was used directly for SDS gel electrophoresis without further treatment. The gel was frozen after electrophoresis, and sectioned into 1 mm slices with a razor blade gel slicer. The radioactivity was eluted by soaking the slices overnight in 0·4 ml. of 0·1 per cent SDS, and counted after addition of 3 ml. NEN 'Aquasol' liquid scintillation fluid. The gel profile shows two major protein peaks, R (molecular weight 60,000–70,000) and A (molecular weight 40,000–45,000). The inset is a photograph of a similar SDS gel run on unlabelled Qβ virus particles dissociated by heating in SDS. The mobility of labelled peak A is the same as that of the largest viral protein; that of R is considerably slower than any of the structural proteins.

* TA medium contains (per l.) 10 g NaCl, 1 g NH_4Cl, 0·25 ml. 2×10^{-2} M $FeCl_3$, 2·5 ml. 0·2 M K_2HPO_4, 1·0 ml. 1 M $MgSO_4$, and 11·5 g TES buffer adjusted to *p*H 7·5 with 1 M NaOH and autoclaved. Then, 100 ml. quantities are completed with separately sterilized solutions of 20 per cent dextrose (2·5 ml.), 1 per cent methionine (1·0 ml.), 1 per cent tyrosine (1·0 ml.), 5 per cent yeast extract (0·05 ml.), 1 M $CaCl_2$ (0·25 ml.), and a mixture of amino-acids in proportion to leucine as they are found in *E. coli*, omitting only those to be used for labelling. The methionine, tyrosine and yeast extract are necessary for growth of strain F11 (tyr$^-$met$^-$, unknown growth factor requiring).

and its autoradiographic image. The same four stained bands seen in Fig. 1 are apparent, although the quantity of protein applied to the gel makes it difficult to resolve bands I and II after slicing and drying for autoradiography. Bands I, III and IV contain ^{35}S, but band II does not. Thus the 70,000, 45,000 and 35,000 molecular weight polypeptides are host specific, whereas only the 65,000 subunit can be phage specific. The fact that the three polypeptides are not purified in the absence of the phage specific subunit argues that they are subunits of the replicase. Moreover, because the source of the ^{35}S-labelled host polypeptides found with the replicase in this experiment was a separate batch of uninfected cells, they must actively exchange during purification or, alternatively, the phage specific protein must be present in large excess in extracts of Qβ amber 12 infected cells.

Subunit II is the Product of the Replicase Cistron

To demonstrate that the phage specific subunit II is the product of the Qβ replicase cistron, I have prepared labelled marker proteins from Qβ amber twelve infected cells the host protein synthesis of which was inhibited by rifamycin. Previous studies on other RNA phages, using either actinomycin[34–36] or rifamycin[37] to inhibit host protein synthesis, have resolved three phage specific proteins on SDS–acrylamide gels. By using amber mutants[36,38], the proteins have been assigned unequivocally to the three known RNA phage cistrons: coat, maturation protein and synthetase (replicase). The largest of the proteins (60,000 molecular weight for phage MS2 (ref. 36)) is the product of the replicase cistron. In a study of the proteins specified by wild type Qβ Garwes *et al.*[39] have reported that four, rather than three, polypeptides are found on SDS gels. Three of these proteins (which I shall call A, A′ and coat) are found in mature Qβ virions. These results have been confirmed in this laboratory (unpublished observations of R. K. and M. Osborn). Whether the second minor virion protein (A′) is in fact the product of a new phage cistron, not present in the other RNA phages studied, is not yet clear. The fourth and largest of the Qβ proteins was assigned to the replicase cistron because it is the only one not found in virions. Although Garwes *et al.*[39] reported the molecular weight of this protein as 49,000, recalculation from the data they presented, using the MS2 proteins they provided as internal markers for molecular weight calibration, indicates that 64,000 would be a better estimate of its size.

Fig. 5 shows the pattern of phage specific proteins synthesized in a Qβ amber 12 infected su$^-$ host labelled

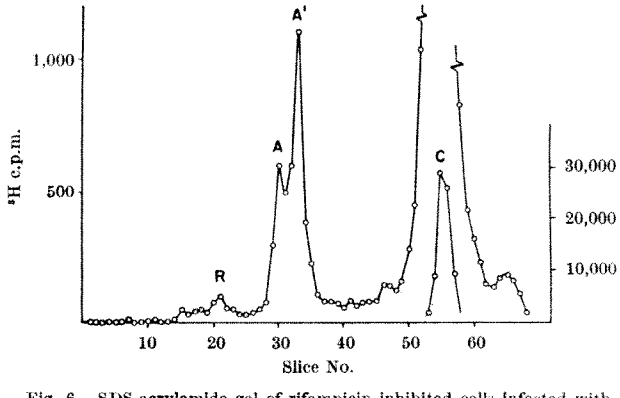


Fig. 6. SDS-acrylamide gel of rifampicin inhibited cells infected with wild type Qβ. 25 ml. of strain F11 in TA medium was grown to 1×10^8 cells/ml. Two 10 ml. aliquots were spun down, and each was resuspended in 1 ml. of fresh medium. One aliquot was infected with wild type Qβ at a multiplicity of infection of 10. After 5 min incubation at 37° C, the cells were diluted with 9 ml. of TA containing 25 μg/ml. rifampicin. They were then incubated until 22 min after infection at which time 10 μCi of ^{3}H-proline (specific activity 26·8 Ci/mmole) was added. At 32 min the cells were chilled and centrifuged. The cell pellet was taken up in 0·1 M Tris (*p*H 7·9), containing lysozyme (100 g/ml.), deoxyribonuclease (10 g/ml.) and ribonuclease (50 g/ml.) and incubated for 10 min at 37° C. SDS (0·5 per cent) and mercaptoethanol (1 per cent) were then added and the extracts heated to 100° C for 10 min. The extracts were then dialysed overnight against 0·1 M sodium phosphate (*p*H 7·2), containing 0·1 per cent SDS, 0·14 M mercaptoethanol, and 10 per cent sucrose. (In long term labelling experiments—see Fig. 5—the enzymatic digestion step was omitted.) The infected cell culture incorporated 25 per cent of the added proline during the labelling period; the uninfected control incorporated less than 0·25 per cent of the label. A gel run on the extract from uninfected cells (not shown) had no visible protein peaks and less than 100 c.p.m. in each slice. The three peaks apparent on the gel from wild type infected cells correspond to the three proteins found in virus particles (see inset to Fig. 5): coat, A and A′. Little if any protein R was labelled in the 22–32 min pulse used.

during the period of replicase overproduction (45–90 min after infection). Two principal protein peaks, called R and A for the larger and smaller respectively, are obtained. No coat protein appears, for the phage contains an amber mutation in this cistron. Peak A has the same SDS gel mobility as the largest of the proteins found in virus particles (see inset in Fig. 5), corresponding to a molecular weight of 41,000, and is probably the Qβ maturation protein. The larger molecular weight protein, R, is identified as the product of the replicase cistron by the following criteria: it is the only Qβ specific protein found in infected cells but not in virions. In wild type infections, in which replicase protein synthesis ceases between 20 and 30 min after infection[1–3,10,13], very little protein with the mobility of peak R is labelled in a 10 min pulse beginning at 22 min (Fig. 6). In cells infected with coat amber 12, maximum synthesis of replicase occurs between 40 and 90 min after infection (to be published). R is the principal phage specific protein made during this period (Fig. 5). It is therefore likely to be the product of the Qβ replicase cistron.

To use the replicase polypeptide labelled in rifamycin inhibited cells as a marker for identification of the phage specific component of purified enzyme, it was necessary to prepare ^{3}H-labelled enzyme sufficiently pure to distinguish its subunits on an SDS gel. Enzyme was therefore purified from a small quantity of wild type Qβ infected cells labelled immediately after phage infection with ^{3}H-proline. As a further control, the cells used had been previously labelled with ^{14}C-proline to identify protein components synthesized before Qβ infection. Fig. 7 illustrates an SDS gel of this double labelled, partially purified enzyme. Peaks with the mobilities of subunits I, II, III and IV are seen, as well as several contaminants. As expected from the ^{35}S experiment, host specific subunits I, III and IV contain ^{14}C as well as ^{3}H-proline, while subunit II contains only ^{3}H-proline. This confirms that subunit II is induced after Qβ infection.

In the gel shown in Fig. 8, ^{14}C-labelled proteins synthesized in rifamycin inhibited cells infected with coat amber twelve are co-electrophoresed with the ^{3}H-labelled purified enzyme. Peak R, identified above as the Qβ replicase gene product, co-electrophoresed with the phage specific component of the enzyme, subunit II. (Note that enzyme subunit III, which is host specific, did not migrate with

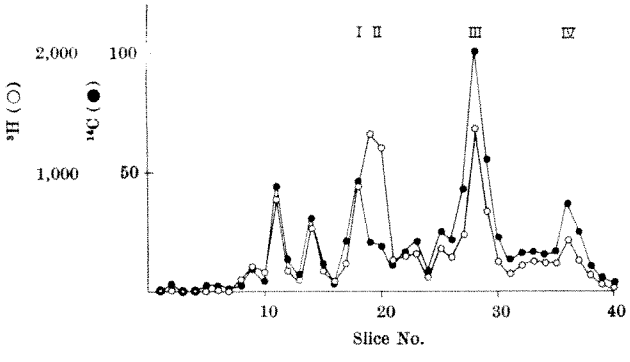


Fig. 7. SDS-acrylamide gel of double labelled Qβ replicase. The ^{14}C curve shows the distribution of protein synthesized before infection. The ^{3}H curve shows protein made after Qβ infection. *E. coli* Q13 (100 ml.) was grown in TA medium (minus proline) at 37° C to a density of about $0{\cdot}5 \times 10^8$ cells/ml. ^{14}C-proline (20 μCi, specific activity 206 μCi/μmole) was then added, and incubation was continued at 37° C. The proline was fully incorporated in 20 min. Two hours after ^{14}C-proline addition, the culture was infected with wild type Qβ (multiplicity of infection of 10) and labelled with ^{3}H-proline (1 mCi, specific activity 26·8 Ci/mmole). 35 min after phage infection the cells were chilled and harvested. After mixing with 2 g of unlabelled Qβ amber 12 infected cells, the replicase activity was purified by a procedure similar to that described in the legend to Fig. 2. Because wild type phage rather than replicase overproducing amber mutant were used, the final enzyme is only about 75 per cent as pure as that shown in Fig. 1. It should also be noted that the proportion of bands I, II, III and IV is different from that previously shown. This is the frequent result of efforts to purify enzyme on a very small scale; it probably arises from partial dissociation of the enzyme complex during purification, and is correlated with a decrease in the final specific activity.

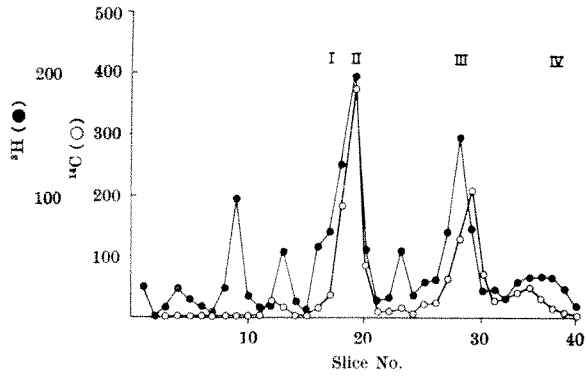


Fig. 8. Co-electrophoresis of ^{14}C-labelled proteins from rifampicin inhibited cells infected with Qβ coat amber 12 and ^{3}H-labelled partially purified Qβ replicase. An aliquot of the ^{14}C-labelled amber 12 proteins (see Fig. 6) was mixed with ^{3}H-proline labelled replicase (see Fig. 7) and run on a 10 per cent SDS gel. Slicing and counting were done as described in Fig. 6. Replicase subunit II co-electrophoresed with the product of the Qβ replicase action (R), but subunit III did not co-electrophorese with Qβ A protein.

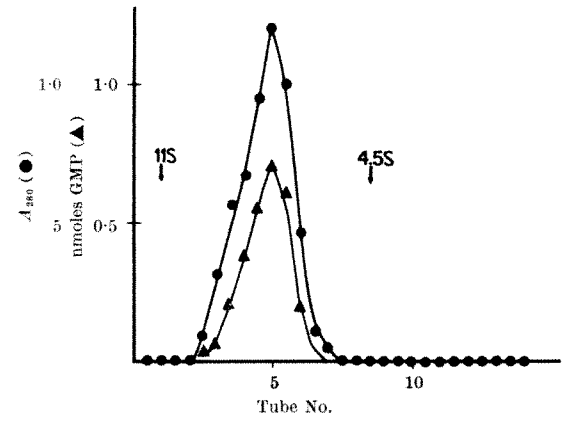


Fig. 9. High salt glycerol gradient of purified Qβ replicase. Approximately 3 mg of purified Qβ replicase (in 0·05 M Tris-HCl (*p*H 7·5 at 25° C)—0·005 M magnesium acetate—0·001 M EDTA—0·5 mM dithiothreitol—0·2 M $(NH_4)_2SO_4$—5 per cent glycerol) was centrifuged on an 11·0 ml. gradient (10–30 per cent glycerol, same buffer) at 40,000 r.p.m. in an International 'B-60' centrifuge, using the 'SB-283' rotor, for 33 h at 5° C. Replicase activity was determined with the standard poly C assay (see Fig. 2) and is plotted as nmole GMP incorporated per 10 min. Haemoglobin and catalase, run in a parallel tube, were used as sedimentation coefficient markers.

the same mobility as the 41,000 molecular weight viral A protein.) I therefore conclude that subunit II, as well as being the only replicase subunit synthesized exclusively after phage infection, is identical in molecular weight to the product of the phage replicase cistron.

Dissociation and Reconstitution of Qβ Replicase

To show that all the host subunits are essential it would be necessary to dissociate the enzyme complex into its four components, and to demonstrate that each subunit must be added to reconstitute enzyme activity. Although this has not yet been achieved, I have fractionated the enzyme into two inactive smaller components, one containing subunits I and II, the other subunits III and IV, and reconstituted activity by mixing the separated components.

The purified enzyme sediments as a single peak on high salt (0·2 M $(NH_4)_2SO_4$) glycerol gradients (Fig. 9) at about 7S. With no added salt, the protein and activity run as an 18S aggregate, with a significant amount trailing to 7S, and a minor peak of inactive material appearing at 4–5S (Fig. 10). SDS gels run on fractions across such "no salt" gradients showed all four polypeptides throughout the activity region, but only bands III and IV in the 4–5S region. Thus the four polypeptides co-sediment in large aggregates at very low salt. The appearance of a small amount of subunits III and IV running slower than

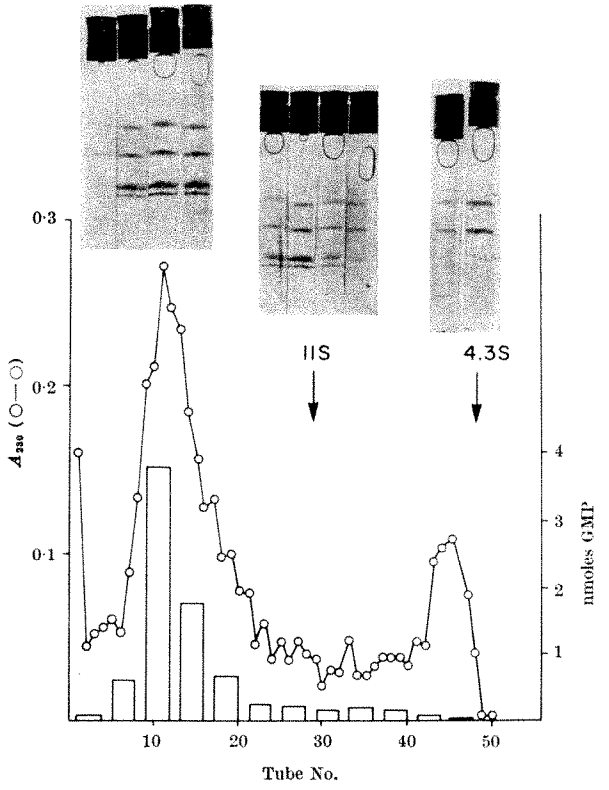
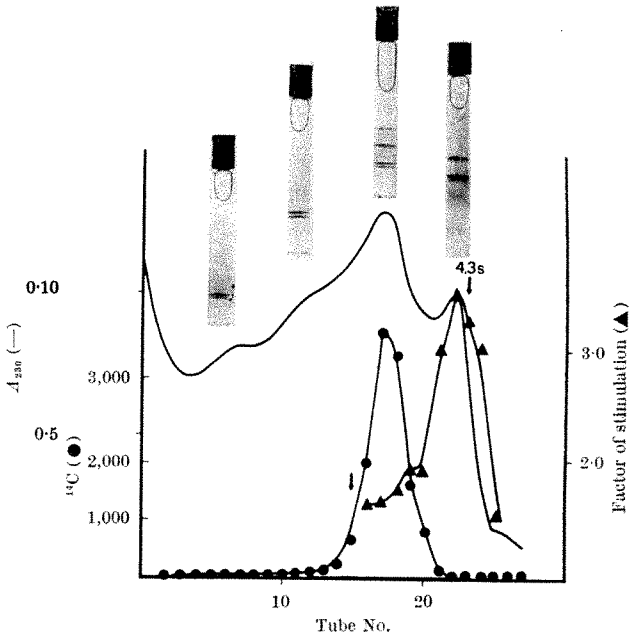


Fig. 10. Low salt glycerol gradient of purified Qβ replicase. Purified replicase (2·2 mg/ml.) was dialysed against 0·01 M Tris (*p*H 7·5), 0·001 M magnesium acetate, 0·0002 M EDTA, 0·1 mM dithiothreitol, 5 per cent glycerol and then 200 μl. was loaded on an 11 ml. 10–30 per cent glycerol gradient in the same buffer. The gradient was centrifuged for 15 h at 4° C in the 'SB-283' rotor of an International 'B-60' centrifuge. Haemoglobin and catalase were run in a parallel gradient as markers. Replicase activity was determined on 5 μl. aliquots from each of three consecutive gradient fractions, using the standard poly C assay, except that incubation was for 30 min at 37° C. SDS-acrylamide gels were run on 100 μl. aliquots of the gradient fractions.

7S suggested that the binding of this pair of subunits might be weak enough to permit more complete resolution in slightly altered conditions. The enzyme was therefore dialysed against low salt (0·01 M Tris-HCl, *p*H 7·5), and then run on separate glycerol gradients with varying $(NH_4)_2SO_4$ concentrations between 0·2 and 0·01 M. As the salt in the gradients decreased from 0·05 to 0·01 M the 7S protein peak shifted to 8–10S, with the simultaneous appearance of a leading shoulder extending to more than 20S, and a trailing peak at 4–5S. The pattern obtained at 0·01 M $(NH_4)_2SO_4$ is shown in Fig. 11. In each case, activity was found exclusively associated with the 8–10S peak. The activity recovered decreased proportionately with the fraction of protein sedimenting in this position. SDS gels run on fractions across such gradients (Fig. 11) demonstrated that the leading shoulder contained subunits I and II, the activity region I, II, III and IV, and the 4–5S peak subunits III and IV. In some experiments, in which very high concentrations of Cleland's reagent (8 mM) were used, a small amount of subunit I, but no II, was also found sedimenting with the 4–5S inactive proteins.

Protein from the leading edge of the activity peak on a 0·01 M $(NH_4)_2SO_4$ gradient, which is composed of subunit I and phage specific subunit II, but little subunit III or IV, was mixed with aliquots of protein from the more slowly sedimenting region of the same gradient. As shown in Fig. 11, protein from the 4–5S region, which is a mixture of host specific subunits III and IV, stimulated activity as much as three times, although it is inactive when assayed separately. In repeated experiments, using enzyme from several separate preparations, between two and seven-fold stimulations were observed. Fig. 12 shows the kinetics of reconstitution at three different temperatures. The reconstitution occurs rather slowly with activity still increasing after more than 3 h at 37° C. This increase in GMP incorporation indicates that active enzyme is being continually generated, for the time required after initiation to finish a product chain (150–200 nucleotides) in the poly C assay is less than 5 min at 37° C and re-initiation does not occur in the assay conditions used. Reconstitution is also temperature dependent; it is more efficient at 37° C than at 30° C, but the net recovered activity decreased markedly at 42° C. The temperature



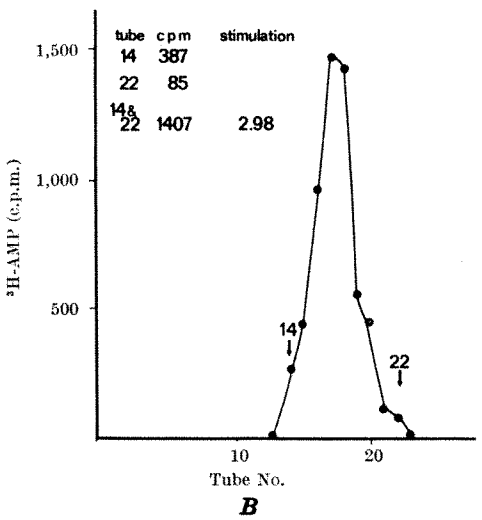


Fig. 11. *A*, Subunit separation gradient. Approximately 1·7 mg of purified replicase in 0·75 ml. was dialysed against 0·01 M Tris-HCl (*p*H 7·5)–0·001 M magnesium acetate–0·0002 M EDTA–0·1 mM dithiothreitol–5 per cent glycerol for 3 h and then loaded on an 11 ml. 10–30 per cent glycerol gradient in the same buffer plus 0·01 M $(NH_4)_2SO_4$. The gradient was centrifuged for 12 h at 41,000 r.p.m., 10° C. Haemoglobin run in a parallel tube served as a sedimentation marker. Replicase activity (of 5 μl. aliquots) was determined in the standard poly C assay, incubating for 30 min at 37° C. Tube 15 (5 μl., 678 c.p.m. assayed alone) was then mixed with 5 μl. from each of tubes 16–26 and re-assayed at 37° C for 30 min. ▲, The factor of stimulation (observed incorporation divided by the sum of the incorporation seen in separate assays). In *B*, the gradient was assayed using denatured double stranded Qβ RNA as template, and ^{3}H-ATP (13,800 c.p.m./mole) as radioactive substrate. The standard reaction mixture, in this case, was modified by the elimination of poly C and the addition of ATP, CTP and UTP to 0·2 mM. The data in the table shown in *B* are the results of a single point reconstitution assay in which aliquots of tubes 14 and 22 were assayed separately and together with the denatured double stranded RNA template.

requirement, combined with the apparent instability of the separated components at slightly higher temperature, probably accounts for the limitation of the net recovery of activity to about 30 per cent of that expected on a protein basis. The extent of reconstitution is only slightly enhanced by increasing the salt concentration (either $(NH_4)_2SO_4$ or KCl), or by using 8 mM dithiothreitol throughout dissociation and reconstitution. High molecular weight aggregates of subunits I and II (>14S) cannot be reproducibly stimulated. This may reflect the denaturation of the subunits in the complete absence of the two low molecular weight polypeptides or, more simply, the difficulty of disaggregating the highly multimerized material. The observed effects are not limited to the poly C activity of the replicase. With denatured double stranded Qβ RNA (a convenient source of minus strands) as template, the same activity profile and extent of reconstitution in mixing experiments was observed (Fig. 11).

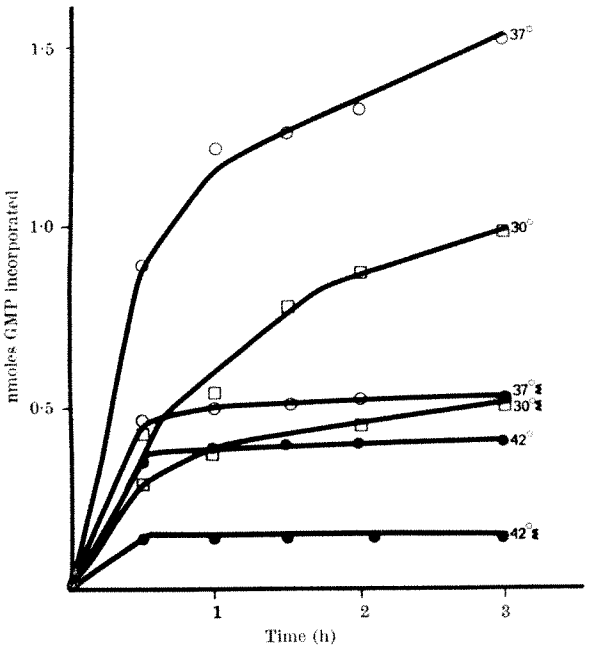


Fig. 12. Kinetics of activity reconstitution. Two fractions of replicase proteins were used. One contained subunit I, subunit II and approximately ten-fold reduced amounts of subunits III and IV. The other was pure subunits III and IV. The fractions were assayed separately and together as a function of time at 30° C, 37° C and 42° C. The curves labelled 30° CΣ, 37° CΣ and 42° CΣ are the sums of the separate assays at the three temperatures, while the curves labelled 30° C, 37° C, and 42° C are the actual observed incorporations with the mixed fractions.

The co-aggregation of all four polypeptides in the absence of salt indicates that there is some physical interaction between them. The aggregation and partial dissociation at low $(NH_4)_2SO_4$ suggest that host subunit I binds tightly to Qβ specific subunit II, while subunits III and IV, either separately or in combination, bind less tightly at this salt concentration and are therefore partially left behind at 4–5S. The stimulation of activity observed when host specific subunits III and IV are added to enzyme deficient in them shows that at least one of these subunits is required as well as the phage specific subunit. Proof of the involvement of the remaining two subunits awaits further physical and functional studies of the enzyme.

The chemical roles of the host subunits in the replicase reaction are not yet known. One or more of the host subunits could be involved in initiation, and may, like the polymerase sigma factor, dissociate from the rest of the enzyme after the initiation event[40]. Another may be involved in specific chain termination. Indeed, the phage specified subunit may serve primarily as a specificity determinant, with the locus of the active site for phosphodiester bond formation residing on one or more of the host subunits. Although none of the subunits is identical to a known component of the DNA-dependent RNA polymerase, it is quite possible that replicase uses some as yet unidentified part of the host's transcriptional machinery. The known relationships between phage and cellular RNA synthesis may be the result of a competition between replicase and RNA polymerase for a common protein factor.

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Dissociation of Ribosomes on Polypeptide Chain Termination and Origin of Single Ribosomes

by
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After polypeptide chain termination, ribosomes are released from polysomes in the form of ribosomal subunits. Single ribosomes, which differ from ribosomes found in polysomes, are formed by association of the released subunits.

In bacteria[1,2] and yeast[3] there is a continual exchange of the two ribosomal subunits between the translating ribosomes and a pool of free subunits. This cyclic process seems to involve the dissociation of ribosomes after each round of translation of messenger RNA[4] and the association of subunits from the pool during initiation of protein synthesis[5,6]. The precise point at which the ribosomes dissociate is not known; in particular we do not know if ribosomes, on completing one or more polypeptide chains, are released from messenger RNA in an undissociated form or as ribosomal subunits.

Single ribosomes accumulate at the expense of polysomes in bacteria and in eukaryotic cells in conditions that decrease the overall rate of protein synthesis. Results of various studies on these ribosomes have been interpreted as evidence that following completion of polypeptide synthesis, ribosomes are first released from polysomes in the form of stable, undissociated single ribosomes and are only subsequently converted to subunits[7-9].

Here we show that on completion of polypeptide synthesis, *Escherichia coli* ribosomes are released from polysomes in the form of ribosomal subunits. These join a pool of subunits which, in sub-optimal conditions for protein synthesis, reassociate readily to form single ribosomes. This class of ribosomes is distinct from ribosomes found in polysomes.

Ribosome Dissociation follows Translation

When an extract containing polysomes from cells grown in heavy isotopes (^{13}C, ^{15}N, ^{2}H) is added to an approximately thousand-fold excess of polysomes extracted from cells grown in light isotope medium (^{12}C, ^{14}N, H) and the mixture is incubated in conditions permitting protein synthesis, rapid ribosomal subunit exchange occurs; this is shown by conversion of fully heavy ribosomes to ribosomes of hybrid density[4,10] that contain one heavy and one light ribosomal subunit[1]. The simplest explanation of this finding is that at or shortly after termination of polypeptide synthesis, ribosomes dissociate into 30S and 50S subunits which enter a pool of free subunits. Because of the predominance of light particles in the pool, heavy subunits recombine mostly with light subunits to form new ribosomes of hybrid density.

This scheme predicts that if reformation of ribosomes, but not the completion of nascent polypeptide chains, is prevented free subunits should accumulate at the expense of ribosomes in polysomes. This was tested by diluting the polysomes and free subunits of the heavy and light extracts a hundred times while keeping the concentration of the supernatant components constant by addition of S-100 extract from light cells. The rationale of this procedure is to attain subunit concentrations that limit the rate of reformation of ribosomes during protein synthesis. Fig. 1 shows that whereas only few free subunits are present in the original heavy polysome extract (*a*), incubation for up to 5 min results in a progressive accumulation of free subunits (*b*, *c*). A slight shoulder of hybrid ribosomes is present in Fig. 1*c*, and further incubation shows that of the ribosomes remaining after 15 min (*d*) approximately half were reformed to yield hybrids. To prevent such residual hybrid formation, the polysome extracts were diluted a further ten times (total dilution, thousand-fold). In these conditions large amounts of heavy and light ribosomal subunits accumulate (Fig. 1*e*) and there is no evidence for hybrids among the remaining ribosomes, which presumably have not yet finished a complete round of translation. The accumulation of subunits is completely blocked by sparsomycin (Fig. 1*f*). A zero time control sample of this incubation yielded the same profile as that in Fig. 1*a*. The results of this experiment show that ribosome dissociation follows translation, but they do not exclude the possibility that a single ribosome stage intervenes between the polysomes and the free subunit pool. If single ribosomes are formed on termination of polypeptide synthesis, however, they must dissociate without much delay.

Single Ribosomes arise from Subunits

A 1 : 10^3 mixture of isotopically heavy and light *E. coli* polysome extracts was incubated as described in Fig. 1, except that the extracts were diluted only twenty times with respect to polysomes and ribosomal subunits. At

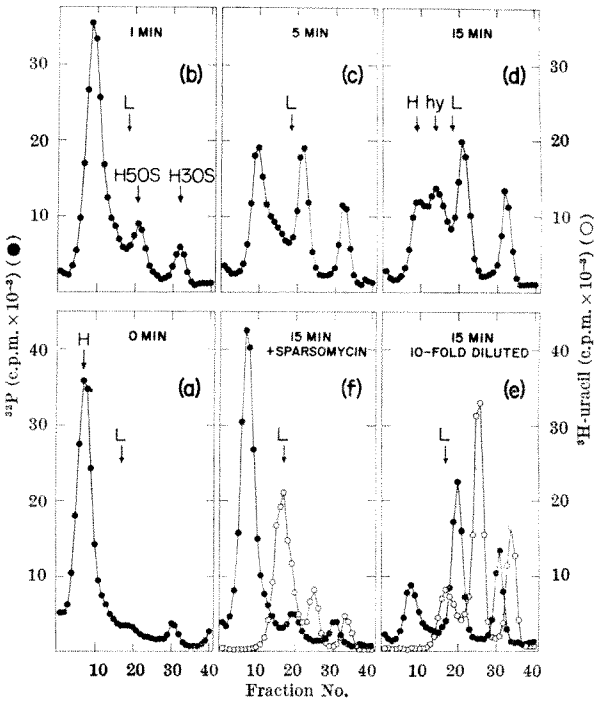


Fig. 1. Accumulation of ribosomal subunits in dilute polysome extracts. *E. coli* D10 (ribonuclease I⁻) was grown in heavy isotope medium[1] with $^{32}PO_4$ or in light isotope medium (0·5 per cent bactotryptone and 0·25 per cent NaCl) with ^{3}H-uracil. When the cultures reached a titre of approximately 10^8 cells/ml., polysome extracts were prepared by neutral detergent lysis at 0° C (refs. 4, 11). The reaction mixture (0·25 ml.) was 0·0067 M magnesium acetate, 0·06 M NH_4Cl, 0·1 mg/ml. *E. coli* tRNA, 0·0002 M ATP, 0·0002 M GTP, 0·005 M PEP, 20 μg/ml. pyruvate kinase, 4×10^{-5} M for each of eighteen amino-acids, 0·005 M β-mercaptoethanol, 0·05 M Tris-HCl (*p*H 7·8). It contained 25 μl. S-100 extract prepared from *E. coli* D10 and 20 μl. (*a*–*d*) or 2 μl. (*e*, *f*) of each polysome extract. The reaction was carried out at 37° C and terminated by rapidly cooling the mixture to 0° C. Eight μg each of ribonuclease and deoxyribonuclease were added in the cold. Sparsomycin (10^{-4} M) was present in sample *f*. The extracts were analysed on 12·5 ml. isokinetic sucrose gradients[1] (12·5 to 19·6 per cent sucrose in 0·01 M Tris-HCl (*p*H 7·4), 0·01 M magnesium acetate, 0·06 M KCl, 100 μg/ml. gelatin). They were centrifuged for 3·8 h at 41,000 r.p.m. ^{3}H-Labelled light ribosomal particles were present in gradients *a*–*d* but for simplicity their profiles are not shown. Instead, an arrow (L) indicates the position of light ribosomes in each gradient[10].

the time of mixing, the extracts contained few ribosomes sedimenting near 70S (Fig. 2*a*). Twenty minutes later, single ribosomes (Fig. 2*c*, region II) had accumulated at the expense of polysomes. The incubation mixture contained 0·06 M NH_4^+ as the dominant monovalent cation. Whether the extracts were centrifuged in sucrose gradients containing 0·06 M K^+ (Fig. 2*a*, *c*) or 0·06 M Na^+ (Fig. 2*b*, *d*), identical profiles were obtained.

Single ribosomes that accumulate *in vivo* and *in vitro* in a variety of sub-optimal conditions for protein synthesis could represent ribosomes released from polysomes on chain termination that are unable to dissociate into subunits. If so, single ribosomes generated from heavy polysomes in extracts containing excess light polysomes should be fully heavy.

The density of the single ribosomes was measured by sedimentation velocity analysis in isokinetic sucrose gradients in which heavy ribosomes and subunits sediment approximately 20 per cent faster than their light counterparts[1]. The density distribution of ribosomes obtained from region I in the gradient shown in Fig. 2*c* serves as a control. This region is expected to contain small polysomes remaining after 20 min of incubation, presumably because their ribosomes have not yet terminated polypeptide synthesis. If correct, this region should contain only fully heavy and fully light ribosomes. Fig. 3*a* shows that these are indeed the dominant species, while almost no hybrid ribosomes are present, an indication that little, if any, successful reinitiation of protein synthesis by newly formed ribosomes occurred in this experiment.

The density distribution of the single ribosome peak (Fig. 2*c*, region II) is shown in Fig. 3*b*. This distribution contrasts sharply with that of region I: almost all label originally present in heavy polysomes is found in ribosomes of hybrid density, sedimenting in a position between fully heavy and fully light species. This demonstrates that the single ribosomes observed in Fig. 2*c* are not derived directly from polysomes, but have arisen by the association of ribosomal subunits.

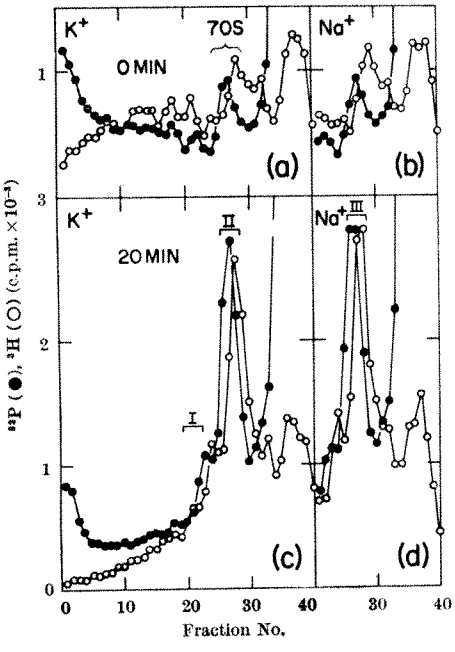


Fig. 2. Accumulation of single ribosomes during cell-free protein synthesis. A mixture of ^{32}P-labelled heavy and ^{3}H-labelled light polysome extracts was incubated as in Fig. 1*a*–*d* except that the concentration of polysomes was raised five-fold. After incubation for 0 min (*a*, *b*) or 20 min (*c*, *d*), the samples, cooled to 0° C, received sparsomycin (10^{-4} M) and deoxyribonuclease (8 μg/ml.) and were centrifuged for 0·6 h at 50,000 r.p.m. in 4 ml. sucrose gradients (see legend to Fig. 1). In gradients *b* and *d*, 0·06 M NaCl replaced KCl. Portions of 15 μl. were analysed for radioactivity.

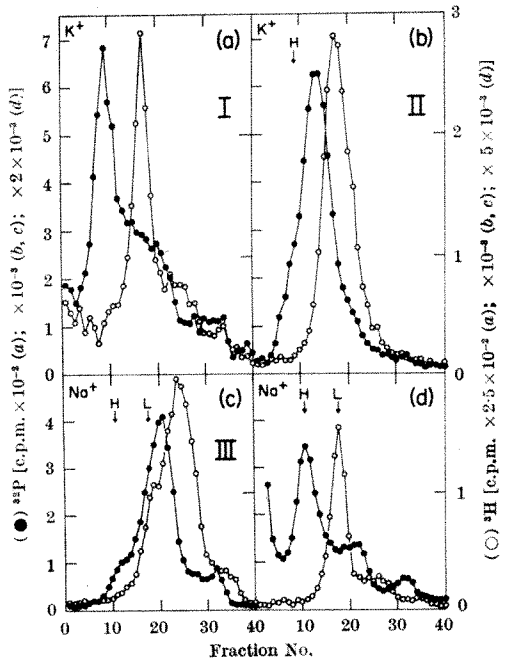


Fig. 3. Density distribution of single ribosomes and the effect of Na^+ ions. Sedimentation velocity analysis of material in regions I, II and III (Fig. 2) in isokinetic sucrose gradients (see legend to Fig. 1) containing 0·06 M KCl (*a*, *b*) or NaCl (*c*). Before centrifugation, the material of region I was incubated with 3 μg/ml. ribonuclease at 0° C. Gradient *d* (NaCl) contains heavy and light cell extracts mixed in the presence of 8 μg/ml. ribonuclease and deoxyribonuclease and kept at 0° C. Centrifugation was for 3·8 h at 41,000 r.p.m. Gradient *a* was centrifuged in a separate rotor. The expected position of heavy (H) ribosomes is shown by an arrow in *b*; arrows in *c* refer to positions of heavy and light ribosomes in *d*.

Formation of Ribosomes is not Freely Reversible

The accumulation of subunits during protein synthesis by diluted polysomes (Fig. 1) and the hybrid nature of single ribosomes (Figs. 2 and 3) are readily explained if ribosomal subunits are released from polysomes on polypeptide chain termination and subsequently recombine to form single ribosomes. The data could also be interpreted, however, in terms of a model in which single ribosomes are first released from polysomes but then equilibrated rapidly with a pool of free subunits. To explain the accumulation of ribosomal subunits, but not ribosomes in dilute polysome extracts (Fig. 1), the latter model requires that in such a system single ribosomes dissociate irreversibly.

To test this, ^{32}P-labelled light single ribosomes isolated from a sucrose gradient such as that of Fig. 2*c* and ^{3}H-labelled polysome extract were incubated with S-100 in conditions that convert most polysomes to ribosomal subunits, as in Fig. 1*e*. Although extensive accumulation of ^{3}H-labelled subunits occurred (Fig. 4, open circles), there was little, if any, dissociation of ^{32}P-labelled single ribosomes (filled circles) compared with the unincubated control (△ — △). The concentration of single ribosomes was approximately one-fifteenth of that of all ^{3}H-labelled ribosomal particles (polysomal and non-polysomal) in Fig. 4, and one-fifth of that of all ribosomal particles present in the experiment of Fig. 1*e*. The Mg^{2+} ion concentrations were identical in all experiments reported here: they were 7 mM during incubation and 10 mM during sucrose gradient centrifugation.

This experiment demonstrates the essential stability of single ribosomes in conditions of protein synthesis leading to the conversion of polysomal ribosomes to subunits, and shows that the formation of single ribosomes is not freely reversible.

Properties of Single Ribosomes

Single ribosomes sediment slower than monosomes obtained by ribonuclease digestion of a polysome extract

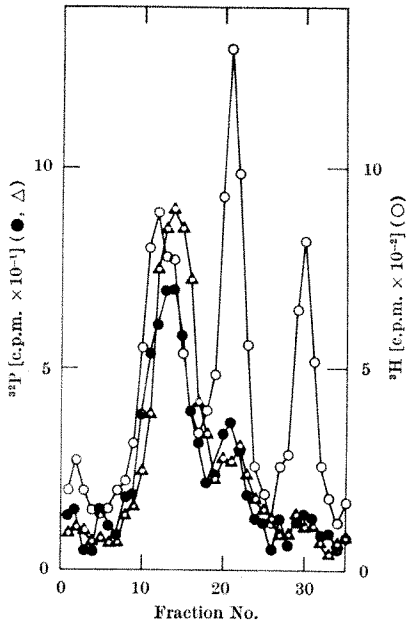


Fig. 4. Stability of single ribosomes in dilute polysome extracts. ^{32}P-labelled single ribosomes (●) isolated from light cells as in Fig. 2*c* (region II) were incubated with ^{3}H-labelled polysome extract (○) as in Fig. 1*e*, except that the concentration of ^{3}H-labelled polysomes was approximately three times higher and no ribonuclease was added after incubation. The sample was centrifuged as in Fig. 3*a* for 4·1 h. A sedimentation profile of unincubated single ribosomes (△), centrifuged in parallel, is superimposed.

(Fig. 5*a*). If the monosome peak is taken as 70S, the sedimentation coefficient of single ribosomes in this figure is 65S. Other experiments gave values varying from 64–66S. The leading edge of the single ribosome peak in Fig. 5*a* most likely contains contaminating monosomes.

Single ribosomes, unlike polysomes, are dissociated by ribonuclease into subunits. ^{32}P-labelled single ribosomes were mixed with ^{3}H-labelled polysome extract and incubated in the presence of 5 μg/ml. ribonuclease for 15 min at 37° C. While this treatment reduced polysomes to monosomes but no further, single ribosomes dissociated almost entirely into 30S and 50S subunits (Fig. 5*b*). The ^{32}P-labelled material sedimenting with the main ^{3}H peak presumably reflects the presence of some monosomes in the single ribosome preparation. Likewise, some of the ^{3}H-labelled subunits may be derived from single ribosomes. Incubation at 22° C rather than 37° C gave identical results but little or no dissociation by ribonuclease occurred at 0° C.

While the yield of single ribosomes is identical during short centrifugation (0·6 h) in K^+ or Na^+ gradients (Fig. 2), the material in region III (Fig. 2*d*) is dissociated during prolonged centrifugation (3·8 h) in an Na^+ environment (Fig. 3*c*). In these conditions, single ribosomes from region II (Fig. 2*c*) yielded the same pattern (not shown) as those from region III. By contrast, a ribonuclease digest of heavy and light polysome extracts sedimented mostly as ribosomes, not subunits, in Na^+ (Fig. 3*d*). This clearly shows that the presence of Na^+ ions leads to the gradual dissociation of single ribosomes, but not of ribosomes found in polysomes.

The extensive ribosomal subunit exchange occurring during protein synthesis in concentrated cell extracts[4] is not merely because of formation of single ribosomes. Most newly formed ribosomes are not dissociated by ribonuclease or by Cs^+ ions, which behave like Na^+ ions with respect to single ribosomes[14].

Single Ribosomes and the Ribosome Cycle

The results presented show that on completion of polypeptide synthesis, ribosomes are released from polysomes in the form of ribosomal subunits. Possibly, the dissociation of ribosomes is not concomitant with their release but occurs immediately after this event. Undissociated intermediates, however, if they exist, must be so short-lived that their presence would not be detectable by the techniques used here. It is also shown that stable single ribosomes, which accumulate in sub-optimal conditions for protein synthesis, arise from the pool of ribosomal subunits released from polysomes. Under conditions conducive to this process, the formation of single ribosomes is not freely reversible.

The nature of single ribosomes is not understood, but they can be distinguished from ribosomes found in polysomes by their properties just described. Others[15,16] have shown that single ribosomes require a higher Mg^{2+} ion concentration to prevent their dissociation than do polysomal ribosomes.

The absence of single ribosomes in gradients containing 0·06 M Na^+ was first shown by Phillips, Hotham-Iglewski and Franklin[14], who suggested that single ribosomes are artefacts created in the presence of K^+ or NH_4^+ ions, but not Na^+. This interpretation was based on the claim that single ribosomes present in cell lysates containing 0·06 M K^+ are stable when centrifuged in Na^+ sucrose gradients[14]. In fact, however, about half of the material sedimenting in the 70S region dissociated in these conditions (ref. 14, Fig. 6*a*). The 70S region analysed came from polysome distributions of early log phase cells (comparable with Fig. 2*a*) which contain few ribosomes sedimenting near 70S. Much of this material probably represents monosomes engaged in protein synthesis, and they are therefore stable in Na^+-sucrose gradients (see also Fig. 3*d*). The remainder represents Na^+ sensitive single ribosomes. Indeed, almost all ribosomes sedimenting near 70S in Na^+ sucrose gradient are active in protein synthesis[17]. Our conclusion that Na^+ but not K^+ causes the specific dissociation of single ribosomes has also been reached by Beller and Davis (personal communication). Mammalian single ribosomes have a specific salt sensitivity as well[18].

In growing *E. coli*, the concentrations of K^+, ranging from 210 to 250 mM, are four to five times higher than those of Na^+, and in late log phase, when increasing levels of single ribosomes are found in cell extracts, K^+ remains greatly in excess of Na^+[19]. Ionic conditions *in vivo* therefore favour the stability of single ribosomes, but these conditions alone cannot account for single ribosome formation.

Incubation with ribonuclease causes selective dissociation of single ribosomes in conditions when polysomal

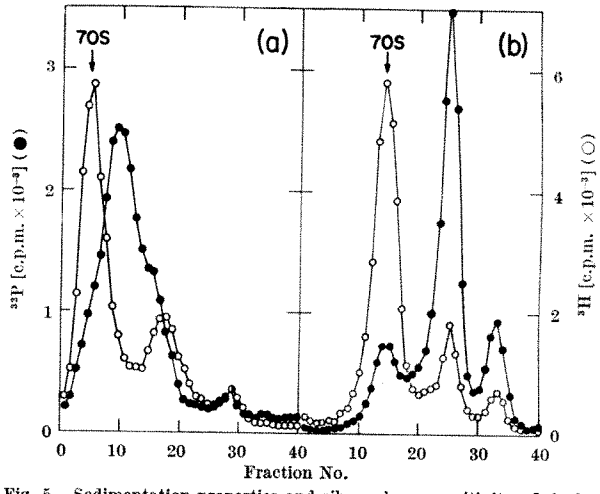


Fig. 5. Sedimentation properties and ribonuclease sensitivity of single ribosomes. *a*, ^{32}P-labelled single ribosomes from light cells, isolated as in Fig. 2*c*, were sedimented as in Fig. 3*a* for 4·7 h. ^{3}H-Labelled polysome extract was incubated for 15 min with 5 μg/ml. ribonuclease at 37° C and centrifuged in parallel. Peak positions did not vary more than one fraction in multiple parallel gradients. *b*, ^{32}P-labelled single ribosomes were incubated for 15 min with ^{3}H-labelled light polysome extract in the presence of 5 μg/ml. ribonuclease at 37° C, and centrifuged as in Fig. 3*a* for 4·0 h.

ribosomes or initiation monosomes bound to phage RNA[12] remain intact. Apparently, the two subunits in a single ribosome are held together by RNA, but because monosomes obtained after ribonuclease digestion of polysomes contain a protected fragment of messenger RNA[12,13], our results suggest that either such a fragment is exposed in single ribosomes, or it is absent altogether. The latter interpretation is also supported by data showing that in polysome profiles of log phase cells labelled for 10 s with ^{32}P, the specific radioactivity of the 70S region in Na^+ sucrose gradients exceeds that in K^+ gradients at least by a factor of two[20]. The suggestion that single ribosomes are artefactual monosomes[21], defective initiation complexes carrying messenger RNA, is not supported by these facts. Moreover, the defect in such complexes would have to be easily reversible, for single ribosomes accumulated in bacterial[22] or mammalian[23,24] cells can rapidly re-enter polysomes in conditions favouring a higher rate of protein synthesis. The relationship between single ribosomes and artificial 60S ribosomes containing poly U[31] remains to be established.

There is no evidence to suggest that single ribosomes are obligatory intermediates in the ribosome cycle. Indeed, in mammalian cells single ribosomes are not in equilibrium with subunits or polysomal ribosomes[23,25,26]. Similar data are not available for bacteria. In these cells, control of the rates of ribosome synthesis and cell division may preclude the accumulation of significant amounts of single ribosomes during steady growth.

The results presented suggest the following sequence in the ribosome cycle. On polypeptide chain termination, ribosomes dissociate into subunits. During rapid protein synthesis, freshly released subunits are immediately recycled into polysomes. When protein synthesis slows down, increasing numbers of subunits accumulate after their release from polysomes; these reassociate into single ribosomes which do not participate in protein synthesis. In conditions requiring a higher rate of protein synthesis, this storage pool of synthetically inactive ribosomes is mobilized and returned to subunits, possibly through the action of one or more factors[27–29].

According to this view, single ribosomes constitute side-track products of the ribosome cycle which are bypassed by ribosomes and subunits active in protein synthesis. Consequently, during low rates of protein synthesis, when the return of single ribosomes to the active ribosomal pool is not favoured, subunits newly released from polysomes would be expected to recycle preferentially. Such preferential recycling seems to occur in a reticulocyte cell-free system[30].

It is possible that in order to initiate a new round of protein synthesis, one or both species of subunits are modified after their release from polysomes. The modification may entail removal of C-terminal tRNA from the 50S subunit. When such tRNA is not removed, 50S subunits may reassociate with 30S subunits to yield single ribosomes stabilized by a tRNA molecule. Accordingly, ribonuclease would act to destroy the integrity of this tRNA and thus cause the specific dissociation of single ribosomes observed *in vitro*.

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Two New Fossil Primates from the Lower Miocene of Kenya

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Relationships of Miocene Hominoidea are re-evaluated in the light of two new fossil pongids from the early Miocene of Rusinga and Songhor, one an undetermined species of *Proconsul* possibly ancestral to orang-utans and the other an undetermined species of *Aegyptopithecus*.

THE Lower Miocene pongids of East Africa have long been recognized as the most likely ancestral group to modern pongids[1]. They were living at a time very close to the divergence of the hominid and pongid lineages, when the prototypes of the two families must still have been very similar[2,3]. In order to understand more clearly the relationships of these Miocene hominoids all of the specimens housed at the Centre for Prehistory and Palaeontology in Nairobi are being re-examined in the light of modern zoological knowledge of population variation and speciation. The size of the collection of primate material in Nairobi has more than doubled since 1951 through the efforts of Dr L. S. B. Leakey and his colleagues. Two of the new specimens are described here as being particularly relevant to the discussion of Lower Miocene primate radiation, a discussion stimulated by the preliminary work of Simons and Pilbeam[4]. A large scale revision of the Miocene Pongidae of Kenya

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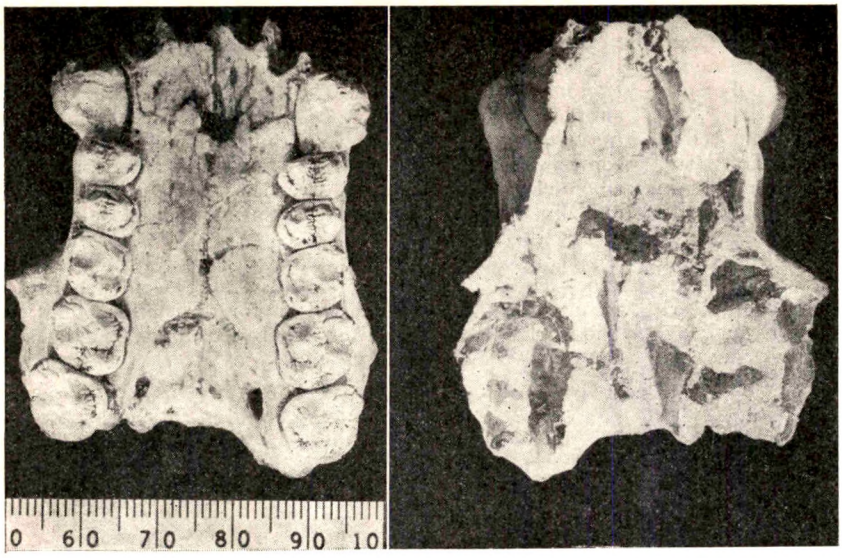


Fig. 1. Songhor palate, *KNM-SO* 700. Palatal view on the left and superior view on the right. Natural size.

is in progress, and until the new diagnoses can be more strictly defined the original taxonomic divisions of Le Gros Clark and Leakey[1] will be retained.

The two specimens consist of a palate with all the teeth except for the incisors (*KNM-SO* 700); and a symphysis and left body of the mandible of a much smaller primate with all the incisors and the left canine to first molar (*KNM-RU* 900). The first was recovered by members of Dr L. S. B. Leakey's team in 1966 from the calcified tuff underlying the red beds at Songhor in Western Kenya. This site has been dated as younger than $19{\cdot}7 \pm 0{\cdot}5$ m.y. (ref. 5). The second was recovered in 1956 from site *R*2 on Rusinga Island in Lake Victoria. The principal deposits on Rusinga Island have now been determined to represent one horizon which has been given a preliminary date of about 18 m.y. (ref. 6). The Songhor specimen has previously been figured[2], but neither specimen has yet been described.

Specimen *KNM-SO* 700

The specimen consists of the bony palate and the alveolar processes of the maxilla. All the teeth except for the incisors are preserved. The tips of both canines are broken off, and it is probable that the canines are not quite fully erupted. The third molars are fully erupted but only slightly worn, which suggests a subadult age for this individual. The floor and inferior lateral walls of the nasal cavity are present back to the palatine bone. The floor of the maxillary sinus is present on both sides, but the medial and lateral walls are broken down except for a few remnants. The medial palatine, incisive and transverse palatine sutures can be clearly seen (see Fig. 1).

The premaxilla is relatively large, extending all the way up the anterior margin of the nasal cavity. The size of the nasal cavity is similar to that of *Proconsul africanus* Hopwood 1933 (ref. 7), as is the bony palate, which is, however, relatively longer in *KNM-SO* 700. In contrast to this is the evidence for shortening of the face in the Songhor specimen: the anterior position of the greater palatine foramen, the prominent ridge for attachment of the soft palate, and the short alveolar tuberosities. The floor of the maxillary sinus is relatively greatly expanded. In the dentition, the canines are large blade-like teeth with a pronounced distal flange. The premolars are more molariform than usual in *Proconsul*, the talon and large lingual cingulum being indications of this. The fourth premolar is unusual in being longer than the third, a condition only found, in pongids, in some specimens of gorilla. The molars are elongated, carrying on the trend seen in the premolars. They increase in length and size from first to third. The trigon is relatively expanded, the hypocone large on all the molars, and the lingual cingulum is massively developed. There is no protoconule, and the occlusal ridges are poorly developed. Measurements of the dentition are given in Table 1.

The taxonomic position of the Songhor palate is not yet clear, and further work is necessary before it can be assigned with any confidence to a species. Its closest affinities are with *Proconsul africanus*, both by size and morphology, and some specimens which are similar to it have actually been described as this species[1,8]. It is possible that these specimens will turn out to be variants of *Proconsul africanus*, but the differences are not completely in line with the variation seen within one species of pongid. Rather, the differences parallel to a remarkable extent those between *Pan troglodytes* and *Pongo pygmaeus*. In the dentition, the blade-like canines with a prominent mesial groove and the molariform premolars are much closer to the orang-utan than to the chimpanzee, while *Poconsul africanus* is closer to the latter. The molar proportions are like those of some orang-utans, with the increase in length and area from the first to the third molar; and on the occlusal surface the low ridges and increase in wrinkling are also like the condition in the orang-utan. *Proconsul africanus* is closer to the chimpanzee in these features. The shortening of the face in the orang-utan is carried out to a greater extent: the alveolar tuberosities are very small relative to the length of the tooth row, the ridge for attachment of the soft palate is very prominent, and the greater palatine foramina are located anteriorly. In the African great apes all these features are developed in a way that is linked

Table 1. MEASUREMENTS OF DENTITION OF *KNM-SO* 700

	C		
Maximum length (L)	12·4		
Perpendicular breadth (B)	8·0		
Index B/L × 100	64·5		
	P3	P4	
Mesiodistal length (buccal) (L)	5·8	6·0	
(lingual) (L″)	5·5	—	
Buccolingual breadth (B)	8·3	8·0	
Index B/L × 100	143·1	133·3	
Index L″/L × 100	94·8	—	
Index L + B/2	7·0	7·0	
	M1	M2	M3
Mesiodistal length (L)	8·5	9·6	10·3
Buccolingual breadth (B)	8·1	10·0	10·7
Index B/L × 100	95·3	104·2	104·0
Index L + B/2	8·3	9·8	10·5

Table 2. MEASUREMENTS OF DENTITION OF *KNM-RU* 900

	I1	I2
Mesiodistal length (L)	2·7	2·9
Buccolingual breadth (B)	3·7	4·3
Buccal height (H)	5·1	5·6
Index B/L × 100	137·0	148·3
Index L/H × 100	52·9	51·8
	C	P3
Maximum length (L)	7·5	6·6
Perpendicular breadth (B)	5·0	4·2
Buccal height (H)	12·0	7·0
Index B/L × 100	66·6	63·6
Index L/H × 100	62·5	94·3
	P4	M1
Mesiodistal length (L)	4·6	6·2
Buccolingual breadth (B)	5·2	5·3
Index B/L × 100	113·0	85·5

with facial lengthening rather than with shortening, and as far as they can be seen the same is true for the three species of *Proconsul*. The evidence for the expansion of the maxillary sinus also compares with the greatly enlarged sinus of the orang-utan[9] whereas the smaller sinus in the published *Proconsul* specimens is more like that of the chimpanzee and gorilla.

The resemblances to the orang-utan shown by specimen *KNM-SO* 700 are interesting in that they provide the first record in the Lower Miocene of Kenya of a possible member of the orang-utan lineage. Possible ancestors to the chimpanzee and gorilla have already been recognized in the Lower Miocene[3] and it is very probable that ancestral orang-utans were also living at this time. Three of the distinctive functional complexes of the orang-utan can be recognized in the Songhor palate; these are: (1) the lengthening and molarization of the cheek teeth; (2) the shortening of the face (in spite of lengthening of the teeth); and (3) the expansion of the maxillary sinus (in spite of the shortening of the face).

These features are to a certain extent functionally independent, although their significance in the present day orang-utan is not known for certain; and being functionally independent it is less likely that the resemblances shown have resulted from a parallel functional development in the Kenya specimen and in the orang-utan. Parallelism is still a strong possibility, however, and only the discovery of more complete Miocene and Pliocene material will show which explanation is the correct one. Phylogenetic considerations apart, the choice of naming this specimen will be left open at this stage to await the description of further material and the results of the statistical analysis now in preparation. Specimen *KNM-SO* 700 is therefore referred to *Proconsul* species *indet.*

Specimen *KNM-RU* 900

This specimen consists of the symphysis and left body of the mandible as far as the first molar. The right lateral incisor to the left first molar are intact, but the crowns of the canine to first molar have been slightly displaced posteriorly over each other. The incisor row has also been slightly displaced, the right side having been shifted anteriorly. The mandible seems to have been subjected to anteromedial pressure during fossilization to produce these distortions, and this has also produced a fracture on the medial aspect of the body of the mandible beneath the third premolar and has slightly crushed the cortex posterior to this. The symphysis has been eroded in the region of the genial fossa, but apart from this it is intact and the contour undistorted. The erosion continues posteriorly to below the roots of the fourth premolar. The alveolus is broken buccally, exposing parts of the roots of all the teeth, and the surface of the rest of the mandible has been fissured and cracked by weathering (see Fig. 2).

Specimen *KNM-RU* 900 is characterized by its very deep narrow symphysis. The breadth/depth index is very low, with a value of 42·3. The mental region is compressed from side to side so that the incisor alveolus is short. It is also curved, the roots of the central incisors being placed anterior to those of the lateral incisors. The anterior border of the symphysis is steep and it curves gently from the alveolar border to near the inferior, at which point it curves abruptly posteriorly. The exact conformation of the inferior part of the symphysis cannot be determined, but from the curve of the bone on either side of the crushing it would seem that the region of the genial fossa was indented strongly. Inferior to this the symphysis is prolonged posteriorly into an inferior transverse torus, and this constitutes the most posterior part of the symphysis. The body of the mandible is also relatively deep and narrow like the symphysis, but becomes much shallower posteriorly.

The dentition of specimen *KNM-RU* 900 is about the same general size as *Limnopithecus legetet* Hopwood 1933 (ref. 7), and the first molar is like that of this species. The third premolar is more like that of *Limnopithecus macinnesi*[1], but the fourth premolar and lower canine are unlike those of either species. The fourth premolar is very short mesio-distally, so that it is broader than long. This condition is seen in *Proconsul* but not in *Limnopithecus*. The canine has a high slender crown that is more reminiscent of the lower canines of cercopithecoid monkeys than of pongids. It has a considerable degree of distal curvature and an elongated mesial ridge. Measurements of the dentition are given in Table 2.



Fig. 2. Rusinga mandible, *KNM-RU* 900. Top left, buccal view; bottom left, lingual view; right, occlusal view. Natural size.

Before creating a new genus for a new specimen it is axiomatic that it should be compared with previously described taxa and assigned to one of them if the degree of similarity warrants it; and because it is not like any other of the Kenya Miocene specimens, it must be compared with taxa from other localities and from other periods. To find the closest similarity in terms of overall morphology it is necessary to go back to the Oligocene of Egypt, to *Aegyptopithecus zeuxis* Simons 1965 (ref. 10). This has a deep narrow symphysis, a "simian shelf", and a nearly vertical ascending ramus, all of which are very similar to the condition seen in the Kenya specimen. The size of the symphysis is less in *Aegyptopithecus zeuxis*, but the shape, as shown by the thickness/depth index of 40·7 is similar. The contour of the posterior slope of the symphysis is very similar, the most posterior part being at the inferior border. The dimensions of the body of the mandible[10] are slightly less than the corresponding values for *KNM-RU* 900, but again the indices are similar. Similarly the size and morphology of the fourth premolar and first molar are also like those of *Aegyptopithecus zeuxis*. Specimen *KNM-RU* 900 is provisionally assigned to the genus *Aegyptopithecus* to indicate its affinities, but no species name can be given until more work is done both on extending the diagnosis of *Aegyptopithecus* and on describing the whole complex of East African forms.

The upper dentition belonging with the mandible *KNM-RU* 900 is not known, but the upper dentition of *Limnopithecus macinnesi* is known in great detail, and it is of interest that there are only slight differences between it and *Aegyptopithecus zeuxis*. It is therefore possible that the upper dentition belonging with the

mandible of *Aegyptopithecus* species is similar in appearance to that of *Limnopithecus macinnesi* and cannot be distinguished from it at present.

The relationships of the Egyptian Oligocene and the Kenyan Miocene hominoids, and hence their phylogenetic affinities, are not yet clear. Simons has suggested[10] that *Aegyptopithecus zeuxis* has its closest affinity with *Proconsul* on the basis of their similar mandibular molar proportions, but the same proportions are seen also in *Limnopithecus legetet*. The differences he mentions between *Aegyptopithecus zeuxis* and *Limnopithecus* in the lower dentition are valid for *Limnopithecus macinnesi* but not at all for *Limnopithecus legetet*, which differs from *Limnopithecus macinnesi* (apart from being much smaller) in having third molars relatively longer than the first and second, more distinct buccal cingula on the lower molars, relatively reduced molar and premolar trigonids, and less distinct trigonid and talonid ridges. In these features *KNM-RU* 900 is closer to *Limnopithecus legetet*. The differences between both *Limnopithecus* species and *Aegyptopithecus zeuxis* are to be found chiefly in the morphology of the mandible and fourth premolar, and it is here that *KNM-RU* 900 differs from *Limnopithecus* and resembles *Aegyptopithecus*. It seems likely therefore, in view of the close similarities between *Aegyptopithecus zeuxis*, *Aegyptopithecus* species (*KNM-RU* 900), and *Limnopithecus legetet*, that all these taxa belong to the same phylogenetic lineage.

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Galactic Soft X-rays and the Alignment of Interstellar Grains

by

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An excess flux of soft X-rays in the galactic disk could result in the alignment of interstellar grains which is required to explain the observations of interstellar polarization.

It is widely believed that the observed polarization of starlight is caused by nonspherical dust grains which are systematically aligned, the sense of this alignment being such that the long axes of the grains are lined up preferentially perpendicular to the galactic plane. The theoretical problem of explaining grain alignment has been discussed for nearly two decades but no entirely satisfactory solution has yet been reached. The most widely discussed alignment theory invokes the process of magnetic relaxation within dust grains which are spinning in the magnetic field of the galaxy[1]. Although this process could produce the correct sense of alignment of grains a high value of the magnetic field strength, greater than 10^{-5} G, seems to be required to produce sufficient alignment of most plausible grain models[2]. This minimum field strength requirement is in considerable excess of recent estimates of $\sim 10^{-6}$ G for the mean magnetic field in normal interstellar clouds. Two other mechanisms, also capable of producing the correct sense of grain alignment, were recently discussed by Salpeter and myself[3] and by Harwit[4]. The former mechanism requires a rather high value of the flux of low energy cosmic rays—a flux that now appears unlikely in view of recent arguments by Fowler, Reeves and Silk[5]. The mechanism proposed by Harwit[4], involving the transfer of internal angular momentum of stellar photons to grains, would appear to be effective only for relatively small-sized particles of a size distribution. Strong alignment effects are restricted to particles with radii $a \lesssim 5 \times 10^{-6}$ cm. The observed wavelength dependence of interstellar polarization, on the other hand, requires the alignment of larger grains with radii $a \gtrsim 10^{-5}$ cm (refs. 6 and 7). The several processes which have so far been discussed may thus be inadequate to accomplish the necessary alignment of interstellar grains.

It will be argued in this article that a galactic X-ray component in the energy range $\sim 0{\cdot}2$–$0{\cdot}4$ keV could play an important part in the alignment of dust grains. The requirement is that the total flux of soft X-rays at low galactic latitudes includes a modest local excess over the flux from an isotropic background. Observations by Cooke, Griffiths and Pound[8] have indicated that such an excess exists in the energy range 1·4–18 keV. Although observations at lower energies are at present somewhat uncertain the possibility of an excess flux arising from unresolved weak sources (in at least some directions in the galactic plane) cannot be ruled out. The alignment mechanism discussed here would, in any case, be expected to dominate in regions sufficiently near to galactic X-ray sources.

We first explore the effects of an excess galactic X-ray flux (of a prescribed intensity) in relation to the alignment of grains. We then derive an estimate of the magnitude of this excess flux which is required in order to accomplish the desired grain alignment.

The alignment process envisaged here involves photoelectric encounters of X-rays with grains, followed by the complete ejection of the resulting photoelectrons. It will be shown that the momenta of emitted photoelectrons are predominantly perpendicular to the direction of propagation of the original photons for the case of highly elongated grains. The resulting torques on the grains will be shown to be largely in the plane of the galaxy. This process would clearly tend to line up the angular momentum vectors of grains parallel to the galactic plane. Consequently the long axes of grains may be aligned preferentially perpendicular to the galactic plane—in accord with the observations of interstellar polarization.

I restrict my discussion to grains composed of silicate material ($MgSiO_3$) because there are arguments to suggest

that such grains may be responsible for interstellar polarization[6]. (My final conclusions will not be sensitive to this assumption, however.) I begin by noting that X-ray energies in the range $\sim 0{\cdot}2$–$0{\cdot}4$ keV—which would be of chief importance to the present process—lie between the K and L_1 edges of each of the constituent atoms in silicate material[9]. The atomic cross-section for photoejection of L electrons by such X-rays may be calculated from formulae derived by Hall[10]. For X-rays with energy $E \gg h\nu_{L_1}$ where ν_{L_1} is the L-edge frequency, the photoejection cross-section of an atom of atomic number Z may be shown to be

$$\sigma_L \cong 6{\cdot}4 \times 10^{-23}(Z-4{\cdot}15)^5\,(E/\mathrm{keV})^{-3{\cdot}5}\ \mathrm{cm}^2 \qquad (1)$$

Summing the contributions due to Mg, Si and O determined by equation (1) the total photoejection cross-section of $MgSiO_3$ then turns out to be

$$\sigma_L(MgSiO_3) \cong 8 \times 10^{-18}\,(E/\mathrm{keV})^{-3{\cdot}5}\ \mathrm{cm}^2 \qquad (2)$$

The stopping distance, D, of a photon of energy E which is intercepted by a grain is determined by

$$\sigma_L \cdot (N_0\,\rho/A) \cdot D \approx 1 \qquad (3)$$

where N_0 stands for the Avogadro number $\sim 6 \times 10^{23}$ cm^{-3}, ρ is the density and A is the molecular weight of the grain material. Setting $\rho \cong 3{\cdot}3$ g cm^{-3}, $A \cong 100{\cdot}38$ for $MgSiO_3$, equations (2) and (3) give

$$D \cong 6{\cdot}3 \times 10^{-6}\,(E/\mathrm{keV})^{3{\cdot}5}\ \mathrm{cm} \qquad (4)$$

Photons of energy $E \cong 1$ keV traverse a distance close to 10^{-5} cm before being stopped, while photons of energy $E \simeq 0{\cdot}3$ keV are absorbed within a few atomic radii of the grain's surface.

The absorption of a photon of energy in the range $E \sim 0{\cdot}2$–$0{\cdot}4$ keV results in the ejection of an L-shell electron from the grain material. The initial energy of the photoelectron is $E-h\nu_L$, where $h\nu_L$ is its binding energy in the L shell. To a sufficiently good approximation we may assume $E-h\nu_L \approx E$. The electron momentum, for the range of photon energies considered here, is predominantly transverse to the photon propagation vector[11]. The initial angular distribution of this momentum about the electric vector of the X-ray is $\sim\cos^2\xi\,d\Omega$ where $d\Omega$ is an element of solid angle in a direction making an angle ξ with the electric vector.

A further condition has to be satisfied for X-ray absorption to result in the transference of the required torques to the grains. The ejected photoelectron must leave the entire grain without being significantly slowed down or stopped within the grain. The characteristic stopping distance, d, of the electron in material possessing a mean excitation potential I, density ρ, mean atomic number $\bar{Z}$, and mean atomic weight $\bar{A}$ is given by[12]

$$d \approx \frac{E^2}{4\pi e^4 (N_0\,\rho \bar{Z}/\bar{A})\,.\,\ln(E/2I)} \approx 7{\cdot}6 \times 10^{-6}(E/\mathrm{keV})\ \mathrm{cm} \qquad (5)$$

where we set $\bar{A}/\bar{Z} \cong 2$, $\rho \cong 3{\cdot}3$ g cm^{-3} and $\ln(E/2I) \cong 0{\cdot}5$.

The condition that the ejected photoelectron escapes from the grain with a high probability, with an energy close to its initial value, is that the photon absorption depth D is much less than the electron stopping distance d. From equations (4) and (5) this condition yields $E < 1$ keV. We confine our attention in the subsequent discussion to the energy range $E \sim 0{\cdot}2-0{\cdot}4$ keV which satisfies this requirement and also ensures the validity of equation (2). For such X-ray energies photoejection of L electrons from silicate grains occurs within a few atomic layers of the grain surfaces. X-rays with energy much below 0·2 keV are unlikely to be important except near sources, on account of the strong absorption properties of the interstellar gas at these energies.

Consider X-ray photons of energy E propagating in a direction $\mathbf{x}$ close to the galactic plane and incident on a grain. I assume a needle-shaped grain with thickness small compared with its length, similar to the models used to discuss the wavelength dependence of interstellar polarization[6]. The orientation of the grain axis may be described by spherical polar coordinates taken at the centre of mass G, with the direction $\theta = 0$ taken to be the direction of the X-ray source $\mathbf{x}$ and the plane $\varphi = 0$ being the plane through $\mathbf{x}$ which is perpendicular to the galactic plane. The direction $\mathbf{x}$ is also taken to be the x-axis, GX, and the normal to the galactic plane through G as GY in a right-handed set GX, GY, GZ of rectangular cartesian axes. Let an X-ray photon be absorbed at a point on the grain surface the position vector of which is $\mathbf{r}$. We first suppose that the momentum of the ejected photoelectron is confined entirely to the YZ plane and may be represented by the coordinates $\mathbf{p} = (0,\ p\cos\alpha,\ p\sin\alpha)$. The angular momentum imparted to the grain is given by the vector product $\mathbf{L} = \mathbf{r} \times \mathbf{p}$ the components of which are

$$[L_x, L_y, L_z] = [rp\sin\theta\,(\cos\varphi\sin\alpha - \sin\varphi\cos\alpha),\ -rp\sin\alpha\cos\theta,\ rp\cos\alpha\cos\theta]$$

We may now evaluate the average values $\langle L_x^2\rangle$, $\langle L_y^2\rangle$, $\langle L_z^2\rangle$ assuming equal *a priori* probabilities for all grain orientations, allowing for a geometrical projection factor proportional to $\sin\theta$, and for all photoejection angles α. Thus we obtain

$$\frac{\langle L_x^2\rangle}{\langle L_y^2\rangle} = \frac{\iiint \sin^2\theta(\cos\varphi\sin\alpha - \sin\varphi\cos\alpha)^2 \cdot \sin^2\theta\,d\theta\,d\varphi\,d\alpha}{\iiint \sin^2\alpha\cos^2\theta \cdot \sin^2\theta\,d\theta\,d\varphi\,d\alpha}$$

where the integrations over θ, φ, α are respectively over the ranges $(0,\pi)$, $(0,2\pi)$, $(0,2\pi)$. Performing these integrals we get $\langle L_x^2\rangle/\langle L_y\rangle = 3$. A more realistic estimate of this ratio may be obtained by taking into account the actual angular distribution of photoelectrons about the electric vector of incident X-rays. For the case where this distribution function is of the form $\cos^2\xi\,d\Omega$ it may be shown by a similar analysis that $\langle L_x^2\rangle/\langle L_y^2\rangle = 12/7$. This result will be assumed in the following discussion.

For a single absorption event of an X-ray propagating in a direction close to the galactic plane the increase in the grain's angular momentum in the galactic plane is by an amount

$$|\Delta L| \approx [2(E-h\nu_L)m_e]^{1/2}\,a \simeq (2Em_e)^{1/2}\,a \qquad (6)$$

where a is the r.m.s. distance from a typical point on the grain surface to the centre of mass of the grain. The component of the grain's angular momentum in the direction of propagation of X-rays therefore grows, by a random walk process, at a rate given by

$$\frac{d}{dt}\langle L_x^2\rangle \cong 2Em_e a^2 A \times \text{flux of X-ray photons of energy } E \qquad (7)$$

where A is the average target area presented by the grain. If $\varphi(E)dE$ denotes the differential energy spectrum of galactic X-rays, equation (7) may be written as

$$\left.\begin{aligned} \frac{d}{dt}\langle L_x^2\rangle &\cong 2m_e\,a^2\,A \cdot I \\ I &= \int_{0{\cdot}2\ \mathrm{keV}}^{0{\cdot}4\ \mathrm{keV}} E\varphi(E)\,dE \end{aligned}\right\} \qquad (8)$$

For the case considered above, the rates of growth of $\langle L_y^2\rangle$ and $\langle L_z^2\rangle$ are related to $\langle L_x^2\rangle$ by

$$\frac{d}{dt}\langle L_y^2\rangle = \frac{d}{dt}\langle L_z^2\rangle = \frac{7}{12}\,\frac{d}{dt}\langle L_x^2\rangle \qquad (9)$$

To assess the likely importance of the present alignment mechanism let us consider a situation where it operates independently of other alignment processes. The main disaligning mechanism is taken to be the effect of gas collisions which causes the square of any angular momentum component (in any direction) to increase at a rate

$$\frac{\mathrm{d}}{\mathrm{d}t}(L_g^2) \cong (m_H v_{th} a)^2 n_H v_{th} A \qquad (10)$$

where v_{th} denotes the r.m.s. velocity of gas atoms. The parameter Q_J (defined by Purcell[2]) which signifies the extent of angular momentum alignment in a steady-state situation may now be computed. Using the definition of $Q_J[=\frac{1}{2}(3\langle\cos^2\beta\rangle-1)]$ together with equations (8–10), we obtain

$$Q_J = \frac{\langle L_x^2\rangle - \frac{1}{2}(\langle L_y^2\rangle + \langle L_z^2\rangle)}{\langle L_x^2 + L_y^2 + L_z^2\rangle + L_g^2} = \frac{5\langle L_x^2\rangle}{26\langle L_x^2\rangle + 12L_g^2} = \frac{5I}{26I + 12\varepsilon} \qquad (11)$$

with

$$\varepsilon = \frac{(m_H v_{th})^2 n_H v_{th}}{2m_e} \qquad (12)$$

where the last equality in equation (11) is obtained by cancelling a common damping time τ. Taking $v_{th} \cong 10^5$ cm s^{-1} we obtain $\varepsilon \cong 10^{-6} n_H$ from equation (12) so that equation (11) gives

$$I \cong 10^{-6} n_H \frac{12Q_J}{5-26Q_J} \text{ erg cm}^{-2}\text{ s}^{-1} \cong 7{\cdot}5\times10^3\, n_H \frac{Q_J}{5-26Q_J} \text{ keV cm}^{-2}\text{ s}^{-1} \qquad (13)$$

The excess flux of X-rays in the galactic plane is thus related to the extent of alignment defined by Q_J and to the gas density n_H. The required value of Q_J depends on the optical asymmetry of the grains which is at present somewhat uncertain. Adopting $Q_J \cong 0{\cdot}1$ as a plausible estimate we obtain from equation (13)

$$I \sim 300\, n_H \text{ keV cm}^{-2}\text{ s}^{-1} \qquad (14)$$

With $n_H \sim 10$ cm^{-3}, in a typical interstellar cloud, the required galactic X-ray flux in the energy range 0·2–0·4 keV is thus $\sim 3\times10^3$ keV cm^{-2} s^{-1}. The galactic X-ray flux at low galactic latitudes must be in excess of the flux measured at high latitudes by this amount.

As we have already noted, Cooke *et al.*[8] have recently reported an excess X-ray flux in the energy range $E \sim 1{\cdot}4$–18 keV, of intensity comparable with that of the isotropic background, in directions close to the galactic plane. At lower energies there seems to be considerable uncertainty in the flux of diffuse X-rays. Several recent measurements of the diffuse flux at 0·25 keV differ by a factor of as much as five, with a mean value close to 450 cm^{-2} s^{-1} sr^{-1} keV^{-1} (refs. 13–17). There is evidence of an increase in flux with galactic latitude (for some galactic longitudes), suggestive of an extragalactic origin for this radiation. For our purposes we would thus require an excess flux $\sim 3{\cdot}10^3$ keV cm^{-2} s^{-1} in at least one direction close to the galactic plane so as to offset the effect of the reported extragalactic background which dominates at high galactic latitudes. Bunner *et al.*[17] have already found evidence to indicate a galactic flux at the Sun amounting to ~ 10 keV cm^{-2} s^{-1} at 0·25 keV from a region in the galactic plane close to $l^{II} \cong 155°$. The possibility cannot be excluded that a higher total flux amounting to $\gtrsim 3\times10^2$ keV cm^{-2} s^{-1} arises from other, yet unobserved directions in the galactic plane. This flux could occur either over a wide waveband or in the form of narrow emission lines.

In the absence of a total X-ray flux in the galactic plane exceeding ~ 300 keV cm^{-1} s^{-1} dust grains near the Sun would be aligned with their long axes preferentially parallel to the galactic plane. The required excess galactic flux would certainly exist in the vicinity of soft X-ray sources, however. The soft X-ray source Vela XR-2 (ref. 18) may be a prototype for such sources, and the excess galactic radiation observed by Bunner *et al.*[17] may also be attributed tentatively to unresolved sources of a similar kind. It would seem reasonable to suggest that the density of the appropriate type of X-ray sources may be sufficiently large to ensure that the required excess X-ray flux exists over a large part of the galactic disk. Such a situation would produce grain alignment in the sense required by the available polarization data while also explaining the observed heating and ionization of the interstellar gas[19,20].

The alignment mechanism discussed here differs from the usual magnetic theory in certain respects which may permit an observational distinction to be made. Magnetic alignment theories naturally predict a very close coincidence between the direction of symmetry of grain alignment (that is, the direction of maximum electric vector of starlight) and the direction of the local galactic magnetic field. In the present theory, on the other hand, a close coincidence of these directions is unlikely to be generally valid—although an approximate coincidence could arise indirectly due to a distribution of sources which is confined within the spiral arms. The relevant astronomical observations are yet too scanty to permit a conclusive distinction to be made. In a fairly localized region of the galaxy it appears that the two directions in question coincide only approximately to within $\sim 25°$ (ref. 21). A more detailed comparison between the directions of the maximum electric vector of starlight and that of the galactic magnetic field at various distances is clearly of crucial importance in deciding between the two alignment theories. It is also of interest to search for a possible relationship between the stellar polarization vectors and the distribution of X-ray sources in regions of the spiral arms which can be viewed from a transverse direction. Regions completely devoid of X-ray sources might be expected to show polarization vectors indicating the alignment of grain axes parallel, rather than perpendicular, to the direction of the local spiral arm. These effects may be distorted, however, if interstellar polarization in most cases arises from local dust clouds in the solar neighbourhood.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Galactic Scattering and Discrete Sources as Possible Origins of the Diffuse Galactic X-ray Background

Cooke, Griffiths and Pounds[1] have recently reported an excess in the diffuse X-ray background intensity observed at low galactic latitudes, compatible with a line source of radiation in the galactic plane. Wickramasinghe[2] has put forward an explanation based on small angle scattering of the universal isotropic flux of X-rays by grains in the galaxy. The purpose of this article is seriously to question this hypothesis. Briefly, Wickramasinghe first argues that the albedo of the galaxy is near unity in the X-ray band, that is, the scattering cross-section of galactic material is quite high in certain directions, while the absorption cross-section is quite low. The second point in the argument is a derivation of a first order solution to the scattered component of the diffuse X-ray background. The answer obtained is

$$I_1(\theta) \simeq I, \ \theta \gtrsim 3^\circ \qquad (1)$$

where θ is the angle of the detector axis with the galactic plane, I_1 is the intensity of once-scattered X-rays observed in the direction θ, and I is the intensity of extra-galactic diffuse X-rays. Hence the observed intensity in a direction close to the plane would be about double that observed at large θ.

Although in the light of previous work by Bowyer, Mack and Lampton[3] it seems that the scattering cross-sections used by Wickramasinghe are overly optimistic, my argument is based on geometry only. The intention of this approach is to avoid the considerable uncertainties in the present state of knowledge about interstellar grains. I suggest that, if interstellar absorption of X-rays is neglected, the proper expression for the observed diffuse intensity in any direction is

$$I_{obs}(\theta) = I_1(\theta) + I_2(\theta) \qquad (2)$$

Here I_1 and θ are as in relation (1) and $I_2(\theta)$ is the intensity of unscattered X-rays arriving from the direction θ. Following Wickramasinghe, we write

$$I_1 \simeq \gamma(1 - e^{-\tau_1 \operatorname{cosec} \theta})\ I$$

where γ is the spherical albedo of a scattering particle, and $2\tau_1$ is the optical depth for scattering of the galaxy, considered as an infinite slab with the Sun on the central plane. Because $\gamma \simeq 1$, as mentioned already,

$$I_1 \simeq (1 - e^{-\tau_1 \operatorname{cosec} \theta})\ I$$

But

$$I_2 = I e^{-\tau_1 \operatorname{cosec} \theta}$$

Therefore $I_1 + I_2 \equiv I$.

In summary, this expression states that if photons are indeed deflected toward the Sun by interstellar grains, an equal number must be deflected away. Another explanation must be sought for the observed excess of diffuse X-rays near the galactic plane.

The discoverers of the galactic excess have argued against an origin in discrete sources, on the grounds that the excess is too smoothly distributed in space. One can calculate the flux due to unresolved sources in various models of the galaxy and compare the results with observation. One model, certainly wrong on the scale of one or two kpc, but possibly realistic on a galactic scale, is a uniform source distribution model. Let us consider the galaxy as a two dimensional disk of radius $R = 15$ kpc. The density of sources is $n = N/\pi R^2$, where $N \simeq 10^3$ (ref. 4) is the total number of sources in the galaxy. A typical source luminosity has been estimated as $L = 5 \times 10^{36}$ erg s^{-1} (ref. 4) in the X-ray band 1–10 Å, which corresponds to about 6×10^{44} photons s^{-1} at 5 keV, approximately the centre of the counter sensitivity,

The intensity to be expected from such a distribution of sources is

$$I = \frac{nL}{4\pi} \ln \frac{r_2}{r_1} = \frac{NL}{4\pi^2 R^2} \ln \frac{r_2}{r_1}$$

where r_2 is the distance to the edge of the galaxy along the line of sight, and r_1 is the distance of the nearest source. In order to estimate the intensity due to unresolved sources, I shall take r_1 as the distance beyond which no galactic sources have been unambiguously located, about 4 kpc.

In the direction $l^{II} = 300^\circ$, $r_2 = 1{\cdot}1\ R = 17$ kpc. Therefore, in the X-ray band

$$I \simeq 10 \text{ photons cm}^{-2}\text{ s}^{-1}\text{ rad}^{-1}$$

This number is almost embarrassingly large, twenty times the flux that has to be explained. The reason that such a large unresolved contribution is not observed might be a lower average source luminosity than previously estimated, or a lower number of sources in the galaxy, or a very strong concentration of sources toward the galactic centre, in contradiction to the uniform model of the galaxy considered here. The fact remains, however, that an estimate of the X-ray intensity due to far-away sources, based on presently accepted ideas about sources, is actually higher than the observed effect. Surely this simple calculation indicates that the discrete source hypothesis cannot be dismissed lightly in discussions of this effect.

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Galactic Component of the Diffuse X-ray Background

WICKRAMASINGHE'S model for the galactic component of the diffuse X-ray background[1], which involves scattering of the isotropic component by dust grains, unfortunately contains a fundamental error which completely invalidates it. Particles along a line of sight may indeed scatter isotropic background radiation into the line of sight, but by the same process they will also scatter out of the line of sight the background radiation originally travelling in this direction (ref. 2, for example). If there is no absorption (that is, the albedo is unity) the two scattering effects are equal; this situation now satisfies the first and second laws of thermodynamics and ensures that the galactic disk is quite indistinguishable from the rest of the background. If the albedo is less than one, then the disk should show up in absorption, in contradiction with the observations. In no conditions can the model increase the flux from the disk relative to that of the isotropic background.

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Reply to Mack and Webster

As pointed out in my article[1] dust grains distributed throughout the disk of the galaxy will scatter X-ray photons from the isotropic cosmic background as well as from discrete sources. While I agree with the comments of Mack and Webster concerning the scattering of the isotropic background by dust I would like to point out that a diffuse galactic X-ray component similar to that observed[2] could arise from the scattering of X-rays from discrete sources. In discussing the contribution from discrete sources Mack neglects scattering by dust. It is likely that this effect is important for distances exceeding a few kiloparsecs. The analysis presented earlier is evidently valid for this case with two provisos: (*a*) discrete X-ray sources are distributed more or less uniformly (with respect to mean volume emissivity) throughout the hydrogen–dust layer of the galaxy, and (*b*) the intensity I (equations (6) and (7) of ref. 1) is defined as the ratio of the mean X-ray emissivity (due to sources) per unit volume to the X-ray extinction per unit distance, and this quantity is assumed comparable with the intensity of the isotropic background.

The data at present available on X-ray sources do not conflict with these requirements. A scattering model of the type considered could produce a fairly smooth galactic X-ray background with the observed concentration towards the galactic plane without requiring an excessive concentration of weak unresolved sources very near the galactic plane.

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Effect of Quantum Conditions in a Friedmann Cosmology

THE observed isotropy of the microwave background and its primordial interpretation has led many to investigate the nature of particle horizons in cosmological models near the initial singularity. The standard Friedmann radiation universe

$$ds^2 = dt^2 - \lambda t[dr^2 + r^2(d\theta^2 + \sin^2\theta\, d\varphi^2)],\ \lambda = \text{constant} \quad (1)$$

has a particle horizon at

$$r = 2\left(\frac{t}{\lambda}\right)^{1/2} \quad (2)$$

at epoch t. Thus near the singularity $t=0$, very limited causal communication is possible between different parts of the universe. The observed isotropy of the microwave background cannot be understood in such a model except as arising from artificially imposed initial conditions. Misner[1] has sought to remedy the situation by looking for solutions of the classical Einstein equations very different from the Robertson-Walker form (to which that given by equation 1 above belongs). These solutions permit unlimited communication near $t=0$.

The purpose of this note is to point out that near the singularity quantum effects are important and may permit unlimited communication near $t=0$, even in the Robertson-Walker form.

There have been many different approaches to a quantum theory of gravitation. Here we adopt Feynman's path integral approach[2], for it best illustrates the difference between the classical and quantum theories. The basic concepts in relation to gravitation have been discussed by Wheeler[3] and are briefly described below. Suppose the system is specified by an action S. Classically, the transition of the system from a state I to a state II is described by a unique path Γ_c given by the principle of stationary action:

$$\delta S = 0 \quad (3)$$

In quantum theory, there is no unique path Γ_c; all paths from I to II are possible. The probability amplitude for the system to adopt a given path Γ is proportional to

$$\exp\{iS/\hbar\} \quad (4)$$

where S is computed along Γ. The classical limit (3) follows when $\hbar \to 0$ or alternatively when $S \gg \hbar$. In this case only paths close to Γ_c contribute any significant amplitude.

In Einstein's theory of gravitation, the action is

$$S = \frac{1}{16\pi G}\int R\sqrt{-g}\, d^4x - \sum_a \int m_a\, da \quad (5)$$

where radiation is temporarily omitted. R is the scalar curvature, g the determinant of the metric tensor, m_a the mass of a typical particle a. The volume integral is over the entire space time and the line integral is over the world line of a. Einstein's equations follow in the classical limit (3); but in the quantum theory this is not the case. We wish to consider equation 5 in relation to a Friedmann cosmology satisfying Einstein's equations

$$R_{ik} - \tfrac{1}{2} g_{ik} R = -8\pi G\, T_{ik} \quad (6)$$

Using the Robertson-Walker form of the metric

$$ds^2 = dt^2 - Q^2(t)\left[\frac{dr^2}{1-kr^2} + r^2(d\theta^2 + \sin^2\theta\, d\varphi^2)\right], k = 0, \pm 1 \quad (7)$$

and specifying the 4-dimensional volume by $0 \leq t \leq t_0$, $0 \leq r \leq r_0$, we get

$$S = -\tfrac{1}{2} t_0\, \rho Q^3 \int_0^{r_0} \frac{r^2\, dr}{\sqrt{1-kr^2}} \quad (8)$$

In arriving at equation 8 we have assumed ρ to be the mean density of matter so that ρQ^3 is constant, and used the fact that from 6

$$R = 8\pi G\rho \quad (9)$$

Evidently, for a specified r_0, we can choose t_0 smal enough to make $S \sim \hbar$. If, for example, we fix r_0 so as to include the part of the universe that we observe at the present day (and which is known to be isotropic from the microwave background observation), $t_0 \lesssim 10^{-102}$ s! At or before this epoch, classical conditions are no longer valid, and we are no longer limited to solutions of Einstein's equations. Any space time metric can be chosen provided the magnitude of S computed for it is comparable with $\hbar$. It is possible, for example, to take the metric

$$ds^2 = dt^2 - \alpha^2 t^2\left[\frac{dr^2}{1+r^2} + r^2(d\theta^2 + \sin^2\theta\, d\varphi^2)\right] \quad (10)$$

$$\alpha = \text{constant},$$

for this gives $R = 0$. Such a universe with trace free T_{ik} would therefore have $S = 0$.

This metric has the desired property of unlimited communication near $t = 0$. A light signal emitted at $t = t_1$ from $r = 0$ reaches $r = r_0$ at time $t = t_2$, where

$$r_0 = \sinh\left\{\alpha^{-1} \ln \frac{t_2}{t_1}\right\} \quad (11)$$

Thus for any t_2, r_0 can be made arbitrarily large by taking t_1 near enough to $t = 0$. Because the restriction of Einstein's equations is removed, many more Robertson-Walker type models with unlimited communication and $S \sim \hbar$ are possible. The quantum universe could have taken any one of these possible paths.

The question now arises of whether the above argument would be seriously changed by an electromagnetic field, such as the microwave background, assumed primaeval. The action is changed by

$$\frac{1}{8\pi} \int (E^2 - H^2) d^4x \quad (12)$$

where (E, H) is the electromagnetic field. This is zero for a transverse field, so such a field has no action contribution that would affect the argument directly. Nor does the gravitational action change, for T_{ik} is traceless. It follows that a transverse field would have no effect on the argument if it were uncoupled from matter. Near $t = 0$, however, the background would have a very high temperature and a profusion of particle pairs would be created. But such particles would not be comoving with respect to the coordinate system. For pairs produced by very high energy quanta $da \sim 0$ instead of $da = dt$ assumed in arriving at equation 8. In so far as particles move at speeds close to that of light they behave essentially as quanta, in which case the situation is not changed. There would seem therefore to be a strong *prima facie* case that quantum effects are important in establishing communication near the singularity of a Friedmann cosmology.

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Is Ball Lightning a Nuclear Phenomenon?

BALL lightning is characterized by the presence of a luminous mobile globe of high energy density produced in regions of considerable electrical activity. The properties of ball lightning have been outlined in recent surveys[1-4]. We are interested here in the few but significant cases of high energy ball lightning.

In one incident a lightning ball fell into a rain barrel and boiled 18 l. of water for a few minutes[5]. The energy of the ball was estimated as 8×10^6 J with an energy density between 2×10^9 and 5×10^9 J m^{-3} (refs. 4, 6, 7).

Another report[8], brought to our attention by C. M. Botley, describes a red lightning ball about 60 cm in diameter which dug a trench 100 m long and 1·2 m deep in soft soil near a stream, and then tore away another 25 m of stream bank. If the trench was about 1 m wide, the energy expended by the ball must have been at least 3×10^6 J and very likely more than 10^7 J, with an energy density in the ball exceeding 10^8 J m^{-3}. On the other hand, if this ball had an energy density of 4×10^9 J m^{-3}, as in the rain barrel case, the total energy would have been 4×10^8 J.

There are reports of ball lightning exploding or shattering wooden logs[9], cutting metal cables and wires[10], or doing damage requiring surprisingly large amounts of power.

In both of the cases cited, and in several other cases also indicative of high energy ball lightning, an energy density significantly higher than 10^8 J m^{-3} is indicated. This means that if the energy were stored by chemical means, the ball would have to consist of air or gas which is at least singly ionized. Experimental results, however, indicate that an ionized ball of air, ozone or any of the nitrogen oxides could not remain ionized in the atmosphere for longer than a few tenths of a second without an external supply of power[11]. The decay of an ionized ball of air would most likely be explosive. According to the rain barrel report, however, the water boiled for a few minutes, and remained hot even after 20 min. We conclude that the ball lightning supplied at least 5×10^4 W of power to the water in the rain barrel for a few minutes at a relatively steady rate (in other words, not explosively). The case of the trench-digging ball also indicated a large but continuous power release.

Another difficulty with the idea that all the energy of ball lightning is stored in the form of ionized air is the absence of any indications of intense heat. Ball lightning does not rise like hot air nor is it disrupted by convection into a thermal plume as are hot fireballs. Magnetic containment of a hot plasma under normal atmospheric conditions is not possible at the required energy density[12] and in any case would be subject to convective disruption as well. A cold degenerate plasma ball[13] held together by exchange forces or collective plasma modes would not emit enough light to make the ball visible.

If the energy of ball lightning were maintained by the focusing of microwaves[14] or electric currents[11] into a small volume perhaps through standing waves[14] or converging electric fields[11], then ball lightning could not enter metallic enclosures. Nevertheless, we have several reports of ball lightning floating for several seconds down the aisles of metallic passenger aircraft[15,16], as well as into homes[10]. No luminous trails leading to the ball from the outside environment have been reported, as far as we know. Such trails should be visible or noisy if they transmit 10^4 to 10^6 W of power and should affect the geometry and luminosity of ball lightning as it moves around inside the enclosure. This is not the case[15].

An adequate theory of high energy ball lightning must explain (1) the large energy density, (2) the large but non-explosive power release, (3) the absence of convective effects, (4) the small amount of acoustic, visible, infrared, and ultraviolet radiation, (5) the reported electrical effects, and (6) the stability of ball lightning. In our opinion, none of the theories so far proposed[3,17] regarding the nature of high energy ball lightning is satisfactory if we assume the veracity of the reports.

We consider the possibility that ball lightning is a nuclear phenomenon. The most abundant isotopes in a thundercloud are ^{14}N and ^{16}O from the air, and ^{1}H (protons) from water vapour and droplets. If nuclear reactions do occur in a lightning discharge, those reactions induced by electron–nucleon collisions would not be important because of the large mass difference and small energy transfer per collision. A set of nuclear reactions for consideration in lightning is

$$^{14}N + p \rightarrow {}^{15}O + \gamma$$
$$^{16}O + p \rightarrow {}^{17}F + \gamma$$

The protons are supplied by dissociated water molecules in the lightning discharge. If a sufficient quantity of ^{15}O and ^{17}F nuclei could be produced within a relatively small volume (less than a metre in diameter) by a lightning stroke, the beta decay of these nuclei might produce an effect such as ball lightning. The beta decay reactions are

$$^{15}O \rightarrow {}^{15}N + \beta^+ + \nu$$
$$^{17}F \rightarrow {}^{17}O + \beta^+ + \nu$$

The half-lives of the ^{15}O and ^{17}F nuclei are 123 s and 66 s respectively. Electron-capture competes with positron-emission but accounts for only about 0·1 per cent of the beta-decay events[18].

By this hypothesis, to produce high energy ball lightning of 10^7 J would require a blob of air containing roughly 10^{20} nuclei of ^{15}O or ^{17}F. If such a ball had a diameter of 20 cm, one out of every 2,000 nuclei would be either ^{15}O or ^{17}F. The beta decay process produces positrons, most with energies around 0·5 MeV, which dissociate the surrounding air molecules. The visible effects are similar to those of a glow discharge. Of the roughly 10^5 W emitted by the ball, about 100 W might appear as visible radiation. When the positrons annihilate with electrons after traversing less than 2 m in air, the energy is converted into 0·5 MeV gamma rays which dissipate the energy over a radius of several hundred metres. In contact with water, the entire energy dissipates in about 40 cm, causing boiling or explosions from sudden vaporization of water.

The cohesion of the ball would result from a radial electrostatic field in a partially ionized plasma. As positrons leave the centre of the ball, a negative charge remains behind; where the positrons annihilate electrons in the air surrounding the ball, a positive charge is generated. The positron current from the centre to the periphery of the ball is about 0·1 A. (From the rain barrel report, 10^7 J was dissipated in about 100 s, which means about 10^{20} positrons in 10^2 s, or 0·1 A.) It is likely that the radial electric field will increase to a large value in the lightning ball, because free electrons readily attach to oxygen and to halogen contaminants, so that the neutralizing current is a relatively slow ion drift. If the electric field can be limited by ionic drift, the ball will die silently. If the electric field cannot be limited by ionic drift but continues to increase, an avalanche will eventually occur and create a radial arc discharge. The ball will then assume a dazzling brightness, the cohesive electric field will be shorted out and the ball will disappear with an implosive noise.

The primary theoretical difficulty with the nuclear hypothesis is the production of 10^{20} nuclei of either ^{15}O or ^{17}F in a small volume in a time scale of 10^{-3} s. When dissociated, water vapour in a thick cumulus cloud (10^{-5} g cm^{-3}) could supply protons at 7×10^{17} cm^{-3} and ^{16}O nuclei at $3{\cdot}5 \times 10^{17}$ cm^{-3}. In heavy rain the water (10^{-3} g cm^{-3}) could supply 100 times as many protons as water vapour.

A detailed calculation shows it would be possible to create sufficient numbers of ^{15}O and ^{17}F nuclei with lightning parameters, if thermal equilibrium conditions could prevail for 10^{-3} s in a sphere less than 20 cm in diameter at a mean proton energy between 0·1 and 1·0 MeV. Thermal equilibrium conditions, however, seem extremely unlikely.

Consider instead a beam of protons travelling down the lightning channel. The cross-section for the reaction of ^{14}N with a proton at 1 MeV is 2×10^{-28} cm^2. If we assume an ^{14}N background of 4×10^{19} cm^{-3}, the absorption coefficient is only 8×10^{-9} cm^{-1}. If all the available protons, between 7×10^{17} and 7×10^{19} cm^{-3}, were accelerated to 1 MeV (or $1{\cdot}4 \times 10^9$ cm s^{-1}), the reaction rate would be between 8×10^{18} and 8×10^{20} cm^{-3} s^{-1}. In 10^{-3} s there would be between 8×10^{15} and 8×10^{17} reactions cm^{-3}. A volume of between 10^4 and 10^2 cm^3 (that of a sphere between 20 and 5 cm in radius) would be required to create ball lightning. It is likely, however, that only a very small percentage of the protons is accelerated to 1 MeV because the mean free path of a proton in air is small (10^{-5} cm). In fact, even a 1 MeV proton can travel only about 1 cm through air before it becomes thermalized. Even if enough reactions can occur in a lightning flash, there is the problem of localizing and confining the radio-isotopes to a small volume during the creation process.

According to the nuclear hypothesis, an observer standing within 2 m of high energy ball lightning (10^7 J) for a few seconds might receive a radiation dosage of a few hundred rad, enough to cause radiation sickness or death. Most ball lightning, however, is probably 10 to 100 times less energetic, so that the radiation dosage would not cause biological effects.

Although the theoretical objections against a nuclear energy source for ball lightning are numerous and difficult, the nuclear hypothesis can at least be tested experimentally. The isotope abundances of air and water can be measured just after tornadoes, intense thunderstorms and (if possible) ball lightning decay. If the relative abundances of ^{15}N and ^{17}O in the sample are found to be a few tenths of a per cent higher than normal, the result would support the idea of a nuclear origin for ball lightning.

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Plate Tectonics of the Red Sea and East Africa

McKenzie *et al.*[1] have produced a strong case for considering the East African rifts and the Afar triple junction in terms of oceanic plate tectonics. I should like to present some arguments suggesting that modification to plate analysis of continental regions is required, for tensional as well as for compressional zones[2].

The Afar triple junction is unique in being a land area so that its stratigraphical, volcanological and structural evolution is exposed. McKenzie *et al.* seem to pass over what is known about this evolution[3,4], with the result, for example, that they have recourse to a "jumping" of the Red Sea spreading axis across the Danakil horst. The evidence for Quaternary crustal spreading in northern Afar, associated with rotation of the Danakil horst[3-6], indicates that two parallel lines of spreading are pushing apart at the southern end of the Red Sea. This requires uncoupling of the sialic Danakil block from the underlying spreading movements, as does the non-parallelism of the spreading axes with the opposing margins of the Red Sea basin. Furthermore, McKenzie *et al.* ignore the existence of the Aisha horst, another sialic block in Afar, against which the Gulf of Aden abruptly impinges. Both the Danakil and Aisha horsts show evidence of considerable compressional deformation.

A coast-to-coast fit across the Red Sea[1] is unrealistic when the geology of Afar is considered. The visible outcrop of sialic rocks in the vicinity of latitude 14° N limits the width of neo-oceanic crust between the Ethiopian and Yemeni plateaux to 250 km along the postulated spreading direction here, or to only 200 km according to Tazieff and Varet[7]. Although the sialic crust exposed in Afar and the bordering horsts is undoubtedly thinned by normal faulting, calculation shows that continental crust must extend for some tens of kilometres offshore under the Red Sea. In western Afar sialic rocks extend for as much as 60 km eastwards of the Ethiopian plateau escarpment in the downwarped and antithetically faulted margin zone between plateau and rift[8].

The extension of continental crust under the margins of the Red Sea requires a reappraisal of McKenzie *et al.*'s rotational pole for the Nubian and Arabian plates. Phillips *et al.*[8] have shown that in the central Red Sea (vicinity of latitude 20° N) the axial trough and associated magnetic anomalies have a zigzag pattern, which seems to fit best with a Quaternary spreading vector between N and NNE. This vector simplifies the analysis of the Sinai triple junction given by McKenzie *et al.*, the Dead Sea representing a transform fault, and the opening of the Gulf of Suez is reduced to a magnitude more consistent with the geology. The NNE spreading vector, and a 20 km extension of continental crust under the Red Sea margins, are mutually consistent if the Nubia–Arabia pole of McKenzie *et al.* is placed farther south near Benghazi, Libya.

If the validity of the Gulf of Aden (Arabia–Somalia) pole is accepted, adjustment of the Red Sea pole requires a corresponding adjustment of the East African rift pole. Also the geology of the African rift system shows that this latter pole is not placed correctly. The model of McKenzie *et al.* shows a total separation of 60 km between Nubia and Somalia at the north end of the principal Ethiopian rift. This is almost the whole width of the rift at this latitude. Although the basement rocks of the rift are obscured by thick silicic volcanics here, structural evidence suggests that at least the greater part of the rift is underlain by sialic rocks[9]. Further south, in the Lake Margherita basin, the principal Ethiopian rift is visibly floored with Pre-Cambrian gneisses[10]. The model of McKenzie *et al.* requires about 50 km of total spreading here, which is irreconcilable with the known amounts of normal faulting, even including the imposing Amaro horst.

A rotation pole for the East African rifts at 8·5 S, 31·0 E, is incongruous with the apparent unity of the African rift system as a whole, which continues well to the south of the proposed pole. If a single pole could be applied to the African rift system, then structural evidence suggests it would lie south of Beira. This is in keeping with the adjustment of the Red Sea pole discussed earlier.

But McKenzie *et al.* acknowledge, from the evidence of seismicity, that the applicability of a single pole to the rift system is doubtful. This is confirmed by the occurrence of structural triple junctions within the system (for example, in southern Tanzania and central Kenya), of rift bifurcations (for example, at Lake Rudolf), and rift and fracture zones within supposedly rigid plates (for example, at Lake Tana and at Jebel Dumbeir in the Nubian plate[11]). In terms of plate tectonics, at least four small plates must be considered in eastern Africa, all moving apart or transforming with respect to one another. This reduces the value of seismic slip vectors from eastern Africa in analysing the Afar triple junction.

The evaluation of plate boundaries in the African rift system is complicated by the lack of a ridge-transform fracture pattern, from Afar southwards, and the curvilinear plan of the Western Rift, like that of the Baikal rift, seems inexplicable in terms of oceanic tectonics. Gilluly notes[12] precisely the same difficulties in reconciling the Basin and Range tectonics of the western United States with a presumed sub-crustal extension of the East Pacific Rise. As Gilluly remarks, if this presumption is true, then uncoupling must occur, a mechanism also proposed for the Rhine graben[13] in relation to sub-crustal processes.

Does plate theory tend to break down at very small rates of relative motion? Unfortunately, the African rift system is ill suited to answering this question, because the complicating effect of the presence of continental crust over a spreading zone is not yet evaluated. It is striking that very similar rift valleys occur in other continents (for example, the Baikal and Rhine rifts), which are apparently unconnected with sea floor spreading processes. The manner in which some continental rifts peter out laterally (for example, the Kavirondo and Tana rifts) suggests that an analysis of the mechanical properties of continental crust above a spreading layer is required. It is clear that the total vertical and horizontal displacements in continental rift valleys are of a comparable order[14].

Finally, if McKenzie *et al.*'s analysis of the Afar triple junction is correct, the Arabian, Nubian and Somalian plates should fit back against the spreading axes. A triple junction of three spreading axes is theoretically stable[15], but the gross asymmetry of, for example, the spreading axis in the western Gulf of Aden indicates (i) an asymmetric axial spreading[16], which raises problems of the manner of dyke injection, or (ii) lithospheric uncoupling such that the spreading axis has a component of motion independent of the continental margins. Whichever suggestion might be correct, oceanic-type plate tectonics is evidently subject to modification in the Afar region.

The importance of realizing this is underlined where McKenzie *et al.* consider the 1969 Sardo seismicity, and its sinistral shear along NW fracture lines[17], as being related to movement along a major plate boundary. Not only

is this difficult to reconcile with the regional structure of Afar[3], but sinistral shear has affected much of eastern Afar during the Quaternary, across a zone more than 100 km wide. Furthermore, in spite of the evidence of ground deformation and focal-mechanism analysis for a NW–SE fault plane[1,17], the Sardo aftershocks are distributed along an east–west line[18], a structural direction which is not expressed by surface faulting in Afar[7].

Elsewhere[19] I reconcile the bifurcation of spreading axes at the south end of the Red Sea with the tectonics of Afar, in particular with NW–SE sinistral shear in eastern Afar. It turns out that the Danakil and Aisha horsts have played a determining part in the evolution of Afar, and are far from being passive sialic fragments of accidental import. If my model is correct, the regional tectonism of Afar underwent a drastic change in the late Pliocene–early Pleistocene such as would not be inferred from previous studies[1,15].

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Mechanisms for the Oxidation of Fe_3O_4

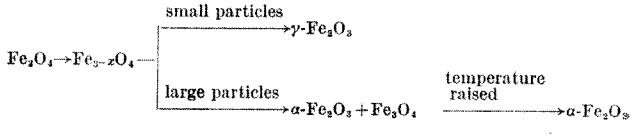
We have published results[1] of a study of the kinetics of the oxidation of a sample of Fe_3O_4 consisting of submicron crystals almost perfectly cubic and almost uniform in size[2]. In agreement with Egger and W. F.[3] and W. F.[4,5], it was found that the reaction was single phase topotactic ($Fe_3O_4 \rightarrow Fe_{3-x}O_4 \rightarrow \gamma\text{-}Fe_2O_3$). The mechanism proposed was based on the diffusion of iron cations, the coefficient of which was found to depend on the Fe^{2+}/total iron ratio. When a model of this mechanism was simulated on a computer, an accurate prediction was obtained of the kinetic behaviour over all composition ranges.

The model also predicted that during oxidation a high concentration gradient of cation holes would develop in the monocrystalline particles, leading to a gradient in the lattice constant. Thus lattice strain would develop, and we found that the broadening of the reflexions in quenched samples increased to a maximum at about 16 per cent Fe^{2+} and then diminished and returned to the original value when the reaction was complete. The effect on large particles must be greater than on small particles. Lattice strain was assumed in earlier work to be the cause of nucleation of $\alpha\text{-}Fe_2O_3$ in large particles to explain the role of particle size in controlling the path taken by the oxidation; in other words, why the end product is $\gamma\text{-}Fe_2O_3$ for small particles, $\alpha\text{-}Fe_2O_3$ for large particles[3–5].

Colombo *et al.*[6] have criticized these views and suggested that $\alpha\text{-}Fe_2O_3$ forms only when nuclei of $\alpha\text{-}Fe_2O_3$ or stacking faults are present in the Fe_3O_4. They maintained that their results indicate clearly the incorrectness of the interpretation of the part played by grain size in the mechanism proposed by K. J. G. *et al.* We wish to point out that this claim is based on a misunderstanding; grain size as such played no part at all in our mechanism for cation transport in the reaction $Fe_3O_4 \rightarrow \gamma\text{-}Fe_2O_3$, and thus Colombo's results are irrelevant to any discussion of the validity of this mechanism.

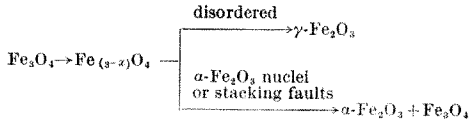
On the question of oxidation to $\alpha\text{-}Fe_2O_3$ their results disagree with evidence previously presented[3–5], which established that particle size was chiefly responsible for determining whether $\alpha\text{-}Fe_2O_3$ would appear in the end product. Our view may be summarized as follows. In all preparations of Fe_3O_4 the first step of the oxidation of Fe_3O_4 is a single phase topotactic reaction leading to the intermediate solid solution phase, $Fe_{3-x}O_4$, which is a solid solution phase. If the particles are small (less than about 3000 Å), the reaction rate is great even at low temperatures and the oxidation comes to an end in a reasonable time. With increasing size, the temperature has to be raised to complete the reaction. As $\gamma\text{-}Fe_2O_3$ undergoes a phase transition to $\alpha\text{-}Fe_2O_3$ at higher temperatures[8] a point is reached when nuclei of $\alpha\text{-}Fe_2O_3$ appear spontaneously in the surface layer of $\gamma\text{-}Fe_2O_3$. The underlying intermediate solid solution phase then disproportionates to $\alpha\text{-}Fe_2O_3$ and Fe_3O_4, the former building up a layer at the surface, hindering further oxidation.

We formulate the reaction path



The first step in the reaction path of Colombo *et al.* is the same as ours. But they maintain that during the second stage only disordered samples oxidize readily to $\gamma\text{-}Fe_2O_3$ and that $\alpha\text{-}Fe_2O_3$ is formed only when $\alpha\text{-}Fe_2O_3$ nuclei or stacking faults are present.

Their reaction path can be formulated



We would like to make the following comments on their arguments. The sensitivity of X-ray diffraction for the detection of traces of $\alpha\text{-}Fe_2O_3$ is given by Colombo *et al.* as "$\pm$0·05 per cent" (they refer to accuracy; we assume they mean sensitivity which is more relevant to the detection of one phase in the presence of another); this is much lower than anything we and other authors have found, using the same technique and time of exposure. The sensitivity with which traces of a second phase in another can be detected depends much on the crystal size of the former. To be detected the trace component would have to be considerably larger than 300 Å. For a sample of Fe_3O_4 with a size of 3000 Åa content of 0·05 per cent $\alpha\text{-}Fe_2O_3$ would mean that on the average there would be just one $\alpha\text{-}Fe_2O_3$ particle in one Fe_3O_4 crystal. We do not therefore understand how the autocatalytic growth of $\alpha\text{-}Fe_2O_3$ nuclei could lead to a continuous layer of $\alpha\text{-}Fe_2O_3$

on the surface and prevent further oxidation inside the particle.

Contrary to Colombo *et al.*[6], samples of Fe_3O_4 prepared by the wet oxidation method never showed traces of α-Fe_2O_3 in X-ray diagrams. To test whether samples prepared by this method[7], but in conditions which produce large particles ($\sim 0{\cdot}5$ μm) and thus oxidize to α-Fe_2O_3, might contain initial traces of α-Fe_2O_3 which had escaped detection, we have carried out a few simple experiments (after the appearance of the report by Colombo *et al.*). We did this by heating a sample for 60 h in a CO/CO_2 (1 : 9) mixture at 550° C. The oxidation behaviour was similar to that of the untreated sample, that is, disproportionation of the intermediate phase at 320° C and the formation of α-Fe_2O_3 without any appearance of γ-Fe_2O_3.

Furthermore, the isothermal oxidation of two samples of Fe_3O_4 prepared by reduction of α-Fe_2O_3 in an H_2/H_2O mixture[3] (particle size $\sim 0{\cdot}6$ μm), one with traces of α-Fe_2O_3 and one without, yields in both cases, after oxidation at 220° C for 4 h, about the same small amount of α-Fe_2O_3 in addition to $Fe_{3-x}O_4$. When these two samples were then treated in the same CO/CO_2 mixture at 550° C for 60 h they also produced small amounts of α-Fe_2O_3 and $Fe_{3-x}O_4$ after oxidation, also at 220° C for 4 h.

These new results confirm that spontaneous nucleation of α-Fe_2O_3 occurs and that it is not necessary for nuclei to be present in the original sample for the formation of α-Fe_2O_3. During isothermal oxidation, nucleation of α-Fe_2O_3 can occur at a much lower temperature in large particles. For example, nucleation was observed in a particle size of $\sim 0{\cdot}5$ μm at 180° C after about 96 h. We are convinced that, if Colombo *et al.*[9] had oxidized their sample described as "stoichiometric Fe_3O_4 particle size 0·4" of their Fig. 2, somewhat more than 4 h at 220° C, nucleation of α-Fe_2O_3 would have taken place.

Colombo *et al.* mention that stacking faults in the Fe_3O_4 preparations produced chiefly by ball milling yield nuclei of α-Fe_2O_3. We do not find in any of the articles of Colombo *et al.* mentioned in the letter to *Nature*[6], nor in an earlier communication[9], a clear proof by, for example, electron diffraction, that any of their preparations of Fe_3O_4 contained stacking faults.

Colombo *et al.*[6] conclude from their Fig. 1 "that oxidation of magnetite to yield solid solution does not lead to any lattice strain". We have observed, however, that during the oxidation leading to the solid solution phase the shift of the Fe_3O_4 lines is accompanied by a small broadening in quenched samples. For samples with small particles which yield γ-Fe_2O_3 as the end-product the width of the reflexion reaches its maximum at about half-way through the oxidation, then diminishes, and in the γ-Fe_2O_3 the lines become as sharp as those of the initial Fe_3O_4, again in quenched samples. As pointed out in the previous article[1], the line broadening disappears when samples are annealed in the absence of oxygen. Colombo *et al.*[6] do not state whether their samples were quenched during the reaction. Quenching is essential for preserving the concentration profile for cation vacancies which develops during the reaction. As the crystal size does not change, line broadening in samples quenched after oxidation can be caused only by the lattice parameter varying with the concentration gradient; this means that strain must be present. It is irrelevant which method is used to calculate the degree of strain from the shape and width of the reflexions. The method we used was due to Kochendörfer, which Colombo *et al.* allege to be inaccurate.

We agree with Colombo *et al.* that considerable strain develops when α-Fe_2O_3 forms during isothermal oxidation. This is to be expected in any situation where a new phase the lattice of which is different from the host lattice nucleates *in situ*. This strain, however, does not require quenching to be observed.

Colombo *et al.* write that disordered samples oxidize readily to γ-Fe_2O_3. No details are given[6,10] what kind of disorder is meant; in other words, whether it is irregular lattice disorder[11], faulting on certain *hkl* planes, dislocations or clusters of dislocations[12]. No direct evidence is given for such disorder.

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BIOLOGICAL SCIENCES

Thermodynamics of the Reversible Oxygenation of *Bis*(Acetylacetone)-Ethylenediiminecobalt (II) in Pyridine

The reversible reaction of certain cobalt chelates with molecular oxygen can be considered as a simple model of the reversible oxygen binding by haemoglobin and myoglobin[1,2]. It was recently pointed out[3–6] that in

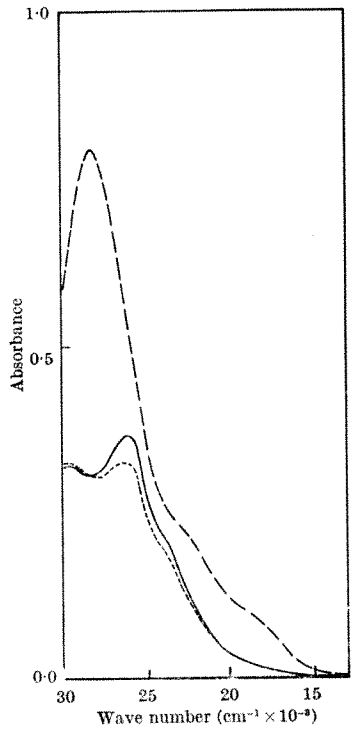


Fig. 1. Visible spectra of: Co^{II}BAE in $6{\cdot}7 \times 10^{-4}$ M pyridine (———); the same solution saturated with oxygen (– – –); after 20 min bubbling nitrogen (- - -).

suitable experimental conditions synthetic oxygen carriers show a reaction stoichiometry corresponding to one oxygen molecule per metal atom. In both cases the six-coordinated complex with oxygen can be formed by an equatorial tetradentate chelating agent and a donor ligand in the axial position *trans* to oxygen[4]. The reversibility of oxygenation seems to depend on a non-polar environment of the metal provided by the aprotic solvents in the simple complexes and by the hydrophobic pocket accommodating the haem in haemoglobin[7].

In spite of numerous descriptions of cobalt complexes in recent years[1], there has been no detailed analysis of the equilibrium and kinetic aspects of the reaction with oxygen. A preliminary survey of several oxygen binding cobalt(II) complexes indicated that *bis*(acetylacetone)-ethylenediiminecobalt(II) (Co^{II}BAE) was the most suitable for a study of the thermodynamics of oxygen binding, the results of which we report here.

The solid complex was prepared according to Morgan and Smith[8] and stored under nitrogen. The oxygen equilibrium curves were determined in pure pyridine by a spectrophotometric method[9], and in all the experimental conditions we tested the complex was stable as judged by the constancy of spectra after equilibrium was reached (Fig. 1). In all the intermediate saturation states, the spectral properties of the solution could be accounted for in terms of two absorption species, one corresponding to the fully deoxygenated, the other to the fully oxygenated, compound.

Fig. 2*A* shows the oxygen equilibrium curves of Co^{II}BAE at different temperatures. In all cases the shape of the curve corresponds to a rectangular hyperbola, as expected for a reversible equilibrium of the simple form

$$Co^{II} + O_2 \rightleftarrows Co^{II}O_2$$

This is brought out more clearly in the double logarithmic plot (Fig. 2*B*). The data correspond to a value of $n = 1$ in the Hill equation, commonly used in work with haem proteins:

$$\frac{Y}{1-Y} = \frac{[Co^{II}O_2]}{[Co^{II}]} = K\ pO_2^n$$

Thus the reversible reaction of Co^{II}BAE with oxygen differs from that reported for other Co^{II} complexes, where several distinct equilibria had to be formulated to account for the shape of the equilibrium curves and stoichiometry[10,11]. At 20° C the value of $p_{1/2}$ (the oxygen pressure for half saturation) is 2·75 mm Hg, corresponding to $K = 6{\cdot}6 \times 10^4\ M^{-1}$ (taking the solubility of oxygen in pyridine as $8{\cdot}6 \times 10^{-4}$ M at 20° C and 156 mm Hg).

The dependence of the oxygen affinity on temperature (see Fig. 3) corresponds—in the temperature range investigated—to the Van 't Hoff relationship with $\Delta H = -15{\cdot}03 \pm 1{\cdot}35$ kcalories/mole of oxygen (gas) at 20° C. From these values the thermodynamic data at 20° C for the reaction of Co^{II}BAE with oxygen are as follows:

$\Delta G = -6{\cdot}45$ kcalories/mole; $\Delta H = -15{\cdot}03$ kcalories/mole; $\Delta S = -29$ e.u.

The oxygen affinity of Co^{II}BAE in pyridine is much greater than that reported in the presence of N,N-dimethylformamide, confirming the suggestion that the tendency to form the oxygen adduct increases with increase in base strength[1]. Our preliminary data indicate that complexes like *bis*(salicylaldehyde)ethylenediimine-cobalt(II) and *bis*(salicylaldehyde)phenylenediiminecobalt (II) have an oxygen affinity in pyridine lower by about two orders of magnitude than Co^{II}BAE.

As far as the comparison can be made, it is interesting to note a parallel between the data for the reaction of Co^{II}BAE with oxygen and those for the same reactino of whale myoglobin. For the latter, in dilute buffer solutions, at *p*H 7 and 20° C, the values of ΔG, ΔH and ΔS are $-8{\cdot}08$ kcalories/mole, $-14{\cdot}8$ kcalories/mole and -23 e.u., respectively (unpublished results of G. A., E. A., M. B. and E. Magnusson).

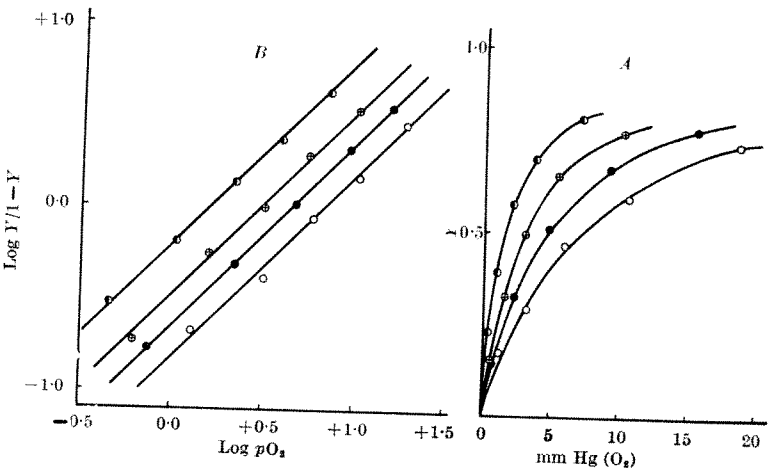


Fig. 2. Oxygen dissociation curves of 2×10^{-3} M Co^{II}BAE in pyridine at different temperatures. *Y* is the fractional saturation with oxygen. ◐, 15° C; ⊗, 20·7° C; ●, 26° C; ○, 31·5° C.

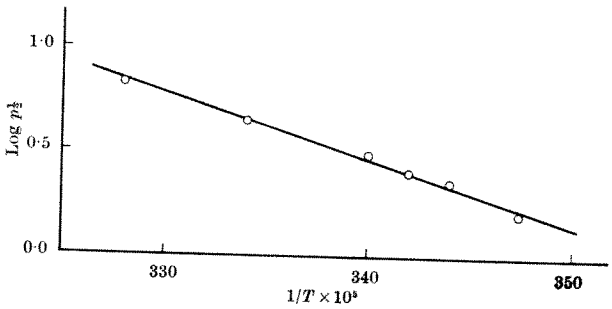


Fig. 3. Influence of temperature on the oxygen affinity (as log $p_{1/2}$) of 2×10^{-3} M Co^{II}BAE in pyridine.

Thus the known similarities between respiratory proteins and cobalt(II) complexes can be extended to the thermodynamic parameters of the oxygen reaction. A systematic study of the effect of the chelating agent, the *trans* axial ligand and the solvent on the reaction of Co^{II} complexes with oxygen might offer insight into the mechanisms by which the polypeptide chains of haem proteins control the reactivity of the haem iron.

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Three Dimensional Fourier Synthesis of Horse Deoxyhaemoglobin at 2·8 Å Resolution

THE deoxyhaemoglobin of horse forms orthorhombic crystals with the space group $C222_1$ and four molecules in the unit cell, which lie on two-fold symmetry axes. A Fourier synthesis at 5·5 Å resolution showed the tertiary structures of the individual subunits to be closely similar to that of oxyhaemoglobin, but the quaternary structure of the tetramer to be markedly different[1,2].

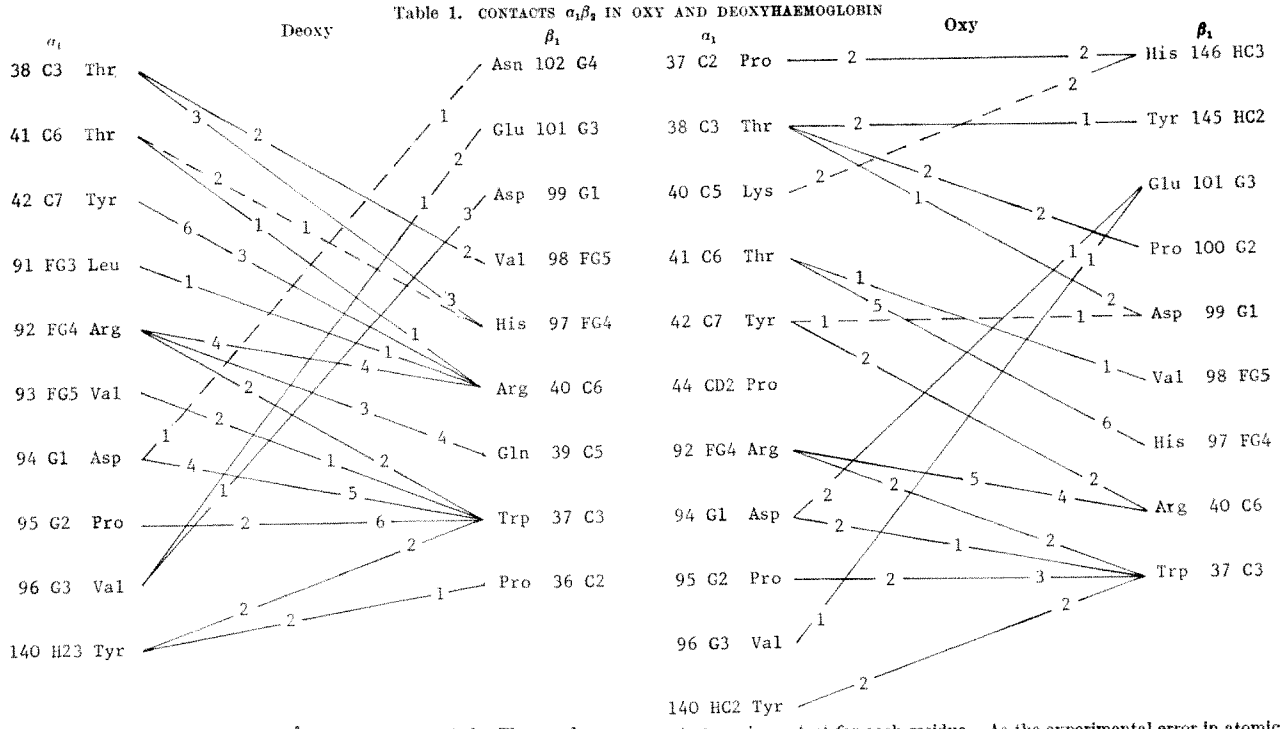
X-ray reflexions were recorded on precession photographs and measured on a flying spot integrating microdensitometer[3] for the native protein and four heavy atom derivatives. Heavy atom positions were first determined from centrosymmetric projections and refined in three dimensions after phase angles had been determined separately for each plane of data as described by Matthews *et al.*[4]. The mean figure of merit was 0·63. An electron density distribution was calculated using "best" angles and plotted on the scale of 1 Å = 2 cm. An atomic model of the molecule was constructed on the same scale using Richards's reflecting box[5]. Detailed comparisons of the atomic models of horse oxy- and deoxyhaemoglobin will take some time to complete. Here we wish to describe some significant differences between the two structures.

In methaemoglobin the iron atoms are displaced by 0·3 Å from the mean plane of the porphyrin ring on the side of the proximal histidine (F8). In both the α and β chains of deoxyhaemoglobin this displacement is increased to 0·75 Å. The porphyrin ring probably deviates from planarity, as it does in other iron porphyrin derivatives, but the extent of such deviation is hard to judge at 2·8 Å resolution. In the α and β subunits of methaemoglobin the cavity between the haem group and helix E is sufficiently wide to accommodate oxygen, while in the deoxy form this is true only of the α chain. In the β chain one of the γ-methyl groups of valine E11 blocks the ligand site. There are many small differences between the tertiary structures of the two subunits in the oxy and deoxy forms which will be described elsewhere.

We now come to the contacts between neighbouring subunits. The β chains are not in contact with each other in either the oxy or deoxy forms, but the gap between them is much wider in the deoxy form (see Fig. 11 of ref. 6). There is no contact between the α chains in the oxy form, but in the deoxy form they are linked by salt bridges. The α carboxyl group of arginine HC3(141) of one α chain is in contact with the α amino group of valine 1 and the ε amino group of Lys H10(127) of its partner chain. The C-terminal guanidinium group of one α chain forms a salt bridge with Asp H9(126) of its partner chain.

The contact $\alpha_1\beta_1$ undergoes slight changes which seem to involve a loosening, rather than sliding, at certain areas of contact. A table of the residues in contact looks very similar to that given for methaemoglobin (Table 2 of ref. 7) except for the omission of twelve lines and the addition of two new ones. There seem to be 32 residues or 98 atoms in contact in deoxy, as compared with 34 and 110 in the oxy form. The number of hydrogen bonds has remained constant at four or five. The contact $\alpha_1\beta_2$ undergoes drastic changes as the two subunits turn relative to each other by 13° (Table 1). Note that the hydrogen bond linking Asp G1(94)α_1 to Asn G4(102)β_2 in oxy is replaced in deoxy by another linking Tyr C7(42)α_1 to Asp G1(99)β_2. There are 20 residues or 69

Table 1. CONTACTS $\alpha_1\beta_1$ IN OXY AND DEOXYHAEMOGLOBIN



All contacts at distances of 4 Å or less were counted. The numbers represent atoms in contact for each residue. As the experimental error in atomic positions is at least 0·5 Å, these figures are only approximate.

atoms in contact in deoxy, as compared with 19 and 80 in oxy. If we omit residues involved in salt bridges, the number of interatomic contacts between α and β subunits seems to be less in deoxy than in oxyhaemoglobin. This point will have to be checked by further refinement of the two structures.

Human and horse haemoglobin differ chemically by 43 out of 287 residues in the half-molecule. Many of these differences show up clearly in comparisons of the electron density maps of human[8] and horse haemoglobin, but they are without influence on the tertiary or quaternary structure, which seem to be identical within the limits of our resolution. The two proteins crystallize in two completely different lattices dictated probably by the different patterns of side chains on the surface of the two species. The identity of the internal structures shows that these are not affected by the nature of the crystal lattice.

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Higher Plant Cytochrome *c*

Zuckerkandl and Pauling[1] have pointed out the potential value of proteins as documents of evolutionary history for the species from which they have been extracted, and various aspects of the structure of proteins have been used for this purpose. These include serological cross-reactivity, amino-acid composition, peptide maps, electrophoretic mobility, catalytic activity and chromatographic characteristics[2]. It is generally agreed, however, that the greatest insights in this area are liable to come from comparisons of the primary structure of proteins, that is by partial or complete amino-acid sequence determination, for only these comparisons are likely to indicate accurately enough the mutation distance between the homologous genes involved.

There are two principal methods for the mathematical treatment of sequence data from a large number of species[3,4]. The matrix method (for example ref. 5) is used to construct a tree so that the reconstructed matrix derived from it is the closest approximation to the original matrix obtained from the observed sequences. Alternatively, it is possible to construct trees using inferred ancestral sequences[1,4], and because the structures of all sequences are used in the derivation of the nodes, this method is preferable; in the common ancestor method the derived tree should involve the smallest possible total number of amino-acid replacements.

In the animal kingdom classical comparative methods and a reasonable fossil record allow considerable confidence in the suggested relationships of many groups; for example, a generally acceptable phylogenetic tree for the vertebrates has been established from the fossil record[6]. Plants, however, do not have an adequate fossil record from which a tree could be constructed. By the Upper Cretaceous, when flowering plant fossils became abundant, many of the major present-day orders were already represented. In the absence of a fossil record indirect methods have been used to construct phylogenies, and as a consequence there are several conflicting schemes[7]. The use of more objective data from plants could therefore be extremely important in this connexion, and we hope to establish a phylogenetic tree based on amino-acid sequence data.

For the initial study we chose cytochrome *c* from the many proteins which are widespread in plants for several reasons. (1) It is ubiquitous in aerobic organisms; (2) its relatively small molecular weight makes it very suitable for amino-acid sequence determination, for although present techniques can be applied to proteins of molecular weight up to 40,000 other things being equal, progress is more rapid the smaller the molecule; (3) the sequence of cytochrome *c* has already been established from various animals, bacteria, fungi and for wheat germ[8]; (4) although the synthesis of cytochrome *c* is directed by only a small part of the genome, the fact that the phylogenetic tree constructed from animal cytochrome *c* sequence data is essentially similar to that constructed from the fossil record[5] means that the cytochrome *c* information is representative of the whole genome. It is likely, however, that before a complete phylogenetic tree can be constructed, proteins other than cytochrome *c* will have to be used, particularly in determining the final delimitations of the branches, because compared with other proteins cytochrome *c* appears to be relatively conservative; at present it is not clear what will be the most suitable choice of more rapidly evolving proteins in plants.

We wish to present here the sequences of cytochrome *c* from mung bean (*Phaseolus aureus* L.), sunflower (*Helianthus annuus* L.), sesame (*Sesamum indicum* L.) and castor (*Ricinus communis* L.), and to make a preliminary comparison of these four dicotyledonous plant sequences with that of the monocotyledon wheat (*Triticum aestivum*)[9], the only higher plant sequence determined at the start of this investigation.

The methods used to purify and sequence the cytochromes *c* have been described before[10–12]. Paper electrophoresis combined with the Dansyl/Edman sequence

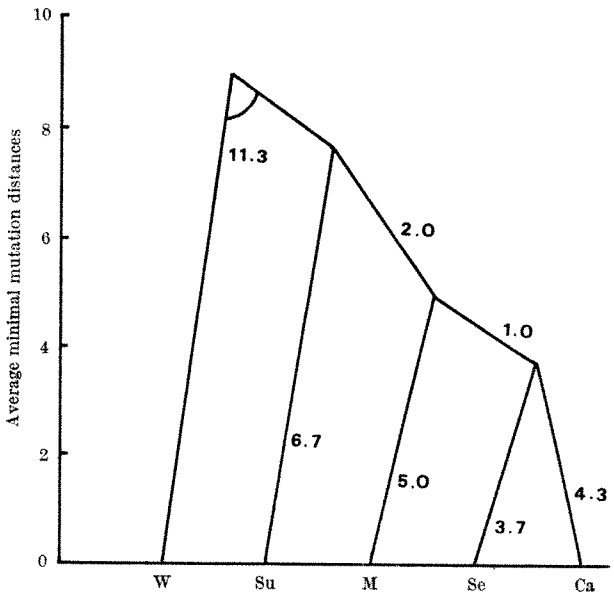


Fig. 1. Topology based on the minimal mutation distance method.

Table 1. AMINO-ACID SEQUENCES OF HIGHER PLANT CYTOCHROME *c*

	(1)									(10)									(20)									(30)									(40)
Wheat germ[9]:	Acetyl	A	S	F	S	E	A	P	P	G	N	P	D	A	G	A	K	I	F	K	T	K	C	A	Q	C	H	T	V	D	A	G	A	G	H	K	Q	G	P	N	L
Mung bean[11,16]:	Acetyl	A	S	F	N	E	A	P	P	G	D	S	K	S	G	E	K	I	F	K	T	K	C	A	Q	C	H	T	V	D	K	G	A	G	H	K	Q	G	P	N	L
Sunflower[12]:	Acetyl	A	S	F	A	E	A	P	A	G	D	P	T	T	G	A	K	I	F	K	T	K	C	A	Q	C	H	T	V	E	K	G	A	G	H	K	Q	G	P	N	L
Sesame[16]:	Acetyl	A	S	F	S	E	A	P	P	G	D	V	K	S	G	E	K	I	F	K	T	K	C	A	Q	C	H	T	V	D	K	G	A	G	H	K	Q	G	P	N	L
Castor[16]:	Acetyl	A	S	F	N	E	A	P	P	G	D	V	K	A	G	E	K	I	F	K	T	K	C	A	Q	C	H	T	V	E	K	G	A	G	H	K	Q	B	P	N	L

	(50)									(60)									(70)									(80)									(90)
H	G	L	F	G	R	Q	S	G	T	T	A	G	Y	S	Y	S	A	A	N	K	N	K	A	V	E	W	E	E	N	T	L	Y	D	Y	L	L	N	P	X	K	Y	I	P	G	T	K	M	V	F
N	G	L	F	G	R	Q	S	G	T	T	A	G	Y	S	Y	S	T	A	N	K	N	M	A	V	I	W	E	E	K	T	L	Y	D	Y	L	L	N	P	X	K	Y	I	P	G	T	K	M	V	F
N	G	L	F	G	R	Q	S	G	T	T	A	G	Y	S	Y	S	A	A	N	K	N	M	A	V	I	W	E	E	N	T	L	Y	D	Y	L	L	N	P	X	K	Y	I	P	G	T	K	M	V	F
N	G	L	F	G	R	Q	S	G	T	T	P	G	Y	S	Y	S	A	A	N	K	N	M	A	V	I	W	G	E	N	T	L	Y	D	Y	L	L	N	P	X	K	Y	I	P	G	T	K	M	V	F
N	G	L	F	G	R	Q	S	G	T	T	A	G	Y	S	Y	S	A	A	N	K	N	M	A	V	Q	W	G	E	N	T	L	Y	D	Y	L	L	N	P	X	K	Y	I	P	G	T	K	M	V	F

	(100)									(110)								
P	G	L	X	K	P	Q	D	R	A	D	L	I	A	Y	L	K	V	A	T	S	S
P	G	L	X	K	P	Q	D	R	A	D	L	I	A	Y	L	K	E	S	T	A	
P	G	L	X	K	P	Q	E	R	A	D	L	I	A	Y	L	K	T	S	T	A	
P	G	L	X	K	P	Q	E	R	A	D	L	I	A	Y	L	K	E	A	T	A	
P	G	L	X	K	P	Q	D	R	A	D	L	I	A	Y	L	K	Q	A	T	A	

The single letter code used is that given in the tentative rules of the IUPAC–IUB Commission on Biochemical Nomenclature. X represents ε-N-trimethyllysine.

technique[13] makes it possible to obtain a complete sequence on 2 μmoles of pure cytochrome *c* and, because cytochrome *c* occurs in only small amounts in plants, the use of these methods has considerably extended the range of possible plant sources.

The amino-acid sequences of cytochrome *c* from the five higher plants are given in Table 1, and Table 2 gives the number of differences in amino-acid residues, from which the matrix of minimum mutation distance differences, as defined by Fitch and Margoliash[5], is derived; these data have been used to construct a topology based on the minimal mutation distance method (Fig. 1). This topology places castor (family Euphorbiaceae) closest to sesame (Pedaliaceae), then mung bean (Leguminosae), then sunflower (Compositae) and then wheat (Gramineae).

Table 3 gives the minimal mutational distances interrelating pairs of cytochrome *c* sequences as calculated from the leg lengths of the data of Fig. 1, together with the input data from the sequences themselves. These are in good agreement as shown by the percentage standard deviation of the calculated to input data[5] of 8.

Alternatively the data can be processed using the common ancestor method of Dayhoff and Eck[4]. In this case, where five species are being considered there are five possible ways of constructing a topology in which the nodes have three branches. Four topologies (Fig. 2*a*) are chosen from these fifteen because they involve the smallest total number of mutations, that is thirty-three.

Table 2. TABLE OF DIFFERENCES

	W	Su	M	Se	Ca
W	×	15	16	15	14
Su	15	×	11	11	11
M	16	11	×	8	9
Se	15	11	8	×	7
Ca	14	11	9	7	×

Amino-acid residues.
W, Wheat; M, mung bean; Su, sunflower; Se, sesame; Ca, castor.

Table 3. MINIMUM MUTATIONAL DIFFERENCES TO INTERRELATE PAIRS OF CYTOCHROME *c*

	W	Su	M	Se	Ca
W		18	18	18	19
Su	18		14	13	14
M	19	13		10	10
Se	19	13	9		8
Ca	17	15	11	8	

Figures at the top are calculated from leg lengths (values rounded off). Figures at the bottom are input data.

After the best closed topologies have been established with the data from the five flowering plants, the next question is what is the relationship of these sequences to those of the fungi and animals already established? The flowering plant cytochromes *c*, like those of the fungi and animals, are basic and of the eukaryotic, mitochondrial type. They have an N-acetylated tail of 8 residues, giving a total length of 111–112 residues, which is not found in any other group. They also have Σ-N-trimethyllysine residues in positions 80 and 94; this unusual amino-acid does not occur in animal cytochromes, but does occur in position 80 in the cytochrome *c* of the fungus, *Neurospora crassa*[14]. It has been found in only two other proteins[15]. There are ninety-one residues common to all the five flowering plant sequences, of which eighteen are unique in their positions compared with all other cytochromes *c*; they can therefore be accepted as a closely knit group.

In considering all the known eukaryotic cytochrome *c* sequences, there are thirty-five residues which are un-

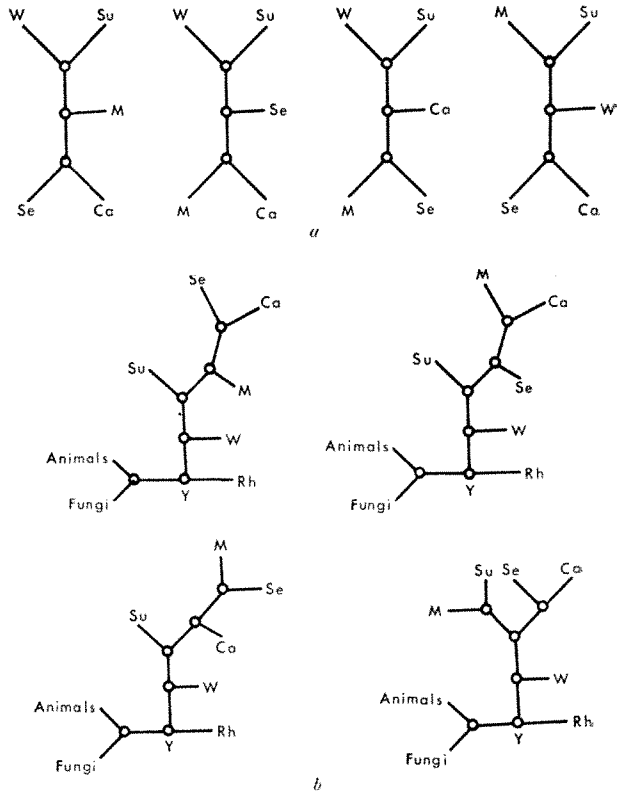


Fig. 2. Various possible topoolgies obtained using the common ancestor method of Dayhoff and Eck[4]. See text. Rh, Rhodospirillum.

varied, and the new flowering plant data do not reduce this number. It is quite clear therefore that at an early stage in evolution there was an ancestor common to plants, fungi and animals. With regard to the point at which the plants diverged as a distinct group, this could have taken place between *Rhodospirillum* and the point of divergence of the fungi, or between the latter and the point of divergence of the animals[8]. Analysis of the data in relationship to the ancestral nodes of the fungi and insects shows that the flowering plants diverged before the fungi.

In joining the flowering plant sequences onto the main phylogenetic tree between *Rhodospirillum* and the point of divergence of the fungi, the calculation of a common ancestral sequence is subject to error because of the considerable divergence between the adjoining groups and their corresponding cytochrome *c* sequences. A sequence of this node (node Y), however, has been determined (Fig. 3), which suggests the four different ways involving the minimum of mutations in which the five flowering plant sequences could be attached to the main branch of a phylogenetic tree (Fig. 2*b*), taking into account the established closed topologies of Fig. 2*a* and based strictly on amino-acid sequence data. From the position of wheat and from biological considerations, it seems possible that the first flowering plants were primitive dicotyledons from which the monocotyledons diverged before the four dicotyledons which we have examined. All statements based on this preliminary tree are of course liable to amendment as the number of species from which data are obtained and from which trees are constructed increases. For this reason it is not justifiable to discuss differences between the data in Fig. 2 and those of published phylogenetic schemes for flowering plants, for these differences might represent sampling error due to the small number of differences in amino-acids compared.

```
(1)                                  (10)                                  (20)
— — — — — — — — — G  D — — A  G — K — F  K  T  K  C

A  Q  C  H  T  V — — (30) G  G — H  K  V  G  P  N (40) L  H  G  L  F

G  R — — G — — — — G  Y  S  Y  S — A (60) N  K — K  G — T

W — E (70) N  T  L — — Y  L — N  P (80) K  K  Y  I  P  G  T  K  M

(90)
— F — G  L — K — — — R — (100) D  L  I  A  Y  L  K — A (110) T —
```

Fig. 3. Node *Y*.

We are at present determining the sequences of *Zea mays* L. (Gramineae), *Arum maculatum* L. (Araceae), *Fagopyrum esculentum* Moench (Polygonaceae), *Enteromorpha intestinalis* L. Link (Chlorophyceae), *Ginkgo biloba* L. (Ginkgoaceae), *Cucurbita pepo* L. (Cucurbitaceae), *Malva* sp. (Malvaceae), *Spinacea oleracea* L. (Chenopodiaceae).

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DNA Synthesis and Hormone-induced Elongation in the Cucumber Hypocotyl

It has been reported[1] that inhibition of DNA synthesis also inhibited shoot elongation induced by the hormone gibberellin (GA). GA treatment, which induced elongation, was shown to enhance DNA synthesis without any apparent increase in number of cells[2]. On the other hand, elongation induced by indolеacetic acid (IAA) did not seem to depend on DNA synthesis[3]. Katsumi *et al.*[4] have shown that both gibberellin and auxin induce elongation in the cucumber hypocotyl. We therefore chose this system to compare the effects of the two hormones on DNA synthesis as associated with hypocotyl elongation.

Cucumber seedlings (*Cucumis sativus* L., cr. Beit Alpha) were first germinated for 24 h on facial tissues, then grown uniformly in soil-filled flats in a growth chamber which was run at 29°, 16 h light ('Grolux' plus incandescent, about 700 foot candles at plant level) and 8 h dark. This lighting cycle was necessary for an optimal auxin response. 96 h later seedlings had reached the "initial state" very uniformly: the hypocotyls were about 35 mm long and the cotyledons spread out. The hypocotyl section—a 5 mm section starting 5 mm below the cotyledonary node—was marked with ink and measured at various times after hormone treatment. One of the important criteria for choosing the specified developmental stage and hypocotyl section was the absolute lack of mitotic figures at the time of hormone application. Mitotic activity in the elongating organ would complicate any conclusions to be drawn from measurements of DNA synthesis.

Gibberellin (supplied by ICI as a mixture of A_4 and A_7—about 1 : 1—10 mg/l.), IAA (100 mg/l., Calbiochem) and 5-fluorodeoxyuridine (FdUrd 10^{-5} M, a gift from Hoffmann-La Roche) were applied to seedling apices in 10 μl. droplets of solutions containing 0·05 per cent 'Tween 20'. A 1 h pulse of ^{3}H-thymidine (17·5 Ci/mmole, 10 μCi/ml.; Radiochemical Centre, Amersham) was given to freshly harvested hypocotyl sections by shaking them continuously in the labelling solution. The ^{3}H-thymidine pulses were always given at the same time of day, 12 h after the beginning of the light period. This was done to avoid complications caused by diurnal fluctuations (as found before) in rate of DNA synthesis. The sections were then rinsed thoroughly, fixed, dried, extracted, homogenized, washed on membrane filters and counted as described by Jakob and Bovey[5]. Tests have shown that 80 per cent of the radioactivity thus measured is removed by treatment with deoxyribonuclease. Radioactivity in DNA formed in all treatments approximately 10 per cent of

total radioactivity taken up by the tissue. When rate of transport of hormones and FdUrd in the intact seedling was checked, by using labelled compounds (2-^{14}C-IAA and 6-^{3}H-FdUrd, Radiochemical Centre; 3,4-^{3}H-GA, Dr H. Kende, Michigan State University) which were applied in 10 μl. droplets to the apex, all three compounds reached the hypocotyl section in appreciable amounts within 4 h after application. Although GA_1 is less effective than A_{4+7} in this system, there is no indication for difference between the two kinds of gibberellin in rate of transport.

Total DNA was determined by the diphenylamine reaction[6] on material which was homogenized and extracted according to Smillie and Krotkov[7].

Fig. 1 shows the differences in the effects of the two hormones on the rate of hypocotyl elongation: (*a*) the peak of auxin effect occurs earlier and is lower than that of the GA effect; (*b*) the effect of auxin is shorter than that of GA; whereas rate of elongation of auxin treatment falls back to control level by 48 h after treatment, GA effect at that time is significantly higher.

Fig. 2 shows that the effects of the two hormones on rate of incorporation of ^{3}H-thymidine into DNA parallel very closely the growth curves shown in Fig. 1. All differences between treatments (both in elongation and in ^{3}H-thymidine incorporation) were significant at the 5 per cent level.

The relatively early fall in auxin effect could be explained either by rapid inactivation of the hormone in the plant or by a drop in the sensitivity of the tissue to the hormone.

An experiment with repeated hormone treatment showed that whereas a second GA treatment was relatively ineffective, a second auxin treatment, 36 h after the first, significantly raised the rates of elongation and incorporation of ^{3}H-thymidine as measured 48 h after the first treatment. Absolute amounts of DNA in the hypocotyl section increased significantly from initial state to 72 h after hormonal treatment. The largest increase was in the GA-treated material (Table 1).

Table 1. EFFECTS OF HORMONAL TREATMENTS ON DNA/HYPOCOTYL SECTION

	Initial state	After 72 h		
		Control	GA	IAA
μg DNA/hypocotyl section	0·75	1·080	1·845	1·425
Increase in μg DNA/hypocotyl section	—	0·330	1·095	0·675
Percentage increase	—	44·0	140·6	90·0

100 hypocotyl sections were used for each determination.

In view of all these findings it was of interest to check the effect of FdUrd—an inhibitor of DNA synthesis—on the hormone-induced elongation in this system. FdUrd is known to act through inhibition of thymidylate

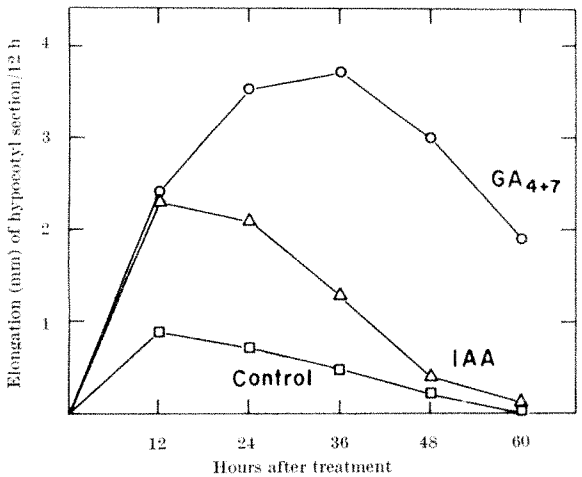


Fig. 1. Auxin (IAA) and gibberellin (GA_{4+7}) induced elongation in the cucumber hypocotyl. Hormonal treatments were given at time 0 (the "initial state"): the hypocotyl section was 5 mm long; length increments were recorded at 12 h intervals. Results represent average of nine seedlings per treatment.

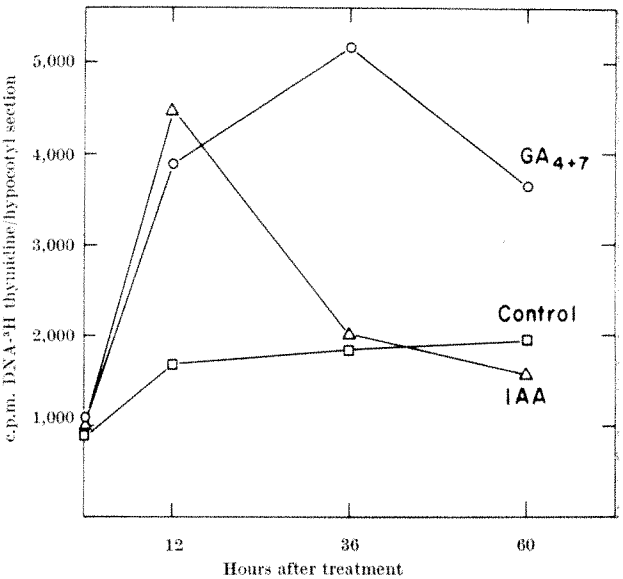


Fig. 2. Effects of auxin and gibberellin on the rate of ^{3}H-thymidine incorporation in the cucumber hypocotyl. 1 h pulses of ^{3}H-thymidine were given 12, 36 and 60 h after the hormone treatments. Results represent average of nine seedlings per treatment.

synthetase[8], causing thymidylic acid starvation, so we applied the FdUrd 12 h before hormone treatment. It was found that FdUrd inhibited very effectively both auxin and GA-induced elongation. This inhibition was largely reversed by thymidine, but not by uridine (both were used at 5×10^{-3} M concentration): it therefore seems likely that it was caused by the inhibition of DNA synthesis. Additional tests showed that ^{3}H-thymidine incorporation was enhanced by the FdUrd treatment, providing evidence that FdUrd acts by depleting the tissue of thymidylic acid. It has been found before[9–11] that the mitotic poison, colchicine, is a very effective inhibitor of AG-induced elongation. There are therefore at least two possible explanations for the inhibiting effect of FdUrd: (*a*) a certain fraction of the DNA synthesis is a prerequisite for hormone-induced elongation; or (*b*) FdUrd, by inhibiting DNA synthesis, acts as a mitotic poison (like colchicine), thus interfering with hormone-induced elongation. Another, parallel, possibility is that most of the hormone-induced DNA synthesis is another manifestation of the enhanced growth, rather than a prerequisite for it. The present data do not allow us to distinguish between all these possibilities. Other observations[11] indicate that most of this enhanced DNA synthesis takes place outside the cell nucleus, in chloroplasts and possibly also in mitochondria.

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Fall in Liver DNA Polymerase Activity in Cortisone-treated Rats

THE physiological properties of glucocorticoid hormones include inhibitory effects on growth and cell division both in intact animals and in tissue culture: administration of these compounds stops net DNA synthesis in the livers of growing rats[1], inhibits mitosis in various cells in tissue culture[2–4], and reduces the burst of DNA synthesis which ordinarily follows partial hepatectomy[5,6]. Administration of glucocorticoid hormones is well known to inhibit DNA synthesis in a number of systems, but the mechanisms by which this might occur remain obscure. While Guzek has shown that the administration of cortisone or ACTH to partially hepatectomized rats quickly inhibits incorporation of radioactive thymidine into liver DNA *in vivo*[7], attempts to demonstrate a direct effect of glucocorticoid hormones on DNA polymerase activity *in vitro* have been unsuccessful[8]. The possibility exists, however, that the administration of these hormones *in vivo* may result in significant changes in enzyme activity, and because of the good correlation between mitotic activity and DNA polymerase activity of liver in a number of different conditions[9–13], we thought it of interest to examine the effects of glucocorticoid administration on the DNA polymerase activity of rat liver. We shall show that the administration of cortisone to young rats rapidly decreases liver DNA polymerase activity. This decline can be demonstrated within a day of the onset of treatment and can be observed at considerably lower doses of hormone than have generally been used in other studies on rat liver.

Enzyme extracts were prepared thus: rats were stunned and exsanguinated. The livers were removed immediately, chilled and homogenized with ten strokes of a hand-driven Dounce homogenizer equipped with a loosely fitting pestle in five volumes of 0·1 M Tris-HCl buffer at *p*H 7·4 containing 1 mM EDTA and 6 mM 2-mercaptoethanol. Homogenization and all subsequent operations were carried out at 0°–4° C unless otherwise noted. The homogenates were centrifuged at 8,500*g* for 10 min and the resulting supernatants subjected to a further centrifugation at 105,000g_{av} for 1 h. The supernatant material from this second centrifugation was used for the subsequent enzyme assays. Protein determinations were performed by a modification of the Folin–Ciocalteu method[14], and enzyme assays were carried out as described by Bollum[15]. Incubation conditions are described in the legend to Fig. 1. When required, native DNA was denatured by heating a solution in a boiling water bath for 15 min followed by immediate chilling in an ice–water mixture.

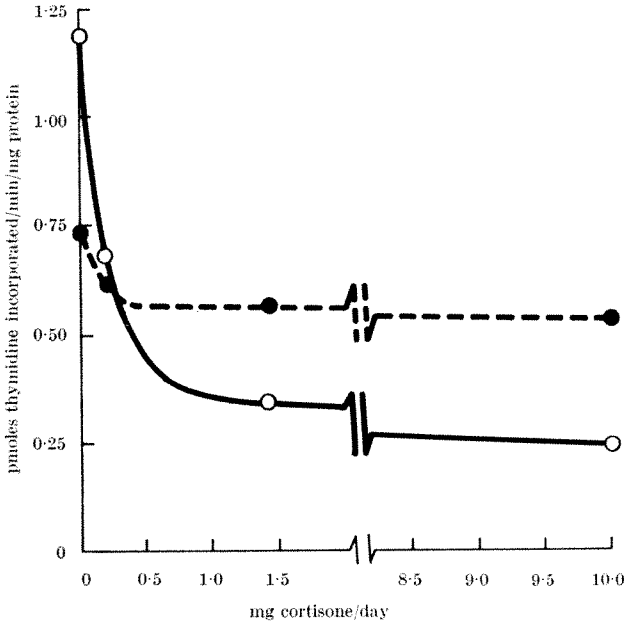


Fig. 1. Decline in DNA polymerase activity as a function of cortisone dose. Rats were treated with cortisone acetate as described in the text and were killed 72 h after the onset of treatment. The enzyme activity of each liver supernatant was tested with both native and denatured DNA as templates. Calf thymus DNA and the four deoxyribonucleoside triphosphates were purchased from the Sigma Chemical Corp. and were of the highest grades; [*methyl*-^{3}H]-thymidine triphosphate (13·3 Ci/mmole) was purchased from the New England Nuclear Corp. Assays were performed with the following components present in a final volume of 0·2 ml.: 50 mM KCl; 3·3 mM $MgCl_2$; 37 mM Tris-HCl at *p*H 7·3; 0·5 mM EDTA; 6 mM 2-mercaptoethanol; dATP, dGTP and dCTP at 150 μM each; 75 μM TTP (7·5 μCi/ml.); 1·6 per cent ethanol (contributed by ethanol present as a preservative in the stock solution of ^{3}H-TTP); DNA, 0·18 mg/ml.; and 0·5–0·6 mg liver protein (added in a volume of 50 μl. of 105,000*g* liver supernatant). Incubations were carried out at 37° C, and at zero-time and after 1 h of incubation 25 μl. samples were withdrawn in duplicate and applied to filter paper disks. Samples were prepared for radioactivity determinations, and enzyme activities (●- - -●, native DNA template; ○—○, denatured DNA template) were calculated as described by Bollum[15]. Incorporation was found to be absolutely dependent upon the addition of both DNA and enzyme extract, and in the conditions used thymidine incorporation was linearly related to the quantity of crude enzyme added.

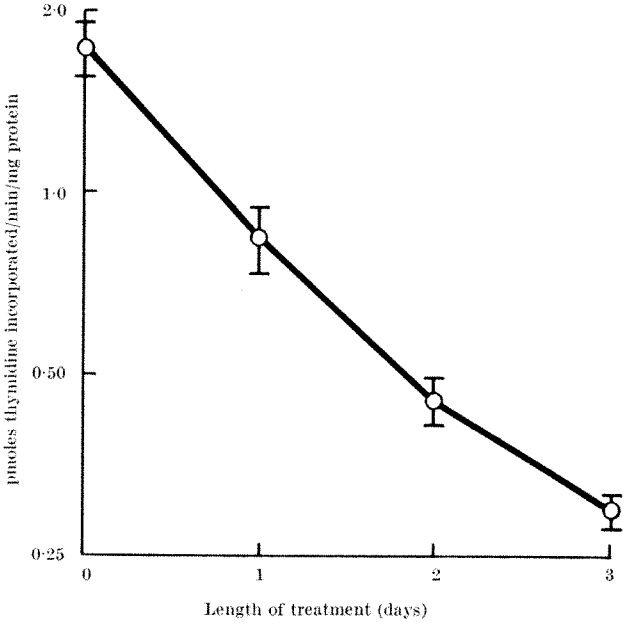


Fig. 2. Decline in DNA polymerase activity as a function of length of cortisone treatment. Rats were injected with 5 mg cortisone acetate twice daily for the number of days shown on the abscissa and were killed 14 h after the last dose. Each point shown above gives the mean ±1 standard error for groups of four to six animals (denatured DNA template). Note that the scale of the ordinate is logarithmic.

In the first experiment twenty male Sherman (Columbia strain) rats weighing 45 to 55 g were subcutaneously injected twice daily, in groups of five, with 0, 0·1, 0·7 or 5 mg cortisone acetate (Merck, Sharp and Dohme) in 0·2 ml. 0·15 M NaCl. Seventy-two hours after the first injection all animals were killed and liver supernatant fractions prepared as described. Fig. 1 shows the DNA polymerase activity of the extracts when tested with both native and denatured calf thymus DNA as templates. While cortisone treatment results in a decline in thymidine incorporation when either native or denatured DNA is used, the decrease in enzyme activity is far more striking when denatured DNA is used as a template and can be observed in rats receiving as little as 0·2 mg of hormone per day. The normal secretory rate of corticosterone, the principal glucocorticoid hormone of the rat with about half the potency of cortisone[16], is estimated at between 0·25 and 1·5 mg per 100 g of body weight per 24 h (refs. 17 and 18).

In the second experiment DNA polymerase activity was studied as a function of the length of cortisone treatment.

Fig. 2 indicates that administration of the hormone results in a decline in enzyme activity within 24 h, and continued administration of the hormone for an additional 2 days results in a further fall in activity to less than 20 per cent of control levels.

Earlier observers have noted that the administration of cortisone not only arrests somatic growth[19,20] and stops the normal increase in DNA content of the livers of growing animals, but also markedly decreases the incorporation of radioactive thymidine in regenerating liver. Our results are consistent with these earlier observations and are, we believe, the first demonstration of an effect of the hormone on an enzyme directly involved in DNA synthesis. Although the relationship between DNA polymerase activity as measured *in vitro* and DNA synthesis *in vivo* has still not been clearly established[21–24], the good correlation between enzyme activity and mitotic activity of liver tissue in a number of different conditions suggests that the enzyme activity measured *in vitro* may be of physiological significance. For example, DNA polymerase activity has been observed by others to be significantly increased in extracts of regenerating liver[9,10], in liver tissue from foetal and weanling animals[11,12] and in liver tissue which has undergone malignant transformation[12,13]. It is not known whether the decrease in polymerase activity described here reflects changes in the rates of synthesis or degradation of the enzyme, or instead a modification of the activity of pre-existing enzyme. Although the effect of hormone treatment was more striking when denatured rather than native DNA was used as a template, the respective roles of the polymerases which respond to native and to denatured DNA as templates remain the subject of much debate[21–24]. Whatever the mechanisms by which the administration of cortisone results in diminished polymerase activity, it is of interest that these effects can be observed with doses of hormone which are close to the "physiological" range.

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Effect of Rate of Cell Division on RNA Synthesis in Developing Frog Embryos

Endoderm cells of developing *Xenopus laevis*[1] and *Rana pipiens* embryos[2,3] synthesize more RNA per cell, including DNA-like RNA, than do the more rapidly dividing ectoderm–mesoderm cells. Slowing the rate of cell division by culturing frog embryo explants in LiCl increases the amount of RNA synthesized per cell, including DNA-like RNA[2], while increasing the rate of cell division by exposure to $NaHCO_3$ has the opposite effect[3]. These data suggest that a reduction in the rate of cell division allows higher levels of RNA (including DNA-like RNA) to be synthesized per cell.

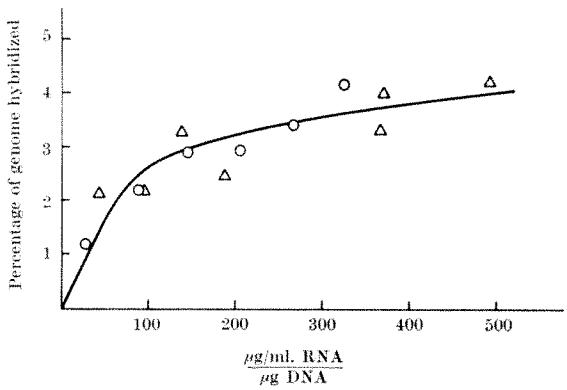


Fig. 1. Increasing amounts of RNA isolated from tailbud (stage 19) embryos raised at 10°–11° C (△) and 20° C (○) were incubated for 48 h in 2 × SSC, 50 per cent formamide at 37° C with 2·8 μg of denatured frog red blood cell DNA immobilized on nitrocellulose filters.

We examined changes in amounts and kinds of RNA synthesized resulting from alteration of the rate of cell division without chemical treatment. Frog embryos were raised at lower and higher temperatures than the normal culture temperature of 20° C. Groups of 200 dejellied blastulae, stage 9 of Shumway[4], were cultured at 10°–11° C and 23°–24° C until they were stage 19 tailbuds that had hatched from their membranes. It took 2–3 days for development to stage 19 at 23°–24° C and 8–9 days at 10°–11° C. Both of these groups of embryos were perfectly normal in appearance. At this time determinations of DNA and RNA were made[5]. The stage 19 tailbuds raised at the two temperatures had similar amounts of DNA (414 ± 8 μg at 10°–11° C and 419 ± 9 μg at 23°–24° C), indicating that they underwent a similar number of cell divisions. But the embryos raised at the colder temperature had more RNA ($1{,}278 \pm 12$ μg) than those raised at the higher temperature ($1{,}040 \pm 10$ μg). This gave a higher RNA/DNA ratio for the embryos raised in the cold (3·09), compared with those raised at the higher temperature (2·48); this is a 25 per cent difference. The results of five similar experiments showed that embryos raised at 10°–11° C had 27 per cent ± 8 per cent more RNA than those raised at 23°–24° C, even though there was a similar amount of DNA per embryo in the tailbuds raised at the two temperatures. This agrees with the work of Krugelis *et al.*[6] who found higher RNA/DNA ratios in salamander embryos raised at 10° C than in embryos developing to a similar larval stage at 20° C.

It has been shown that there is a gradual increase in the kinds of DNA-like RNA which can be detected as development proceeds in whole embryos[7]. In order to determine if the greater amounts of RNA present in embryos raised at the lower temperature are because of synthesis of new kinds of RNA, saturation hybridization experiments were performed. RNA from tailbud embryos raised at 10°–11° C and 20° C was prepared[8], polysaccharide was removed[9], and the RNA was labelled *in vitro* with

^{3}H-dimethyl sulphate[10]. Increasing concentrations of this RNA were incubated with nitrocellulose filters containing 2·8 μg of denatured frog red blood cell DNA, in 0·15 ml. of 2 × SSC (1 × SSC is 0·15 M NaCl, 0·015 M sodium citrate) and 50 per cent formamide at 37° C. It has been shown that in these conditions there is no degradation of RNA[11] and the specificity of annealing is equivalent to incubating at a temperature of 72° C in 2 × SSC (ref. 12). After 48 h the filters were removed, washed with 3 × SSC, treated with ribonuclease (20 μg/ml.) in 2 × SSC, washed again with 3 × SSC and air dried. The nucleic acids were hydrolysed with ribonuclease–deoxyribonuclease (500 μg/ml. each in 0·01 Tris, *p*H 7·5, 2 mmoles $MgCl_2$) for 20 h at 37° C. The hydrolysates were dissolved in scintillation fluid containing Beckman 'Bio-Solv' solubilizer and counted in a scintillation counter. It can be seen that the saturation curve obtained for the stage 19 tailbud embryos raised at 10°–11° is the same as for embryos raised at 20° (Fig. 1), indicating that the same number of kinds of DNA-like RNA are present in both populations in spite of the fact that the embryos raised at the lower temperature have more total RNA per cell.

In order to compare the percentage of DNA-like RNA present in the RNA obtained from tailbud embryos raised at 10°–11° and 23°–24° C, an annealing procedure was used which allowed progressive depletion of the DNA-like RNA. Samples of RNA (100 μg/ml.) prepared from stage 19 tailbuds raised at the two temperatures were incubated with nitrocellulose filters, each having 15 μg of denatured frog red blood cell DNA, in 0·2 ml. of 2 × SSC, 50 per cent formamide at 37° C for 48 h. New filters were added to the same RNA samples and incubated as before. This procedure was repeated six times and the percentage of DNA-like RNA binding to each filter was calculated. This type of depletion experiment, because it is well below the saturation level, does not allow the detection of qualitative differences in the two RNA preparations. It does, however, compare the number of copies of DNA-like RNA present in the two RNA samples. This technique does not permit the detection of RNA from the unique sequences[13].

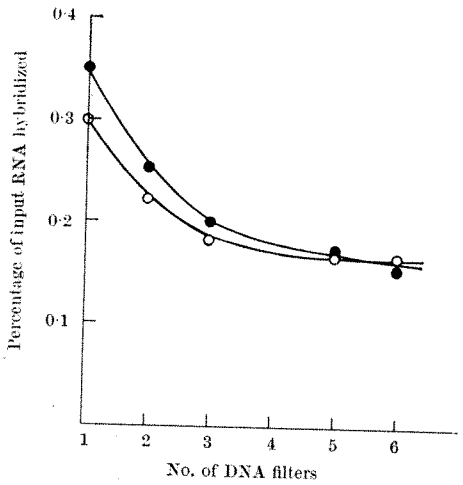


Fig. 2. The depletion of hybridizable RNA from 20 μg RNA samples by the addition of successive nitrocellulose filters containing 15 μg denatured frog red blood cell DNA is shown for RNA isolated from embryos raised at 10°–11° C (●) and 23°–24° C (○).

The percentage of input RNA hybridized plotted against the number of DNA filters added is shown in Fig. 2. It can be seen that the RNA from the embryos raised at 10°–11° C initially has a greater level of hybridization than the RNA from the embryos raised at 23°–24° C. As progressively more of the hybridizable RNA is depleted, however, the levels of hybridization of the two RNA samples approach the same values. This means that the amount of DNA-like RNA hybridized, per equal amounts of RNA added in the two incubations, was greater for the RNA isolated from embryos raised at 10°–11° C (Fig. 2). The simplest explanation for these results is that the percentage of DNA-like RNA is greater in the RNA extracted from the embryos raised at the lower temperature. The apparent plateau is probably caused by ribosomal RNA, transfer RNA and those DNA-like RNAs which are present in excess by comparison with their complementary DNA sequences.

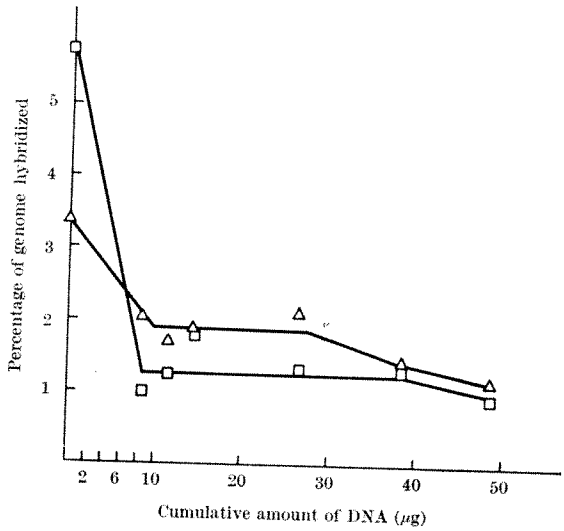


Fig. 3. The depletion of labelled RNA of stage 19 tailbuds raised at 10°–11° C (△) and stage 19$^+$ tailbuds raised at 23°–24° C (□) was measured by successive incubations with filters containing denatured DNA.

In order to compare the number of copies of DNA-like RNA of older embryos raised at a higher temperature with DNA-like RNA of younger embryos raised at a lower temperature, exhaustion experiments were performed using equal amounts of RNA extracted from stage 19$^+$ embryos raised at 23°–24° C and state 19 embryos raised at 10°–11° C. Saturation experiments showed 7 per cent of the DNA had been transcribed in the older embryos, compared with 4 per cent for the younger embryos. In these experiments near saturating amounts of RNA (1,100 μg/ml.) were exposed to consecutive DNA filters for 24 h in 2 × SSC, 50 per cent formamide at 37° C. It can be seen (Fig. 3) that although the initial hybridization level is greater for the RNA extracted from the embryos raised at 23°–24° C, the rate of exhaustion is also much faster. This indicates that although the younger embryos raised at 10°–11° C had fewer kinds of DNA-like RNA than the older embryos raised at 23°–24° C, the younger embryos raised at 10°–11° C had more copies of DNA-like RNA.

These experiments demonstrate that although the amount of RNA synthesized per cell is increased in embryos in which the rate of cell division has been retarded, the number of kinds of DNA-like RNA present are not changed. Culture at lower temperature does change the relative amount of DNA-like RNA as compared with total RNA. This means that although the absolute amount of ribosomal RNA per cell is greater in embryos raised at lower temperatures, the amount of DNA-like RNA is increased even more. Earlier determination of lens[14] and ciliary polarity[15] occurs in amphibian embryos raised at temperatures that are lower than normal. It is possible that the relative increase in DNA-like RNA in these embryos may result in earlier attainment of necessary threshold levels of DNA-like RNA, leading to earlier determination.

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Influence of Mouse Neural Retina on Regeneration of Chick Neural Retina from Chick Embryonic Pigmented Epithelium

In vertebrates such as bony fishes, amphibians, birds and mammals, neural retina can regenerate from the pigmented epithelium early in embryonic life[1]. This capacity persists thoughout life in some salamanders, and is maintained until metamorphosis in some tailless amphibians.

In the early chick embryo Orts-Llorca and Génis-Galvèz[2] showed that neural retina regenerated from the pigmented epithelium when a strand of thread was introduced into the cavity of the optic vesicle. On invagination of the optic vesicle to form the optic cup, the thread locally separated the presumptive neural retina from the presumptive pigmented epithelium. Neural retina regenerated from the pigmented epithelium in such areas of separation.

Until at least as late as 4 days of incubation, pigmented epithelium of the chick embryo can give rise to neural retina. At this age, in a previous study[3], we completely removed the neural retina from embryonic eyes; in some cases we re-implanted a small piece of neural retina. Neural retina did not regenerate when we completely removed the neural retina, but proceeded rapidly if we re-implanted a small piece of neural retina. The new chick neural retina regenerated from the cut edge of the pigmented epithelium at the margin of the eye cup and also in isolated patches in the pigmented epithelium away from the edge of the eye cup. Otocysts from 4-day old embryos also elicited neural retinal regeneration, but pieces of optic tectum from donors of the same age were not effective. So this phenomenon is at least partially tissue specific.

Sologub, using the frog tadpole, also demonstrated that the presence of neural retina was necessary for regeneration of neural retina from pigmented epithelium[4].



Fig. 1. Portion of the eye wall of a fourteen day old chick embryo. The neural retina had been removed at 4 days of incubation and replaced by a piece of mouse embryonic neural retina. The mouse neural retina lies to the right. To the left chick neural retina has regenerated from the pigmented epithelium with which it is still continuous.

To determine whether or not the retinal influence is species specific we replaced the neural retinas of the right eyes of twelve 4-day-old chick embryos with pieces of undifferentiated neural retina taken from mouse embryos with crown-rump lengths of thirteen to seventeen millimetres. At all stages of development the neural retina of the mouse is easy to distinguish from that of the domestic fowl after fixation in Carnoy's fluid and staining by the Masson procedure. Mouse cells have more densely stained nuclei than those of the domestic fowl; also the nuclear and fibre layers of the mouse retina are distinctively different in structure from those of the domestic fowl.

The host animals were killed and their right eyes were examined histologically at 13 or 14 days of incubation. In each of the twelve specimens the implanted mouse neural retina was still present in the vitreous cavity of the host eye. The implants were usually normal histologically. While mitotic figures were still present, the mouse retinas had differentiated a ganglion cell layer and rod and cone precursors in some places.

Neural retina regenerated in all twelve cases in the same way that it had when chick retina had been introduced into the retina-less eye (Fig. 1). In each case a new retinal sheet arose from the cut margin of the pigmented epithelium at the edge of the eye cup. Neural retina originating here grew back into the cup and differentiated so that its rods and cones faced the pigmented epithelium in the normal orientation. In addition to this type of regeneration, in ten of the twelve cases chick neural retina regenerated as isolated patches from pigmented epithelium some distance from the edge of the eye cup. Each patch was surrounded on all edges by, and was continuous with, the adjoining pigmented epithelium. In these cases the neural retina differentiated so that its rods and cones faced the vitreous cavity.

This 180 degree difference in polarity between the neural retina that regenerated as a sheet from the edge of the eye cup and that which regenerated as patches in the pigmented epithelium may be explained by the fact that rod and cone outer segments are in part derivatives of cilia. Early in development the ciliary surface of the presumptive retina is the inner surface of the optic vesicle (Fig. 2). This surface is continuous through the optic stalks with the ciliary surface of the brain wall facing the ventricle. When the optic vesicle invaginates to form the optic cup its ciliary surface becomes the inner wall of the pigmented epithelium of the retina and the

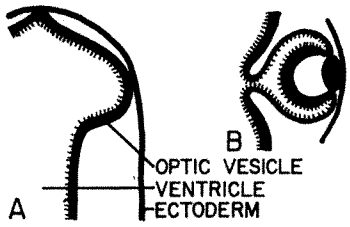


Fig. 2. The ciliary surface of the optic vesicle is continuous with the inner surface of the primitive brain wall (A). After invagination of the vesicle to form the optic cup (B) the ciliary surfaces of the pigmented epithelium and the neural retina face one another.

outer wall of the neural portion of the retina. So the apparent difference in polarity between neural retina which regenerates from the edge of the optic cup and that which regenerates in patches within the pigmented epithelium disappears when ciliary polarity is taken into consideration.

The neural retinae that regenerated in the presence of mouse retinal implants were composed of chick-type cells. They had the histological structure typical of the chick retina and not that of the mouse. The results demonstrate two points. The influence of the neuroepithelium is not highly species specific. The regenerated neural retina arises by metaplasia of the pigmented epithelial cells and not from cells that have migrated from the implant to the pigmented epithelium.

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Separating the Sheep from the Goats on the Basis of their Chromosomes

THE karyotypes of fifty species of ruminants in the family Bovidae have been examined and the fundamental number of sixty chromosome arms has been established for thirty-two of them. Reporting this, Taylor, Hungerford and Snyder[1] pointed out that the distribution of the numbers of metacentric chromosomes among forty-nine species of Bovidae supports the view that Robertsonian[2] translocation has been a common feature of the evolution of the bovid karyotype. There are more Bovidae with few or no metacentrics than there are with many, or a complete complement of them.

The form of the ruminant karyotypes, a series of many acrocentrics which often differ only in size, makes identification difficult. It seems likely, however, that work can be done which exploits the knowledge that these species are the product of evolution from a common ancestor. A meticulous comparative examination of karyotypes, which may indicate translocations and the possibility of different linkages, could be combined with a knowledge of phenotypes to provide a basis for chromosome mapping. In karyotypes with more prominent features, such as that of man, work with one species can be productive. In the case of the Bovidae a comparative study seems likely to be most helpful. With this in mind a comparative study was undertaken of ten goats ($2n = 60$[3]) and ten sheep ($2n = 54$, six metacentrics[4]).

All the sheep in this survey were normal Clun Forest lambs, aged between 2 days and 2·5 months. The goats were British Alpines, Saanens and Welsh × Saanen cross-bred animals, aged between 6 months and 4 years. Five sheep and five goats of each sex were used and all the males were castrated.

Chromosome spreads were prepared from blood lymphocytes by a modification of the method of Basrur and Gilman[5]. The chromosome spreads were photographed with a flat-field × 100 oil immersion objective and a × 15 or × 10 ocular in the photographic tube. Kodak 'Photomechanical' cut film was used for the negatives, which were then mounted between glass sheets for projection onto paper taped to a wall. The size of the projected image was large enough for the observers to measure a difference of at least 0·02 per cent and one particular spread was measured and computed five times to demonstrate that karyotype construction by this method is repeatable. A pencil was used to mark the centromere in each chromosome and to trace its length along the core of each duplicating chromatid. A map-measurer of the "drawing" type was used to measure the length of the pencil lines and the arms arranged in a list of sixty, by length. Adjacent chromosomes were then paired, from the longest to the shortest into thirty homologous pairs and each recorded as a percentage of the total length of chromatin in that spread. Adjustments were made in the male series to bring it into line with the female, by subtracting the length of the Y chromosome and making a "synthetic" X pair by adding the length of the single X chromosome after it had been multiplied by an appropriate factor. The factor was derived from the ratio of the length of the two female X chromosomes and from a preliminary observation that the single male X is a little longer than the longest female X. The longest acrocentric pair in the sheep and the third longest in the goat was assumed to be the X pair as a result of the findings of J. J. Evans[6] in the goat, and in the sheep, because one of these chromosomes is the largest unpaired acrocentric in the male. Measurements of length were used because of their obvious simplicity and the choice of this method is supported by Neurath and Enslein[7] who showed that it provides a quantitative and objective method of assigning chromosomes to groups in the human karyotype. It must be pointed out that the homologous pairs are identified by length only, which is a reasonable but not a fundamental criterion, so that any pair might be displaced in a neighbouring group.

I have assumed that the sheep and goat nuclei contain equal quantities of material[8,9], so that it is satisfactory to

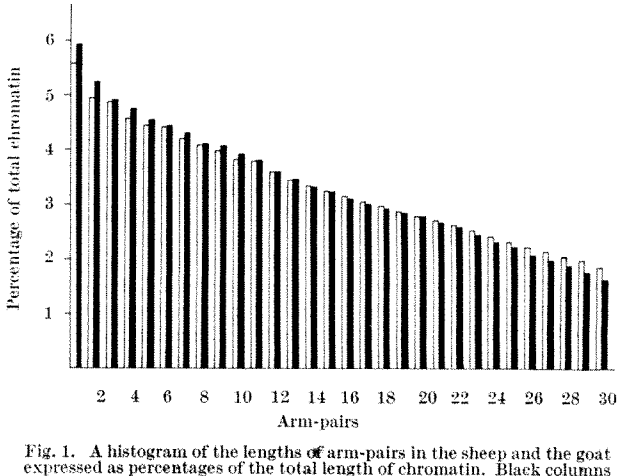


Fig. 1. A histogram of the lengths of arm-pairs in the sheep and the goat expressed as percentages of the total length of chromatin. Black columns represent goat and open columns represent sheep.

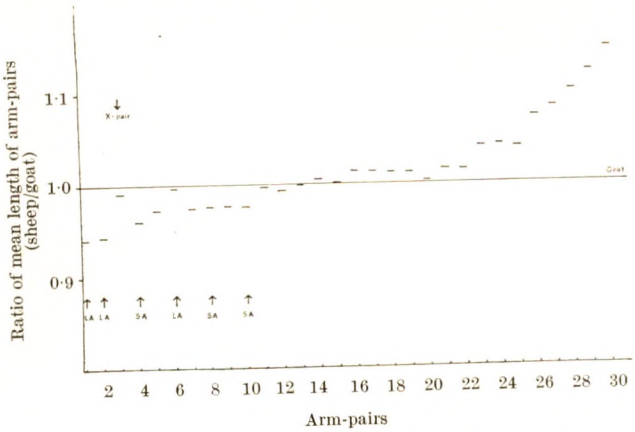


Fig. 2. A graph of the ratio of the mean length of arm-pairs in the sheep to the mean length of corresponding pairs in the goat. The metacentric pairs in the sheep are indicated by arrows with subscripts. SA, Short arms of metacentric; LA, long arms of metacentric.

use percentages in the comparison. A comparison of size is then valid, but real pairing by identity is not yet possible. In view of the close phenotypic and karyotypic resemblance of sheep and goats, there is a fair chance that pairing by size does not give a wild distortion of the true situation. The series of arm-pairs in the sheep was compared with that in the goat (Figs. 1 and 2). The longest arm-pairs of the goat are longer than the longest arm-pairs of the sheep and the shortest arm-pairs of the goat are shorter than the shortest arm-pairs of the sheep.

Robertsonian translocation has been suggested as one of the mechanisms of evolution in the bovid karyotype. Of the two species in question, it is the sheep which shows the form associated with Robertsonian change. The differences in length between the chromosomes, of the sheep and the goat could represent a translocation of chromatin from the large chromosomes, which took part in Robertsonian translocation, to the smallest chromosomes in the complement. Such fusion would have involved the junction of two acrocentrics, each of which had a pair of short arms and a centromere in addition to the long arms. It is likely that the material associated with one pair of short arms and one centromere would be displaced. The differences between the two species at both ends of the series support the theory of a translocation of material from the large chromosomes to the small ones when fusion occurred. If it could be shown, however, that the sheep nucleus contains less DNA than the goat nucleus, a loss of this material could be inferred. Two large acrocentric pairs in the sheep, the sixth and the ninth, also contribute to the differences in length between the species and this suggests that other translocations beside those caused by Robertsonian fusion may have contributed to the evolution of the karyotypes of these two species from a common ancestor. The study is to be extended to the karyotype of the Barbary sheep, *Ammotragus lervia* ($2n = 58$, one pair of metacentrics).

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Immunofluorescent Localization of Chorionic Gonadotrophin in Monolayer Cultures of Human Trophoblast Cells

ALTHOUGH the ability to synthesize chorionic gonadotrophin by human trophoblast cells grown *in vitro* has been reported[1,2], there is still considerable doubt as to which of the cell types is responsible for production of the hormone.

We have attempted to localize this hormone by the "indirect" immunofluorescent method using a commercial preparation of rabbit anti-human chorionic gonadotrophin (Hyland, USA) and a goat anti-rabbit globulin labelled with fluorescein isothiocyanate, kindly provided by Dr Lachmann of the Immunology Division.



Fig. 1. Early trophoblast culture showing large, flattened cells with abundant granular cytoplasm and smaller polygonal cells with one nucleus. ×128.

Villi were dissected off 12–13 week old placentae obtained by hysterotomy from therapeutic abortions. These were minced with a scalpel into 1–2 mm pieces, rinsed three times with Eagle's basal medium at room temperature and then trypsinized for 10 min at room temperature in a 0·05 per cent solution of trypsin (Difco Bacto) in calcium and magnesium free phosphate buffered saline (PBS) using a magnetic stirrer. The tissue was allowed to settle and the supernatant discarded, fresh trypsin being added. This process was repeated three or four times but subsequent supernates were retained, placed in conical centrifuge tubes containing 1 ml. growth medium, and centrifuged at 500*g* for 5 min. The resulting cell pellets were gently resuspended in 2 ml. growth medium (199 containing 20 per cent foetal calf serum) and a cell count made.

The volume was adjusted to give concentrations of 1×10^6 cells/ml. and 4 ml. aliquots of cell suspension placed in flat 30 ml. plastic, screw-capped culture flasks (Falcon plastics), gassed with a 5 per cent CO_2 with air mixture and incubated at 37° C. The cells were examined daily and the medium changed every third day.

After 10 days of growth, the cultures were washed gently with three changes of PBS. After fixation in methanol, the base of the flask with the monolayer was cut away and left to dry at room temperature. This was then incubated for 30 min at room temperature with rabbit anti-HCG diluted 1 : 5 with PBS. The antiserum was washed off and fluorescein isothiocyanate-labelled goat anti-rabbit globulin applied, and left for 30 min at room temperature. The preparation was washed in six changes of PBS and mounted in buffered glycerol. The cultures were examined by a Zeiss Ultraphot fluorescence microscope.

Controls were set up by staining some monolayers with fluorescein-labelled anti-rabbit globulin alone and also by repeating the whole staining procedure on human foetal skin cells.



Fig. 2. Small polygonal cell showing specific fluorescence. ×128.

Initial examination of the cultures revealed three cell types, the most conspicuous being a very large, frequently multi-nucleated cell with abundant granular cytoplasm. Its shape was irregular and the cell appeared firmly flattened against the culture flask. The second type was a much smaller polygonal cell with one nucleus and surrounded by a relatively thin zone of cytoplasm (Fig. 1). The third type was a thin spindle-shaped cell with an elongated nucleus, the appearance of which was not unlike that of a fibroblast. After staining, only the small polygonal cell showed specific fluorescence. This appeared as a bright area within the cell localized around the nucleus (Fig. 2). The other cell types and the controls showed no fluorescence.

The question of the relative importance of cytotrophoblast and syncytiotrophoblast in the production of human chorionic gonadotrophin has still not been settled. In an immunohistochemical study[3] on formalin-fixed placental tissue, it was concluded that the syncytiotrophoblast was the cell of origin of human chorionic gonadotrophin. In a tissue culture study[1], however, it was found that the amount of gonadotrophin present in the culture medium was directly related to the growth of what appeared to be cytotrophoblasts in the culture.

The three types of cells obtained in our culture of human chorionic villi were very similar to those previously described[4,5]. Our results point to the small polygonal cell as the producer of human chorionic gonadotrophin, and their appearance suggests that they may be cytotrophoblasts.

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Unexpected Features of the Action of Dinitrophenol on Cortical Neurones

2,4-Dinitrophenol (DNP) uncouples oxidative phosphorylation by interfering with mitochondrial activity[1,2]. It has been widely used to inhibit the metabolism of central or peripheral neural tissue[3–5]. The reduction in supply of ATP slows ion pumping and so leads to the accumulation of Na^+ inside cells and a corresponding loss of K^+. Blockage by DNP is therefore a criterion for identifying energy-driven processes.

Nishi, Soeda and Koketsu[6], and Kawamura and K. K.[7] recently showed that in sympathetic ganglia and the cerebral cortex, DNP readily blocks the slow excitatory muscarinic action of acetylcholine (ACh). This observation seemed to confirm the hypothesis that in this muscarinic role, ACh has a primary influence on cellular metabolism, associated with only secondary changes in excitability[8]. Such a mechanism would be in sharp contrast to the better-known, quick-acting nicotinic excitation, accompanied by a large increase in membrane permeability. A metabolic involvement was suggested by the long time course of excitation by ACh, and the absence of a clear increase in membrane conductance[8,9].

To understand better how DNP acts, we examined its effects with microelectrodes inserted into cortical neurones. The experiments were performed on cats anaesthetized with a mixture of nitrous oxide and methoxyflurane ('Penthrane', Abbott). To reduce pulsation of the brain, the chest was opened and succinylcholine ('Anectine', Burroughs Wellcome) was infused intravenously. The technique of recording the membrane potential and resistance (tested with 20 ms pulses), combined witd extracellular microiontophoresis, has been describeh before[10,11]. DNP was released from a 0·1 M solution of the sodium salt, at *p*H 9, by an inward current (microelectrode relatively negative). In some experiments a Linc 8 computer was used on-line to apply pulses, sample membrane potentials and derive voltage-current lines[12]. The computer rapidly made available estimates of the membrane resistance and the reversal levels of inhibitory synaptic potentials (IPSPs).

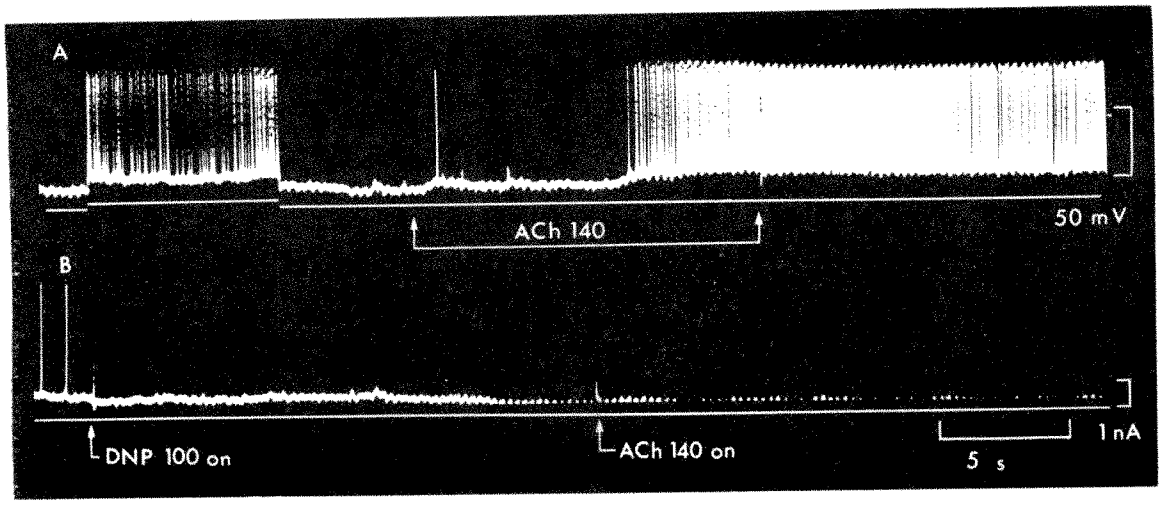


Fig. 1. Intracellular recording from pre-cruciate neurone. *A*, Excitation, first by depolarizing current, monitored on second trace, and then by extracellular application of ACh (iontophoretic current of 140 nA). *B*, Effect of DNP: release by inward current of 100 nA began at arrow and continued to end of trace; at second arrow, start of another application of ACh.

The results of these experiments revealed several unexpected features. The application of DNP leads to a pronounced decrease in electrical excitability, associated with a tendency to hyperpolarization and a lower resistance of the membrane. These effects are illustrated by traces obtained from a pre-cruciate neurone. In Fig. 1*A*, there is strong firing, initiated either by a depolarizing current (at left) or by an application of ACh. The action of the latter has the characteristic slow onset and long duration[13]. The lower trace (*B*) shows the effect of DNP, its release beginning at the first arrow: spontaneous spikes disappear, and the potential trace gradually shifts down (in the negative direction) towards the limit of visibility on the oscilloscope screen. Starting at the second arrow ACh was also applied, as in *A*, but for a longer period. This failed to evoke firing, or even subthreshold depolarization. All the effects of DNP were fully reversible, clear recovery beginning within 1–2 min of ending the application.

Further tests, on the same cell, showed other changes in membrane properties. The oscilloscope traces in Fig. 2 illustrate IPSPs evoked by surface shocks (at arrows in *A*). The IPSP is initially partly reversed, because acetate ions have diffused into the cell from the recording micropipette[14]. In the left-hand row, each trace consists of only a single sweep, with two 20 ms negative pulses of current applied respectively just before and about 30 ms after the surface shock (the current pulses are monitored on another trace). The corresponding voltage pulses clearly demonstrate the following: a lower resistance during the IPSP, a sharp fall in both resting and IPSP resistance during the application of DNP (*B*), and at least partial recovery after the end of the release (*C*). In the right-hand row, several traces were superimposed photographically, and the testing current was systematically reversed to include both positive and negative pulses in each record. The repetitive firing evoked by positive pulses was prevented by DNP (*B*).

Comparable observations have been made on more than twenty neurones, particularly clear effects being recorded from cells having relatively high and stable resting potentials. The hyperpolarizing tendency was seen more often than the resistance changes, possibly because of the technical difficulties involved in measurements of membrane resistance[10,11]. Unlike neurones, cortical neuroglia[15] showed no definite effects of DNP.

This rapid hyperpolarization of cortical neurones differs greatly from the slow depolarizing action of DNP on peripheral nerves[16]. The associated conductance increase points to a rise in membrane permeability as the underlying mechanism. The direction of potential change is consistent with an increase in permeability to K^+ or Cl^-; but the fact that DNP causes a hyperpolarization even when IPSPs are reversed (Fig. 2) makes an increase in anion permeability very unlikely. It is therefore likely that DNP acts by raising the permeability (P_K) of the membrane to K^+. The mechanism of this phenomenon may be comparable with the large increase in proton conductance induced by DNP in artificial phospholipid membranes[17,18]. In view of the high probable cytoplasmic *p*H (ref. 19), the observed negative shift

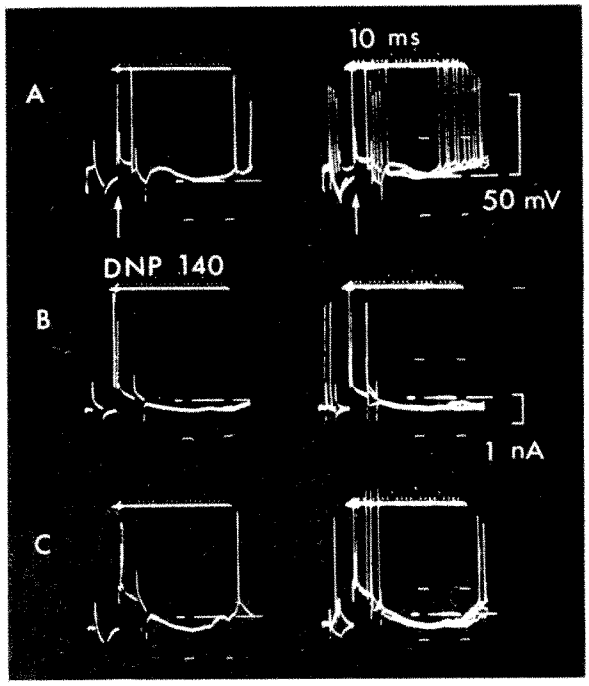


Fig. 2. IPSPs evoked in same neurone by surface shocks (0·1 ms, 12 V, at 3 Hz). In each record, top trace gives time calibration and d.c. reference level; main trace shows, from left to right, pulse monitoring electrode resistance (not visible in *B* and *C*), pulse indicating membrane resistance, stimulus artefact (at arrows in *A*), and IPSP, with second pulse indicating resistance near peak of IPSP; testing current pulses are monitored on third trace. Note single traces at left, with negative pulses only, and several superimposed traces at right, including both positive and negative pulses. *A*, Controls; *B*, during release of DNP; *C*, 1 min after end of release. Recording electrodes contained 1 M potassium acetate.

is probably not generated by a flow of protons; but DNP may act in the membrane of cortical neurones as a carrier of K^+.

Another possible explanation is that the increase in P_K is secondary to metabolic inhibition by DNP. There is some evidence that in red blood cells, metabolic activity is required to maintain a very low internal concentration of free Ca^{2+}, and that a rise in free Ca^{2+} is soon followed by an increase in membrane P_K[20,21]. DNP could augment the internal free Ca^{2+} in two ways: by reducing the Ca^{2+} binding resulting from mitochondrial accumulation[22] or chelation to cytoplasmic ATP[23]. Whatever its mechanism, the enhancement of P_K by DNP is not evident in all tissues (for example, cortical neuroglia or peripheral nerves[16]), but it may occur in cardiac muscle[24].

In conclusion, the depressant action of DNP can be fully explained by the observed changes in membrane properties, which probably result from an enhancement of membrane P_K. The blocking effect of DNP therefore gives no support for the hypothesis of a primarily metabolic action of ACh.

The explanation for the particularly effective antagonism of ACh excitation by DNP seems to be that as a muscarinic excitant, ACh may act in a precisely opposite fashion, that is by reducing P_K. We have some evidence that ACh tends to raise the membrane resistance of cortical cells; a similar muscarinic effect has been observed by Kobayashi and Libet[9] in sympathetic ganglia. In several respects, the action of ACh is analogous to that of Ba^{2+} (ref. 25). It is therefore of interest that Ba^{2+} has a muscarinic excitatory action on a variety of tissues[26–28], including the cerebral cortex (our unpublished observations).

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Mode of Action of 5-Hydroxytryptamine on Isolated Rat Vas Deferens

It has been suggested that 5-hydroxytryptamine (serotonin) might cause the rat vas deferens to contract by interacting directly with the α-adrenergic receptors in the organ[1]. We found that vas deferens isolated from reserpinized rats could not respond to serotonin, so we have investigated the mode of action of serotonin on this preparation, and compared it with the action of tyramine.

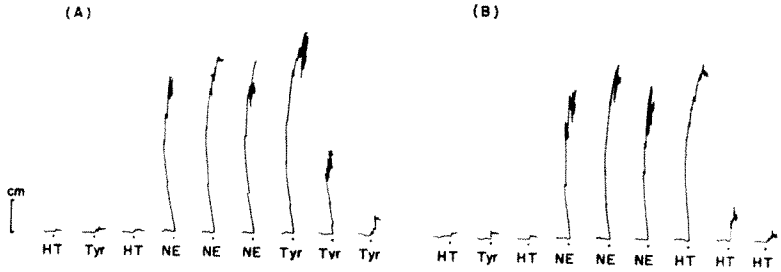


Fig. 1. Responses of vas deferens isolated from reserpinized rat to tyramine (*A*) and 5-hydroxytryptamine (*B*). NE, Norepinephrine 10^{-5} g/ml.; HT, 5-hydroxytryptamine 3×10^{-5} g/ml.; Tyr, tyramine 3×10^{-6} g/ml.

Vas deferens obtained from rats weighing about 200 g was suspended in a 10 ml. organ bath filled with Tyrode solution at 32° C and gassed with a mixture of 95 per cent O_2 and 5 per cent CO_2. The responses of the vas deferens to test drugs were recorded on a smoked paper. In most experiments, cumulative dose–response curves were obtained. The concentrations (g/ml.) of agonists refer to base, and those of antagonists to salt. All values are means of at least ten experiments.

The vas deferens isolated from reserpinized rat (2·5 mg/kg, intraperitoneal, 24 h before death) showed little response to serotonin or tyramine (Fig. 1). But after three successive 2 min exposures of the tissue to norepinephrine 10^{-5} g/ml., the responses of the organ to serotonin (3×10^{-5} g/ml.) or tyramine (3×10^{-6} g/ml.) recovered and equalled the maximum response to norepinephrine. Tachyphylaxis rapidly developed to further doses of serotonin or tyramine.

Contractions of vas deferens from normal rats by norepinephrine, serotonin and tyramine were blocked by a 20 min pretreatment with dibenamine (3×10^{-6} g/ml.). Tolazoline (10^{-6}–3×10^{-6} g/ml.), also an α-adrenergic blocking agent, had a similar effect, the block being competitive in nature. pA_2 values for tolazoline were calculated from parallel shifts in the dose-response curves for norepinephrine, serotonin and tyramine[2] and equalled $6{\cdot}29\pm0{\cdot}13$, $6{\cdot}40\pm0{\cdot}06$ and $6{\cdot}22\pm0{\cdot}14$, respectively.

The response of the vas deferens to serotonin was not affected, but the responses to norepinephrine and tyramine were slightly potentiated by 3-2-benzylaminoethyl-5-methoxyindole hydrochloride (10^{-6} g/ml.) which was reported to be a competitive inhibitor of serotonin. Incubation of the tissue with guanethidine (3×10^{-7}–10^{-6} g/ml.) or imipramine (10^{-7} g/ml.) until the response to norepinephrine was maximally potentiated resulted in an inhibition of the responses to serotonin and tyramine.

The responses to serotonin were more easily inhibited by guanethidine and imipramine than those to tyramine. Contractions produced by serotonin, tyramine and norepinephrine were equally potentiated by 45 min incubation of the vas deferens with procaine hydrochloride (3×10^{-5} g/ml.). Morphine (3×10^{-6} g/ml.) and tetrodotoxin (10^{-6} g/ml.) did not affect the contractions produced by these amines.

We conclude that serotonin causes the rat isolated vas deferens to contract through being taken up into sym-

pathetic nerve terminals[4] and causing the release of norepinephrine from storage.

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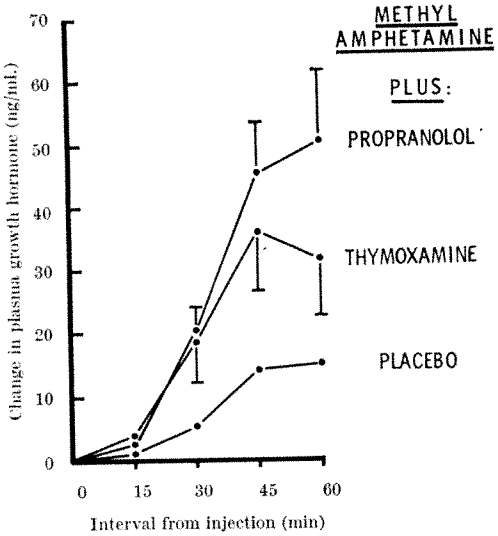


Fig. 2. Changes in mean plasma growth hormone levels after intravenous propranolol (0·15 mg/kg) or thymoxamine (0·10 mg/kg) or a placebo, each given 5 min before 15 mg intravenous methylamphetamine at 1800 h. Vertical bars represent 1 standard error of the differences between active and placebo effects.

Adrenergic Blockade and the Corticosteroid and Growth Hormone Responses to Methylamphetamine

THE observation that the hypothalamus has a high concentration of adrenergic neurones[1] and the highest catecholamine content of any central nervous system structure[2] has focused attention on the possible role of central catecholamines in neuroendocrine regulation. In a previous study[3] we have shown that the sympathomimetic and central stimulant drugs dextro- and methylamphetamine cause significant elevation of plasma corticotrophin, corticosteroids and growth hormone levels in human subjects. The rise in corticosteroids is most pronounced in the evening. It seems likely that the amphetamines act either directly on the hypothalamic neurones responsible for secretion of pituitary hormone releasing factors, or that they result in the local accumulation of catecholamines which then cause these neurones to secrete.

We now report the effects of α and β adrenergic blocking drugs on the corticosteroid and growth hormone responses to methylamphetamine. Thymoxamine, which has relatively specific α blocking activity[4], and propranolol, a β blocking drug[5], were used. Six healthy male volunteers aged 19–34 years were studied on three occasions at 1800 h after a fast of 5 h. A forearm venous cannula was inserted under local anaesthesia. Thirty minutes later either thymoxamine, 0·10 mg/kg body weight, or propranolol, 0·15 mg/kg, or a saline placebo was given, followed in 5 min by 15 mg methylamphetamine. Blood samples were taken before and 15, 30, 45 and 60 min after the methylamphetamine for plasma growth hormone and corticosteroid determinations.

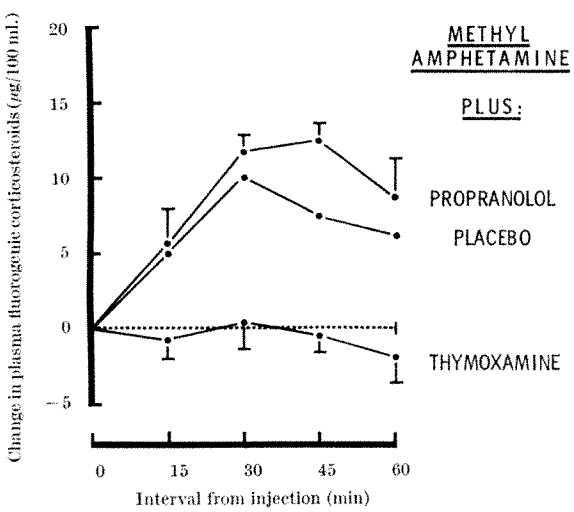


Fig. 1. Changes in mean plasma fluorogenic corticosteroids after intravenous propranolol (0·15 mg/kg) or thymoxamine (0·10 mg/kg) or a placebo, each given 5 min before 15 mg intravenous methylamphetamine at 1800 h. Vertical bars represent 1 standard error of the differences between active and placebo effects.

Treatments were allocated using a double-blind, cross-over method and a "Latin square" design, with 7 day intervals between tests in each subject. The differences were calculated between the control values and those recorded at each time interval after the blocking drugs or placebo. These differences were compared using Student's *t* test (two tailed). Plasma fluorogenic corticosteroids were measured by the method of Mattingly[6] and growth hormone by radioimmunoassay[7].

The administration of the placebo and methylamphetamine combination produced a mean maximum plasma corticosteroid rise of 10·8 μg/100 ml. (s.e. ± 1·43 μg/100 ml.). When the thymoxamine–methylamphetamine combination was given, however, there was no rise in mean plasma corticosteroid concentration (Fig. 1). The differences between the mean rises in plasma corticosteroids produced by thymoxamine – methylamphetamine and placebo–methylamphetamine were statistically significant at each time interval (P values between $< 0{\cdot}01$ and $< 0{\cdot}001$). Methylamphetamine given after propranolol caused a rise in mean plasma corticosteroids which was greater at each time than that produced by the placebo–methylamphetamine combination (Fig. 1), but this reached statistical significance only at 45 min ($P < 0{\cdot}05$).

There was a rise in mean plasma growth hormone with each treatment combination (Fig. 2). Propranolol–methylamphetamine caused a greater rise than that produced by placebo–methylamphetamine ($P < 0{\cdot}05$ at 30, 45 and 60 min). The mean growth hormone rise produced by thymoxamine–methylamphetamine was also consistently greater than that after placebo–methylamphetamine although the differences did not reach the 5 per cent level of significance at any individual time interval. The mean maximum plasma growth hormone increments were 17 (s.e. ± 6·4) ng/ml. after placebo–methylamphetamine and

52 (s.e. ± 11·7) ng/ml. after propranolol–methylamphetamine.

The adrenergic blocking drugs did not significantly change the mean blood pressure responses to methylamphetamine.

This study has shown that the corticosteroid rise produced by the sympathomimetic drug methylamphetamine could be prevented by prior administration of the α blocking drug thymoxamine and increased by the β blocking drug propranolol. By contrast, the effect of both of these adrenergic blocking drugs on the growth hormone increment was to cause an augmentation of the response to methylamphetamine.

The midbrain reticular formation is closely concerned with the control of hypothalamic corticotrophin releasing factor and consequent hypophyseal corticotrophin secretion[8]. Amphetamines act on the reticular formation[9,10], so it is possible that their effect on plasma corticosteroids is a result of an action at this site.

The amphetamine-induced rise in corticosteroids can be prevented by previous administration of thymoxamine, which suggests that this rise results from an α effect of amphetamine. This α effect is enhanced by the β adrenergic blocking drug propranolol. These actions of adrenergic blocking drugs are similar to the changes which they produce in the amphetamine-induced alterations of the electroencephalogram[11].

Because both α and β adrenergic blockade enhance the growth hormone response to amphetamine, it seems that amphetamine-sensitive control mechanisms for growth hormone secretion differ from those involved in corticotrophin release.

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Relation of Occipital Spikes evoked by Intermittent Photic Stimulation to Visual Evoked Responses in Photosensitive Epilepsy

Patients suffering from photosensitive epilepsy show generalized discharges in their electroencephalogram (EEG) during intermittent photic stimulation. In some patients, these discharges are preceded by occipital spikes, suggesting a focal onset. Hishikawa *et al.*[1] have reported that the occipital spikes obtained in photosensitive epileptics have the same latency as some components of the visual evoked responses (VER), and suggested that this might explain the origin of the occipital spikes in photosensitive epilepsy. The spikes were, however, obtained at flash rates of 10–16 s^{-1} (that is, the interval between the flashes was limited to a range of 63–100 ms) and the comparison of the occipital spikes was made not with the VER of the individual patient, but with VERs of a normal population. Further, the latencies for positive and negative components of the VER, as given by the authors, show a very wide range and in fact overlap. The relatively small interval between the flashes and the wide range of the VER components could result in a fortuitous relationship between occipital spikes and VER components. It has also been reported by Rodin *et al.*[2] that spikes preceding generalized seizures, induced by 'Megimide' during intermittent photic stimulation, were related to normal VERs and consisted of a marked exaggeration of the secondary VER components. Their report, however, is concerned with normal animals (cats); and no details of the photic stimuli and the spikes are given. For these reasons we felt that we should re-examine the problem. The existence of a relationship between abnormal and "normal" responses would be critical to any theory attempting to explain the genesis of the occipital "epileptogenic" spikes.

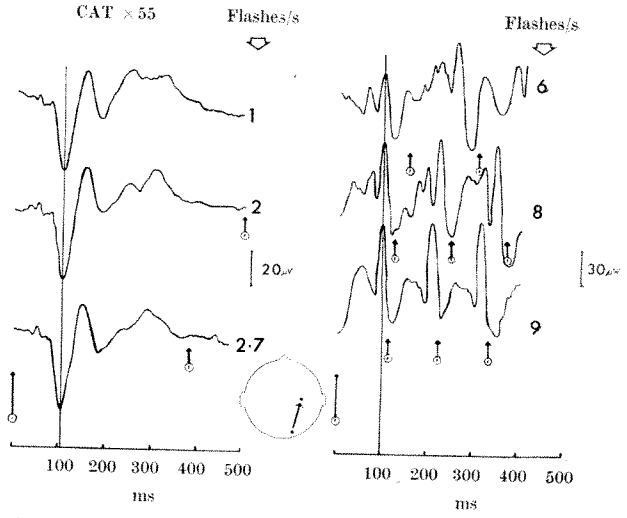


Fig. 1. Responses to photic stimulation in a female patient (R. O'B., code 34/60, aged 18) with photosensitive epilepsy. On the right are the responses to 6, 8, and 9 flashes/s. The negative spike at 100 ms is shown on the right by a vertical line. The only component of the average VER evoked by 1, 2 and 2·7 flashes/s which is of a similar latency is a positive one, indicated on the left of the figure by a vertical line. The horizontal line indicates time in ms. Flash stimuli are indicated by arrows. The CAT write-out is the average at 55 sweeps.

We have examined 23 patients with photosensitive epilepsy. The intermittent photic stimulation was delivered by a Kaiser stroboscope at 0·2 J. This stroboscope contains a metallic grid between a diffuse glass and the light source. The response to intermittent photic stimulation was recorded using a 'Mingograf' EEG machine at increased paper speed (15 and 30 cm/s) and increased gain (25 and 50 μV/cm). A frequency modulated tape recording was made at the same time, and was subsequently played back through an oscilloscope or Technical Measurement Corporation Computer of Average Transients (CAT). A special VER montage[3] was used.

All patients showed high amplitude generalized discharges during intermittent photic stimulation. In 21 of the patients (91·3 per cent) occipital spikes preceded the generalized discharges. This high incidence of occipital origin of the generalized discharges is much greater than has previously been reported[1] and is probably the result of the particular photic stimulus we used.

The responses to flash rates of 1–10 s^{-1} were examined in more detail in ten of these patients. We studied the VER produced by flash rates from 1–4 s^{-1} and occipital spikes occurring in response to 5–9 flashes/s. The occipital spikes[4] were triphasic (Fig. 1). The initial occipital positive component followed the preceding flash with a latency of 85·5 ± 9·5 ms. The most obvious part of the spike was a negative occipital component occurring at a latency of 102 ± 6 ms and followed by a variable positive component. The negative spike exhibited the following characteristics: (*a*) the spike appeared at 0·2–3·0 s after the onset of a train of stimuli and was never seen following the first flash; (*b*) the latency of the spike was constant for the same patient irrespective of the flash frequency over a range of 5–9 s^{-1}; (*c*) in all patients the frequency of the spike was fundamentally related to the flash rate. Some patients showed a gradual increase in the amplitude of the occipital spikes preceding a generalized discharge.

In nine of the ten patients no simple relationship could be found between the latency of the occipital spike and the VER of the individual patient, obtained at the same time and using the same technique. In four of these nine patients the latency of the negative occipital spike was similar to a positive VER component occurring with a latency of 100–112 ms. In only one patient was the occipital spike related in latency to the triphasic VER component V (Fig. 2) described by Gastaut and Regis[5]. No component of the generalized discharges following the occipital spikes showed any consistent or repeatable latency relationship to the flash stimuli.

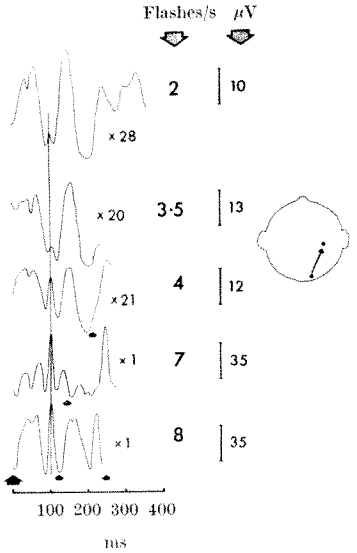


Fig. 2. Responses to photic stimulation in a female patient (M. F., code 33735, aged 24) with photosensitive epilepsy. The three upper traces show the average VER responses to 2, 3·5 and 4 flashes/s. The occipital spike evoked by 7 and 8 flashes/s is shown in the two lower traces and coincides with VER component V. The vertical line crosses the negative component of the occipital spike and the V *b* component of the VER. The horizontal line indicates time in ms. The flash stimulus is shown by a thick arrow.

The comparison of responses obtained at higher frequency intermittent photic stimulation (5–9 flashes/s) with those obtained at lower frequency intermittent photic stimulation (1–4 flashes/s) seems arbitrary. We have therefore compared the responses of ten normal subjects obtained at higher flash rates (5–9 s^{-1}) with those obtained at lower flash rates (1–4 s^{-1}). We found that the components of the VER whose latencies might have been related to those of the negative occipital spike remained unchanged over the range of 1–9 flashes/s. Further, no negative component appeared at latencies related to that of the negative occipital spike of photosensitive patients at 5–9 flashes/s intermittent photic stimulation.

Our observations show that (with the exception of one patient) no simple relationship exists between VER components and occipital spikes. The finding (*a*) that in one patient the occipital spike coincided with the VER triphasic component V, and (*b*) that there was a latency relationship between the positive VER component (equivalent to VER component V) and the negative occipital spike in four other patients, may be significant in that wave V has been related[5] to the scotopic and photopic system of the retina.

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Inhibition of Norepinephrine Stimulated Adenyl Cyclase by Theophylline

Theophylline, at a concentration of 10^{-2} M, has been extensively used as an inhibitor of the enzyme phosphodiesterase, which decomposes adenosine 3′,5′-phosphate (cyclic AMP) to adenosine 5-phosphate[1]. It has therefore found widespread use in the determination of the activity of adenyl cyclase which catalyses the formation of this cyclic nucleotide. A recent assay developed by Weiss and Costa[2] eliminates the need for theophylline by using a pool of unlabelled cyclic AMP to trap that generated from labelled ATP and to protect it from destruction by phosphodiesterase. While using this procedure to study the adenyl cyclase of rat erythrocyte ghosts, it was observed that the addition of theophylline inhibited the enzyme.

Adenyl cyclase activity of rat erythrocyte ghosts was determined by a modification of the method of Weiss and Costa[2] as described previously[3]. The results of three experiments reported in Table 1 demonstrate that 2×10^{-2}–4×10^{-2} M theophylline inhibits both the basal and norepinephrine stimulated adenyl cyclase but has no effect on fluoride stimulation. The decrease in norepinephrine stimulation appears to be greater than can be accounted

Table 1. Inhibition of adenyl cyclase activity

Experiment	Addition	Basal	10^{-4} M norepinephrine	10^{-2} M fluoride
1	None	0·117 ± 0·011	0·373 ± 0·017	0·537 ± 0·082
	2×10^{-2} M Tris	0·103 ± 0·004	0·328 ± 0·015	0·643 ± 0·068
	2×10^{-2} M theophylline	0·069 ± 0·004*	0·176 ± 0·012‡	0·754 ± 0·107
2	None	0·092 ± 0·023	0·148 ± 0·006	0·442 ± 0·015
	2×10^{-2} M theophylline	0·059 ± 0·003	0·105 ± 0·003†	0·440 ± 0·014
3	None	0·063 ± 0·007	0·154 ± 0·009	
	4×10^{-3} M theophylline	0·039 ± 0·004	0·140 ± 0·015	
	2×10^{-2} M theophylline	0·048 ± 0·008	0·078 ± 0·004†	
	4×10^{-2} M theophylline	0·024 ± 0·002*	0·069 ± 0·01†	

The data represent the means of three determinations along with their standard errors and are expressed in nmoles cyclic AMP/0·2 ml. ghosts/30 min.

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

for by a decrease in the basal level. Thus norepinephrine stimulated production of 0·256, 0·056 and 0·091 nmoles of cyclic AMP in experiments 1, 2 or 3, respectively, was reduced to 0·107, 0·047 and 0·03 nmoles by theophylline.

The addition of 2×10^{-2} M theophylline would increase the tonicity of the medium, so it was conceivable that the decrease in production of cyclic AMP resulted from a decreased permeability of the ghosts to ATP. In experiment 1, a comparable increase in tonicity was achieved by the addition of 2×10^{-2} M Tris buffer. As can be seen, no change in norepinephrine or fluoride stimulation of adenyl cyclase was obtained.

When the theophylline concentration was increased to 4×10^{-2} M, the inhibition of basal and norepinephrine stimulated adenyl cyclase was greater. The inhibition disappeared when the concentration of theophylline was reduced to 4×10^{-3} M.

This effect of theophylline becomes apparent only in those conditions where phosphodiesterase activity is not a factor. Such a situation exists by virtue of the fact that the cyclic AMP pool is so large that no significant decomposition of cyclic AMP occurs during the incubation period.

If the pool of unlabelled cyclic AMP is not used, the typical response to theophylline is noted, that is, it enhances the production of cyclic AMP by both norepinephrine and fluoride.

The implications of these observations are at the moment unclear because most, though not all, of the data obtained with theophylline (10^{-2} M) conform to its role as an inhibitor of phosphodiesterase. The recent finding that theophylline inhibited the increase in cyclic AMP produced by electrical stimulation of guinea-pig cortex slices[4] might be explained by an action at the level of the cyclase enzyme. Further work is obviously required to determine the nature of the inhibition of adenyl cyclase, its tissue specificity, and its significance in biological systems.

The results do serve once again to separate fluoride from hormone stimulation of adenyl cyclase as emphasized by Weiss[5] with the pineal gland. One might suggest that theophylline is acting at the level of the norepinephrine receptor or at the coupling between receptor and enzyme. Alternatively, it could act at the level of the cyclase such that the weak activation by norepinephrine, but not the strong activation by fluoride, is inhibited. We are not able to choose from among these suggestions.

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Enhancement of the Pre-irradiation Action of TAB

GRAM negative endotoxin is able either to reduce radiation mortality or at least to enhance the recovery of the haemopoietic system following total-body radiation (TBR)[1-4]. These beneficial effects have been observed in several species, for example mice, dogs, rhesus monkeys. The mechanism whereby endotoxin reduces mortality is obscure. The earlier recovery of haemopoietic cells observed after endotoxin treatment may be secondary to endotoxin-induced changes that may allow these tissues better to tolerate the diverse pathophysiological effects of the acute radiation syndrome.

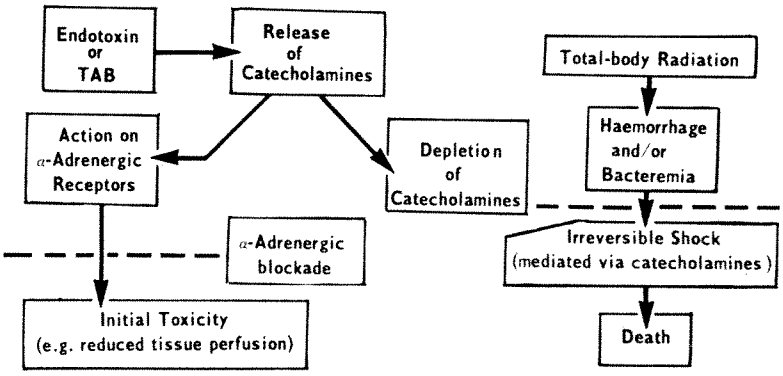


Fig. 1. Hypothesis for the post irradiation effect of TAB and endotoxins.

A recent study[2] suggests that some of the side effects seen shortly after the administration of endotoxin, given as typhoid paratyphoid A and B vaccine (TAB), may result from catecholamine release. Accordingly, the usefulness of TAB vaccine given after TBR could be increased by combining TAB with an α-adrenergic receptor blocking (α-ARB) agent. By contrast, the beneficial effects of endotoxin were lost (Table 1) if acetylation of the endotoxin molecule[5] was used to modify side effects. Substitution of β-adrenergic receptor blockage (β-ARB) for α-ARB also resulted in a loss of the benefits of post-irradiation treatment with TAB[6]. When the dose of TAB is lowered so as to minimize side effects, the mortality-reducing action of TAB is also lowered[3]; some of the beneficial effects of TAB, for example earlier platelet recovery, may be retained[4].

Table 1. ACETYLATED ENDOTOXIN ON RADIATION MORTALITY OF $C_3H \times AKR$ MICE

Treatment	Mortality (per cent)
700 rad only	62/64 (97)
700 rad + endotoxin*	35/63 (55)
700 rad + acetylated endotoxin	36/44 (82)

* *S. typhosa* endotoxin (Difco) 1 mg/kg 1 h after TBR.

The studies dealing with the side effects of TAB vaccine suggest that catecholamine release may, in the long term, play a part in the beneficial actions of TAB. Accordingly, a hypothesis was formulated for the post-irradiation action of TAB; this was based on the possibility that catecholamine depletion from tissues (especially from the adrenals) might allow better tolerance towards bacteraemic and/or haemorrhagic shock during the acute radiation syndrome[2]. The hypothesis is outlined in Fig. 1.

When given before irradiation, gram negative endotoxin more effectively reduces radiation mortality. Catecholamine release (leading to reduced tissue perfusion and hypoxia) might explain, in part, the pre-irradiation action of TAB. On this assumption, the pre-irradiation action of TAB should be enhanced by pre-irradiation treatment with a β-ARB agent and post-irradiation treatment with an α-ARB agent. This study was designed to test this hypothesis.

$C_3H \times AKR$ female mice (*Pseudomonas* free) were given various doses of TBR using 300 kVp X-rays as previously reported[2]. The irradiated animals were divided into four groups. Group I served as a control. One hour before irradiation, group II received 0·1 ml. of

TAB vaccine (Parke Davis) intraperitoneally. Groups III and IV received TAB as described; in addition group III was treated 0·5 h and 0·25 h before irradiation with the β-ARB agent propranolol hydrochloride ('Inderal', ICI). Each dose was 10 mg/kg intraperitoneally. (Given alone, propranolol does not reduce mortality.) In addition to TAB vaccine and β-adrenergic blockade, group IV was treated after irradiation with the α-ARB agent, phentolamine ('Rogitine', Ciba). The dosage schedule for phentolamine was as previously reported[2]. In all groups, animals started to die at about the middle of the second week following TBR. After a 30 day observation period, LD_{50} values and dose modifying factors (DMF) were calculated from the dose-mortality data[7]. Table 2 gives LD_{50} and DMF values for the four groups. β-ARB treatment alone enhanced the mortality-reducing action of TAB vaccine. Further enhancement was obtained when post-irradiation treatment with α-ARB was combined with pre-irradiation β-ARB.

Table 2. ADRENERGIC BLOCKING AGENTS ON PRE-IRRADIATION TAB

Group	Treatment		LD_{50}* (rad)	DMF
	Before TBR	After TBR		
I (Control)	—	—	620	—
II TAB	—	—	750	1·21
III TAB +	β-ARB	—	820	1·33
IV TAB +	β-ARB	α-ARB	860	1·39

* Similar slopes.

Assuming hypoxia (caused by reduced tissue perfusion) is partly responsible for the radioprotective action of TAB, then the enhancing action of the β-ARB agent, propranolol, could be due to further decreases in blood flow. Evidence for such an effect of propranolol was recently reported[8]. In these experiments, propranolol potentiated the ability of adrenaline to reduce blood flow through the superior mesenteric artery of the dog.

An effect of propranolol at the cellular level (for example, a cysteamine like action) is tentatively ruled out because this β-ARB compound does not modify the *in vitro* X-ray survival cure of a mammalian cell line[9]. Furthermore, no such effect would be expected for the known metabolites of propranolol.

TAB vaccine was administered shortly before TBR, so the long-term values of catecholamine depletion (Fig. 1) should add to the hypoxic action of TAB. This may explain why gram-negative endotoxin given before TBR is more effective in reducing radiation mortality than is post-irradiation treatment. For both the pre and post-irradiation actions of TAB, catecholamine release appears as a common factor. The earlier recovery of the haemopoietic system observed after endotoxin is explicable within the hypotheses proposed in this study. Hypoxia would reduce the effective radiation dose to the haemopoietic system, and catecholamine depletion might allow irradiated animals better to tolerate the bacteraemic and/or haemorrhagic shock associated with the acute radiation syndrome. Although additional studies will be required to substantiate the "catecholamine hypothesis", this study indicates that appropriate adrenergic receptor blocking agents may increase the capacity of gram-negative endotoxins to reduce radiation mortality.

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Production of Pyrroles from Pyrrolizidine Alkaloids by Human Embryo Tissue

PYRROLIZIDINE alkaloids are produced by a number of common plants including *Senecio* and *Heliotropium* species. They have been implicated in cases of bread poisoning in man in South Africa[1], and the infusion of certain of these plants to make bush teas is believed to cause veno-occlusive disease in children in the West Indies[2]. Grazing animals are frequently affected by these hepatotoxins, and the disease has therefore been closely studied during experiments on domestic and laboratory animals, especially rats; but relatively little is known of the effects on man. The toxic effects of heliotrine and the resulting depression of RNA, DNA and protein synthesis in human liver cells in culture have been studied recently[3].

The production of pyrroles from pyrrolizidine alkaloids, by rat livers, *in vivo* and *in vitro*, has been reported[4]. A modified Ehrlich's reagent gives a magenta colour with pyrroles, but does not react with the parent alkaloids. The pyrroles are highly reactive alkylating agents and are probably far more toxic than the alkaloids themselves.

Fulvine causes both lung and liver damage in rats[5], and West Indian children given bush tea containing fulvine have been admitted to hospital with veno-occlusive disease and pleural effusions[6]. The tests for the ability to metabolize three alkaloids, lasiocarpine, retrorsine and fulvine, were therefore carried out on both lung and liver tissues from human embryos.

Slices of tissue about 5 mm across and 2 mm thick were added to tubes containing the alkaloids dissolved in 1 ml. Eagle's medium, and rotated at 37° C. The pieces of tissue were heated for 3 min with 1 ml. of the modified Ehrlich's reagent at 85°–90° C. The pieces were then washed in alcohol and examined.

The liver slices all gave positive reactions with all three alkaloids, but the lungs gave negative results, even when 200 μg of alkaloid was used. Liver pieces were incubated with 100 μg of alkaloid for 24 h. Lasiocarpine consistently gave stronger colours than retrorsine; fulvine produced an intermediate intensity. This is interesting because it is the reverse of the order of toxicity in rats, and tissue culture work indicates that retrorsine is more toxic to human embryo liver cells than lasiocarpine (unpublished work). Liver slices incubated with 100 μg lasiocarpine showed a steady increase in the production of pyrroles from 1 h, to at least 48 h. Pieces of liver treated with 100 or 200 μg lasiocarpine for 24 h gave strong reactions of similar colour intensity; a slight colour was obtained with 50 μg, and no colour was detectable with 10 μg. The indicator system is not very sensitive so it was not possible to detect pyrroles in the incubation fluid or in monolayers of hepatocytes.

The results indicate that human embryo liver tissue is able to metabolize pyrrolizidine alkaloids, producing pyrroles. Either lung tissue cannot perform this reaction, or the amounts of pyrroles produced are too small to be detected.

Preliminary results using tissue culture seem to confirm that the alkaloids themselves have little or no effect on lung cells. Trypsinized lung or liver cells were implanted in mouse plasma on polythene disks, forming monolayers[7]. The cultures were incubated with an alkaloid, and then examined with acridine orange, or used for autoradiography[8]. After 3 days' incubation with 100 μg lasiocarpine, 83 per cent inhibition of DNA synthesis was found with hepatocytes. Identical experiments with lung cells showed that neither 100 μg lasiocarpine nor retrorsine had any effect on DNA synthesis. Similarly, it was found that monolayers of lung cells incubated for 7 days with 25 μg lasiocarpine or retrorsine appeared histologically normal, whereas hepatocytes treated in the same way were obviously abnormal.

Chemically produced pyrroles, when administered to rats, cause lung damage similar to fulvine intoxication[4]. The lung oedema in children with veno-occlusive disease could therefore be due to pyrroles produced in the liver and carried to the lungs via the blood stream.

The production of pyrroles *in vitro* does not seem to correlate with the toxicity of the alkaloids. This could be a consequence of the presence of varying proportions of the more hydrolysed pyrrole derivatives, which are non-toxic but which react to give a positive colour.

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Detection of H-2 Antigens on Mouse Spermatozoa by the Cytotoxicity Test

HISTOCOMPATIBILITY antigens of the type H-2 are found on most cells of the mouse, but there are conflicting reports concerning their expression on spermatozoa. Using the indirect immunofluorescence technique, Barth and Russell[1] found no H-2 antigen on spermatozoa. Using the techniques of immunogenicity, antibody absorption, and indirect immunofluorescence, Vojtiskova *et al.*[2] concluded that H-2 antigens were probably detectable on spermatozoa by all three procedures. As these authors point out, however, it is difficult to exclude entirely the possibility that contaminating cells were responsible for the positive findings with the first two techniques.

Fellous and Dausset[3] reported the presence of HL-A antigen on human sperm, and on the basis of the proposed homology between the *HL-A* and *H-2* loci it seems probable also that H-2 antigen is present on spermatozoa. In this investigation we used the cytotoxicity test[4,5] to obtain further evidence on whether or not H-2 antigens are in fact expressed on spermatozoa. The advantages of this test for the presence of cell surface antigens are (1) that individual cells carrying the antigen are seen to be lysed, so that minor contamination with cells of another type is immaterial, and (2) that control of serological specificity is easier than in the case of the indirect immunofluorescence method. It is therefore perhaps the most effective serological test available at the present time for demonstrating the presence of antigen on viable cells. Its application to spermatozoa, however, presented unforeseen technical difficulties. When these were overcome we were able to demonstrate without doubt that H-2 antigen is present on mouse spermatozoa.

The chief difficulty in applying the cytotoxicity test to spermatozoa is the toxicity of the sera used to provide complement. This is because of naturally occurring hetero-antibody. Rabbit serum is superior to guinea-pig serum in most immune cytolytic systems involving nucleate cells (reviewed by Haughton and McGehee[6]), but both are toxic for mouse cells. Thymocytes were already known to be more sensitive than other cells to hetero-antibody, and we soon found that spermatozoa are still more sensitive, making it essential to remove the hetero-antibody by pre-absorption with mouse cells. In our experience, this can only be done effectively (that is, without prohibitive loss of complement) by absorbing the rabbit serum with mouse cells in the absence of free calcium. Removal of calcium prevents the binding of complement and so permits the absorption of the hetero-antibody without concomitant loss of complement[7].

The mice used were from the inbred strains C57Bl/6 ($H\text{-}2^b$); A ($H\text{-}2^a$); C57Bl/H-2^k congenic with C57Bl/6; A.BY ($H\text{-}2^b$) congenic with A; AKR.K ($H\text{-}2^a$) congenic with AKR ($H\text{-}2^k$); the hybrid $(C57Bl/6 \times A)F_1$ ($H\text{-}2^{b/a}$); and the backcrosses $(BALB/c \times C57Bl/6)F_1 \times C57$ ($H\text{-}2^{d/b} \times H\text{-}2^b$) and $(C57 \times AKR)F_1 \times C57$ ($H\text{-}2^{b/k} \times H\text{-}2^b$); all were from the colonies maintained by E. A. B. (C57Bl/6 abbreviated to C57).

Sperm were obtained from different regions of the male reproductive tract. The vas deferens was removed and its contents extruded into a drop of phosphate-buffered saline (*p*H 7·0) containing 0·5 per cent fructose and 5 per cent foetal calf serum previously heated to 56° C for 30 min. The fructose and foetal calf serum were found to improve sperm viability. Epididymal spermatozoa were obtained by cutting the entire epididymis into sections in a few drops of medium; after 5 min the medium containing free spermatozoa was pipetted off. There was less than 5 per cent contamination with cells other than spermatozoa as determined in a haemacytometer by phase contrast microscopy.

Table 1. REMOVAL OF HETERO-ANTIBODY FROM SELECTED RABBIT SERUM BY ABSORPTION WITH MOUSE CELLS IN THE ABSENCE OF FREE CALCIUM

Number of absorptions of rabbit serum	Lowest dilution at which rabbit serum shows no toxicity*	
	For thymocytes	For epididymal sperm
0	1/16†	1/45
1	1/4	1/4
2	1/2	1/2

* Background < 15 per cent spermatozoa dead.
† Dilution before use in the test. Test constituents are equal parts of (*a*) serum dilution; (*b*) spermatozoa or thymocyte suspension (5×10^6/ml.); (*c*) medium 199 (for thymocytes) or phosphate buffered saline with 0·5 per cent fructose and 5 per cent foetal calf serum (for spermatozoa).

The four H-2 antisera used were prepared as described in ref. 8. They are as follows: C57/H-2^k (congenic with C57) anti C57 leukaemia EL4 designated H-2^k vs H-2^b; A anti-C57 leukaemia EL4 designated H-2^a vs H-2^b; C57 anti-A strain leukaemia ASL1 designated H-2^b vs H-2^a; and $(BALB \times C57)F_1$ anti-C3H sarcoma BP8 designated H-$2^{d/b}$ vs H-2^k. These had haemagglutination[9] titres of 1/1,000 to 1/8,000 and cytotoxic titres of 1/640 to 1/1,000 against lymphocytes of the relevant H-2 type.

Rabbit serum was used as the source of complement (C′) in the cytoxicity test. Twenty to thirty rabbits were bled from the ear and their sera titrated individually for toxicity against mouse thymocytes. Sera which were non-toxic at a dilution of 1/16 were then titrated for C′ activity in a known allogeneic cytotoxic system such as H-2. (The dilutions given refer to dilutions made before use in the cytotoxic test.) The rabbit with the highest C′ titre was then bled by cardiac puncture. Even these selected sera were toxic for

Table 2. SUMMARY OF CYTOTOXICITY TESTS ON MOUSE SPERM WITH H-2 ANTISERUM

(*a*) With singly absorbed rabbit serum (1/4) as source of C′

Antiserum	Sperm donor	Source	Per cent sperm dead (stained by trypan blue) Experiment a	b	c	d	e	f	g	h	i	j	k	l
H-2^k vs H-2^b	H-2^b (C57)	Epid	50											
		Vas	39											
	H-2^k (C57/H-2^k)	Epid	12											
		Vas	6											
H-2^a vs H-2^b	H-2^b (C57 or A.BY)	Epid		57	62	79	55	50	49	50	58	51		
		Vas		52	40	55								
	H-2$^{b/a}$ (C57 × A)	Epid					37	44	35					
	H-2^a (A or AKR.K)	Epid		14	15	10	15	16	15				15	
		Vas		10	9	10								
H-2^b vs H-2^a	H-2^a (A or AKR.K)	Epid					54	55	43				46	58
		Vas												51
	H-2$^{b/a}$ (C57 × A)	Epid					25	43	30					
	H-2^b (C57 or A.BY)	Epid					16	15	14	12	15	16		13
		Vas												8
H-2^b vs H-2^a plus H-2^a vs H-2^b	H-2$^{b/a}$ (C57 × A)	Epid					61	65	46					

(*b*) With doubly absorbed rabbit serum (1/2) as source of C′

Antiserum	Sperm donor	Source	Experiment m	n	o
H-2^a vs H-2^b	H-2^b (C57)	Epid	82	70	63
	H-2$^{b/a}$ (C57 × A)	Epid	74	56	53
	H-2^a (A)	Epid	15	10	16
H-2^b vs H-2^a	H-2^a (A)	Epid	76	55	75
	H-2$^{b'a}$ (C57 × A)	Epid	68	30	60
	H-2^b (C57)	Epid	15	16	16
H-2^b vs H-2^a plus H-2^a vs H-2^b	H-2$^{b'a}$ (C57 × A)	Epid	80	62	54

* The dilution of antiserum at which the highest percentage of spermatozoa were killed was usually 1/2 (undiluted antiserum was not tested).

mouse sperm at a dilution of 1/32 or more, and it was therefore necessary to absorb the hetero-antibody. For this purpose EDTA was added to the rabbit serum to a concentration of 0·01 M and the serum was then absorbed with washed viable mouse cells (8 : 1 v/v) for 30 min in an ice bath. The cells used for absorption were prepared by washing in Earle's balanced salt solution lacking calcium and magnesium. Preliminary tests indicated that testicular cells, prepared by gentle homogenization of testis, were most efficient in absorbing hetero-antibody. Spleen cells, leukaemia cells and thymocytes, as available, were also added. After absorption, $CaCl_2$ was added to restore the level of free calcium. These absorbed sera were non-toxic to sperm at a dilution of 1/4. A second absorption decreased toxicity further, allowing the use of the absorbed serum at a dilution of 1/2 (Table 1).

Doubling dilutions of H-2 antisera were prepared. Equal volumes (0·05 ml.) of antiserum dilution, sperm suspension (4×10^6–5×10^6/ml.), and absorbed rabbit serum diluted 1/4 (or 1/2 of doubly absorbed rabbit serum) were incubated for 45 min at 37° C. Then 0·1 ml. of trypan blue solution was added. (1 per cent trypan blue was made up in distilled water, and diluted immediately before use with 42·5 g/l. NaCl, 1 part in 5, to render it isotonic.) The percentage of dead spermatozoa was determined in a haemacytometer. Live spermatozoa do not take up trypan blue whereas dead spermatozoa take up stain in the head region.

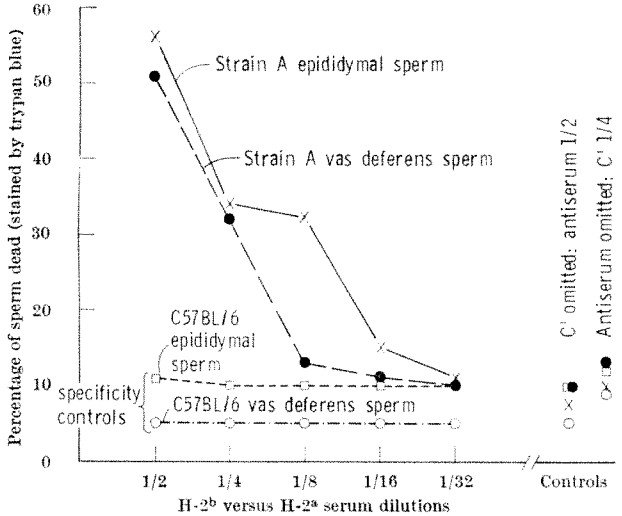


Fig. 1. Detection of H-2^a on epididymal and vas deferens sperm of A strain mice by the cytotoxicity test.

A representative test is illustrated in Fig. 1. Results summarized in Table 2 leave no doubt that spermatozoa are susceptible to H-2 antisera. Specificity of the reaction for H-2 is shown, for example, by the results with the H-2 antiserum H-2^k vs H-2^b prepared in congenic strains of mice (Table 2, experiment a). As a further test of specificity, spermatozoa from backcross mice already typed for H-2 by haemagglutination[9] were tested with H-2 antisera by the cytotoxicity test; these blind tests invariably showed a positive correlation (Table 3).

The proportion of spermatozoa lysed by H-2 antisera was greater when they were from the epididymis than when they were from the vas deferens, suggesting either that H-2 antigens are reduced in amount as the sperm matures, or that they are masked by secretions from the genital tract which are known to become adsorbed on spermatozoa[10]. Indeed, present evidence does not exclude the possibility that the H-2 antigen is actually carried by adsorbed secretions.

Table 3. CYTOTOXICITY TESTS ON SPERMATOZOA FROM BACKCROSS MICE TYPED INDEPENDENTLY FOR H-2 BY HAEMAGGLUTINATION

Backcross: (BALB × C57)F$_1$ × C57 (H-2$^{d/b}$ × H-2^b) Antiserum: H-2^b vs H-2^{a*} H-2 type haemagglutination	Cytotoxicity test on sperm (per cent dead)	Backcross: (C57 × AKR)F$_1$ × C57 (H-2$^{b/k}$ × H-2^b) Antiserum: H-2$^{d/b}$ vs H-2^k H-2 type haemagglutination	Cytotoxicity test on sperm (per cent dead)
db	88	bk	50
db	89	bk	45
db	87	bk	52
db	76		
db	72	bb	< 10
db	87	bb	< 10
db	88	bb	< 10
		bb	< 10
bb	15	bb	< 10
bb	5	bb	< 10
bb	13	bb	< 10
bb	14		

* Relevant H-2 specificities: 3, 4, 8, 10, 13.

The proportion of spermatozoa lysed by H-2 antisera was generally higher in H-2 homozygotes than in H-2 heterozygotes (Table 2, experiments e, f, g, m, n and o). This may indicate that only one H-2 haplotype is represented on epididymal spermatozoa. On the other hand, the cytotoxic reaction of H-2 antisera on spermatozoa obviously represents a weak detection system, and in such systems the reaction with heterozygotes is weaker than that with homozygotes because of the lesser amount of antigen on the former. The sensitivity of the H-2 cytotoxic reaction will have to be increased, or some other method found to distinguish between these two possibilities.

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Sex Difference in Trophoblast Behaviour on Transplantation

STUDIES on conditions resulting in the premature termination of pregnancy indicate that the male conceptus may be less well adapted to survive in the maternal host than the female. Sex chromatin determinations[1] carried out on chorionic villi of spontaneously aborted foetuses have shown that the majority of foetuses lost are male[2,3]. Moreover, more male foetuses than female seem to be involved in premature termination as a result of antepartum haemorrhage[4]. Because the trophoblast is the foetal tissue most intimately associated with the maternal host during pregnancy, we decided to examine the influence of foetal sex on the ability of trophoblast cells to survive as allografts.

Foetuses with attached placentae were obtained by hysterotomy on 6–8 week pregnant guinea-pigs, under anaesthesia. Trophoblast tissue was separated from the placenta, and lung or skin removed from the foetus, and placed in dishes containing warm sterile Eagle's minimal essential medium (MEM). This tissue was washed in warm MEM, cut into 2 mm fragments and trypsinized (0·05 per cent trypsin in calcium and magnesium free PBS) at room temperature. After 5 min, the supernatant was discarded, fresh trypsin added, and the tissue agitated gently for a further 5 min using a magnetic stirrer. The supernatant was collected, 1 ml. growth medium (MEM + 20 per cent foetal calf serum) added and the procedure repeated four or five times. The resultant supernatants were combined and centrifuged at 500*g* for 10 min. The supernatant was discarded and the cell pellet resuspended in MEM to give a final cell concentration of 10^7 cells/ml.

Eight virgin female and twelve mid-term pregnant guinea-pigs (multiparous and primiparous) were used as recipients in this study. Each animal received six intradermal inoculations of 0·1 ml. of each of the following cell suspensions from one pregnant donor animal: (1) trophoblast cells from a male foetus; (2) lung or skin cells from the same male foetus; (3) trophoblast cells from a female foetus; (4) lung or skin cells from the same female foetus. Intradermal injections of cell suspensions were made into marked areas on the back of recipient guinea-pigs, and a sample of skin removed at various intervals for histological examination.

In the virgin female recipients, non-trophoblast foetal cells (skin or lung) were rapidly destroyed by the host, no donor cells being found later than six days post-inoculation. No obvious sex or tissue differences were found in the behaviour of the non-trophoblast foetal cells on transplantation. Trophoblast cells in the same recipients survived considerably longer than the other foetal tissues, the former being present in the recipient for up to 14 days post-inoculation for male trophoblast and 20 days for female trophoblast cells (Table 1).

With pregnant recipients, the non-trophoblast foetal cells were rejected less rapidly than they were by virgin females, healthy cells being present as late as 10 days post-inoculation. Trophoblast cells, however, were present at 35 days post-inoculation in the case of the female tissue, and at 20 days for the male (Table 1). Female trophoblast cells were also seen to be proliferating from 10 days onwards, large numbers of healthy cells, some showing mitotic activity, being present in the dermis of the recipient animals at the end of the experiments 35 days post-inoculation.

The results indicate that allografts of separated female trophoblast cells will survive considerably longer than the corresponding male cells, in both virgin and pregnant hosts, and that the female trophoblast cells are capable of proliferating in an ectopic environment, as late as 35 days after transplantation, in the pregnant host. No such obvious sex difference was found in the behaviour of the non-trophoblast foetal tissue. These results seem to confirm conclusions drawn from earlier studies on tumours of the trophoblast in which it was shown that most trophoblast tumours were chromatin positive[5,6], indicating that the foetuses concerned were genotypic females.

Table 1. SURVIVAL TIMES OF CELLS AFTER INTRADERMAL INOCULATION

Recipient	Allograft	Donor sex	Surviving cells. Days post-inoculation 3	6	10	14	20	35
Virgin	Foetal	Male	+++	+	0	0	0	0
		Female	+++	+	0	0	0	0
Female	Trophoblast	Male	+++	++	+	+	0	0
		Female	+++	+++	++	++	+	0
Pregnant	Foetal	Male	+++	++	+	0	0	0
		Female	+++	++	+	0	0	0
Female	Trophoblast	Male	+++	+++	++	++	+	0
		Female	+++	+++	++++	++++	++++	+++++

Surviving healthy guinea-pig trophoblast and foetal lung cells in serial sections of skin taken from recipient animals at various times after intradermal inoculation of 0·1 ml. of cell suspension (approximately 10^6 cells).

Thus, trophoblast cells arising from a female conceptu seem to be better able to survive as allografts in a maternal host than the corresponding male, and in certain circumstances the cells may even continue to proliferate in the absence of a viable foetus. We shall attempt to characterize the mechanisms underlying the observed sex-associated differences in the behaviour of the trophoblast and its tumours.

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Maturation of the Maternofoetal Transport System for Human γ-Globulin in the Mouse

THE plasma concentration of γ globulin (IgG) in the human conceptus after 22 weeks or less of gestation is a fifth to a twentieth of that in the mother[1,2], but from 26 weeks to the end of gestation the two concentrations are the same[1,2]. Because the foetus, even at term, can synthesize only small amounts of γG, this marked increase in the plasma IgG seems to be a consequence of an abrupt change in the permeability of the maternofoetal barrier to IgG. We have investigated the nature of this change in permeability in the pregnant mouse.

Swiss albino Webster mice were used. During 10 days, seven groups of females were placed with mature males for 1 or 2 days, each group being bred on a different day; two or three mice became pregnant in each group. On the eighteenth day after the first mating, all pregnant mice started to receive drinking water containing 0·25 g per cent of sodium iodide. On the next day, they were given a single intravenous injection containing 2 μCi of human IgG labelled[3] with iodine-131 and 2 μCi of human serum albumin labelled[3] with iodine-125; the amounts of IgG and albumin injected were less than 0·1 mg and 1 mg, respectively. Twenty-four hours after the injection, on the twentieth day after the first mating, mice were anaesthetized and bled. Aliquots of maternal serum were precipitated in 10 per cent trichloroacetic acid (TCA). The foetuses in a given litter were pooled, weighed and homogenized in 10–20 volumes of 10 per cent TCA. The maternal serum precipitates and the precipitates in aliquots of the foetal homogenates were washed twice in 10 per cent TCA, and assayed for radioactivity using a 400 channel spectrometer with a 3 inch sodium iodide crystal. The ratio of the amount of iodine-131 and iodine-125 per g of foetal tissue, F, to the amount of iodine-131 and iodine-125, respectively, per ml. of maternal serum, M, was taken as a measure[2,4] of the extent of maternofoetal transfer of human IgG and human albumin, respectively.

Fig. 1 shows that the F/M ratios for human albumin increased approximately four-fold from 11 days of gestation to term, and that most of this increase occurred after the sixteenth day. The F/M ratios for human IgG at 11 days of gestation were only slightly greater than for albumin, but by 12 days maternofoetal transfer of human IgG had increased dramatically. On the fifteenth day of gestation human IgG F/M ratios were 100 times those on the eleventh day, and remained so until term. Thus in the mouse maternofoetal barrier there is a rapid increase in permeability to human IgG between 11 and 15 days of gestation, much like the rapid increase in the permeability of the human placenta to IgG between 22 and 26 weeks of gestation.

In the pregnant mouse, human serum albumin is transferred from mother to foetus by a first order process[4], that is the amount of human albumin transferred to the foetus is proportional to the maternal serum concentration of human albumin, or F/M for albumin $= k$, where k is the proportionality constant. Human IgG in the pregnant mouse at term is transferred by two processes[4]: one is first order, like that for albumin, and the other is an enzyme or carrier-mediated process. The first order proportionality constant for human IgG transfer is similar in magnitude to that for human albumin[4], so that the ratio of the amount of human IgG transferred only by the first order mechanism, F_f, relative to the maternal human IgG concentration, M, or F_f/M, is closely approximated by the F/M ratio for human albumin[4]. The net amount of human γG transferred to the foetus solely by the enzyme or carrier-mediated process, F_c, relative to the maternal human IgG concentration, M, or F_c/M, is equal to F/M for human γG minus F_f/M; because F_f/M is approximately equal to the F/M ratio for human albumin F_c/M is approximated by F/M for human IgG minus F/M for human albumin. Applying this information to the data in Fig. 1, it is apparent that the sudden increase in the permeability of the maternofoetal barrier to human IgG after 11 days of gestation is attributable to a rapid increase in the activity of the enzyme or carrier-mediated transport system for human IgG.

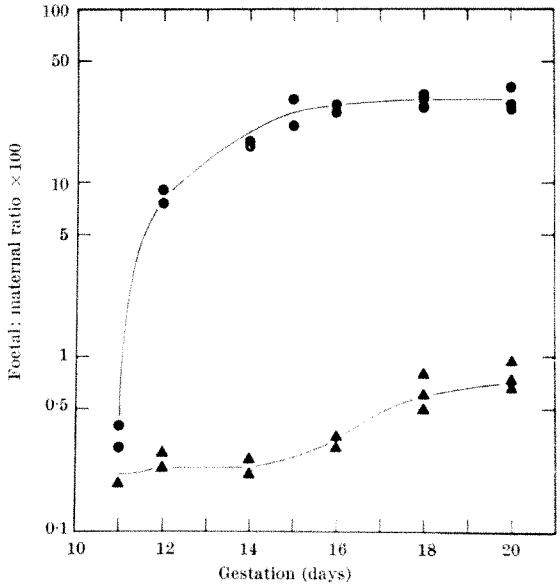


Fig. 1. Ratio of the foetal tissue concentration to maternal serum concentration of human IgG (●) and human albumin (▲) 24 h after intravenous injection into the mother. The period of gestation is that at the time of sampling.

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Assessment of Marginal Malnutrition

MANY tests have been proposed for the early detection of protein–calorie malnutrition (PCM) of early childhood; the latest is based on morphological changes in the root of the hair from the head[1]. The tests may be classed as biochemical (for example, amino-acid ratio in plasma[2], serum transferrin[3], hydroxyproline excretion in urine[4], urinary creatinine/height ratio[5], urinary urea/creatinine ratio[6]); anthropometric (a single measurement like weight, mid-arm circumference, or a ratio—chest/head circumference, weight/height, weight/head circumference[7]); or miscellaneous (buccal mucosal changes[8], auricular cartilage elasticity[9] or hair root morphology[1]). Tests on young children, frequently of uncertain age, pose practical problems. Ideally, any biochemical test should require only a small (capillary) sample of blood or "spot" sample of urine and a minimum of equipment and skilled personnel. Other variables often influence the results of these tests; for example, infections and infestations, age, time and nature of meal before test[10–12] and for the blood tests the degree and type of PCM (refs. 3, 13). Anthropometry has the advantage of being easy to perform, rousing

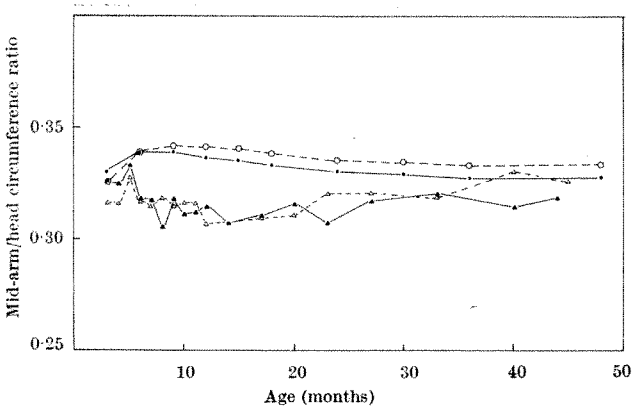


Fig. 1. Mid-arm circumference/head circumference ratio in relation to age and sex. The standard curves are from the data of Wolanski[15] and the Lebanese are mean values for 1,231 children in a field study. ●—●, Standard males; ○ - - - ○, standard females; ▲—▲, Lebanese males; △ - - - △, Lebanese females.

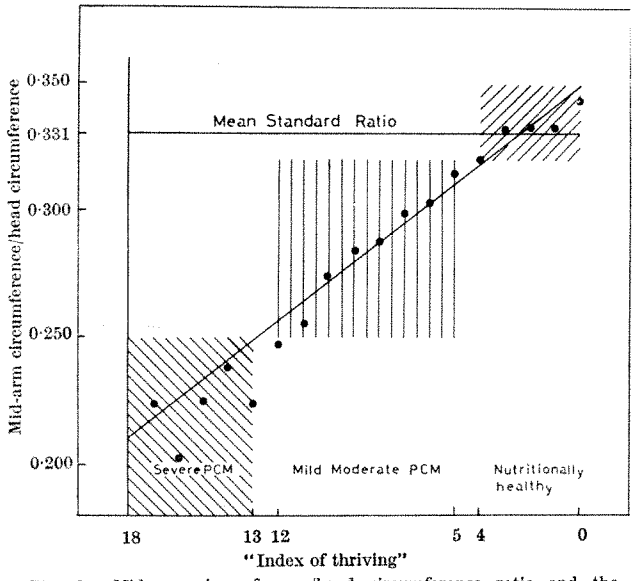


Fig. 2. Mid-arm circumference/head circumference ratio and the "index of thriving"[16] for 1,276 children (1,231 in a field study and 45 with acute marasmus on admission to the metabolic unit). The mathematical regression line for the highly significant correlation of these measurements is shown. The mean standard ratio is from the modified data of Wolanski[15]. ● = Group mean; $r_{1276} = 0{\cdot}75$ ($P < 0{\cdot}001$). $y = 0{\cdot}349080 - 0{\cdot}007688\,x$.

no antagonism and requiring only simple apparatus. But all of the measurements proposed hitherto are to some extent age-dependent. Mid-arm circumference is least affected, because it changes only slightly between 1 and 4 years[7]. Weighing requires a relatively expensive and delicate piece of apparatus that is often used incorrectly by junior personnel.

The hair root morphology test has several disadvantages. Epilation is traumatic to the subject and possibly to the hair. The procedure is tedious and time-consuming. Laboratory equipment and technicians are required. The authors criticize clinical signs for being "difficult to standardize and express quantitatively". The same may be said for their "subjective classification of the incidence of certain abnormalities" of the hair root, the method for which they do not make clear, "to assess the relative severity of protein–calorie malnutrition". They state that the differences in mean hair root diameter "were not highly significant ($P > 0{\cdot}05$)" for the intermediate weight/age groups, but this is the range where assessment is especially sought. Differences with $P > 0{\cdot}05$ are usually regarded as "not significant". It remains to be seen if comparable changes in hair morphology occur in other ethnic groups and where marasmus predominates.

When international standards[14,15] are used certain ratios of somatic measurements proposed by Jelliffe[7] vary considerably with age during the pre-school period and the chest/head ratio also differs for the two sexes. We propose a more satisfactory alternative in the mid-arm circumference/head circumference ratio. The measurements are easy to take and a stout tape measure cheap. As shown here, the ratio can be interpreted directly. This ratio shows virtually no sex difference and between 3 and 48 months is practically constant. The curve of the mean values for a group of 1,231 Lebanese children of low socio-economic status is slightly lower than that of the standard values and is also unaffected by age or sex (Fig. 1). We have developed an "index of thriving"[16], consisting of weight, height, head circumference, and mid-arm circumference measurements in relation to international standards. Fig. 2 shows the relationship between the mid-arm/head ratio and the "index of thriving" for 1,276 children (1,231 in the field study and 45 with acute marasmus on admission to our metabolic unit, each with an "index of thriving" of 13 or above). The following nutritional interpretation is recommended: ratio $> 0{\cdot}310$, nutritionally healthy; $0{\cdot}310$–$0{\cdot}280$, mild PCM; $0{\cdot}279$–$0{\cdot}250$, moderate PCM; $< 0{\cdot}250$, severe PCM.

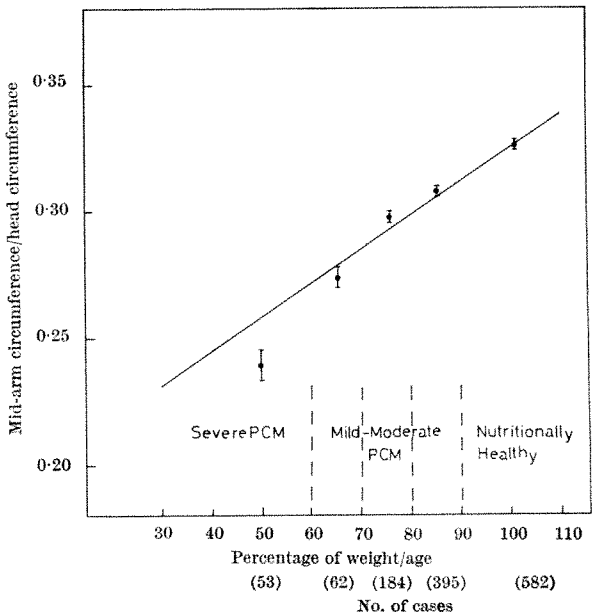


Fig. 3. Relationship between the mid-arm circumference/head circumference ratio and the percentage weight/age. Bars indicate group mean ± s.e.; $r_{1276} = 0{\cdot}68$ ($P < 0{\cdot}001$). $y = 0{\cdot}190905 + 0{\cdot}001341\,x$.

The mid-arm/head ratio data are compared with the weight for age in Fig. 3. Comparison should be made with Fig. 1 of Bradfield and Jelliffe[1]. Overall results are similar for the two tests, but for our data the differences between means of the ratios for all five of the weight/age groups were highly significant ($P < 0{\cdot}001$).

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Enzymatic Destruction of Bone *in Vitro*

THE enzyme phosphoprotein phosphatase catalyses the rapid demineralization of tooth enamel[1,2]. It has no demonstrable proteolytic activity, does not seem to hydrolyse synthetic hydroxyapatite, and is thought to act by dephosphorylating the mineralized phosphoprotein matrix of the enamel.

Bone is destroyed *in vitro* by phosphoprotein phosphatase, and the enzyme could be involved in the resorption of bone in pathological and physiological conditions[3]. The bone specimens after treatment with the enzyme, however, demonstrated a protein-like coagulum. The major protein constituent of bone has been shown to be collagen, and so we have investigated the effect of collagenase plus phosphoprotein phosphatase on specimens of bone.

Fragments of bone were dissected from the mandibles of 21-day-old COBS albino rats and were defleshed and enclosed in dental wax so that an area of bone of approximately 3 × 3 mm was exposed to the test solutions. The specimens were incubated for 3 or 6 h at 37° C with constant shaking speed[4] in 2 ml. of the following solutions: Deionized water, 0·1 per cent trypsin, 0·1 M sodium acetate buffer at *p*H 6·0, 0·01 per cent phosphoprotein phosphatase, autoclaved, heat-inactivated, 0·01 per cent phosphoprotein phosphatase, 0·1 per cent collagenase or a combination of 0·1 per cent collagenase and 0·01 per cent phosphoprotein phosphatase. All enzymes were prepared in the acetate buffer.

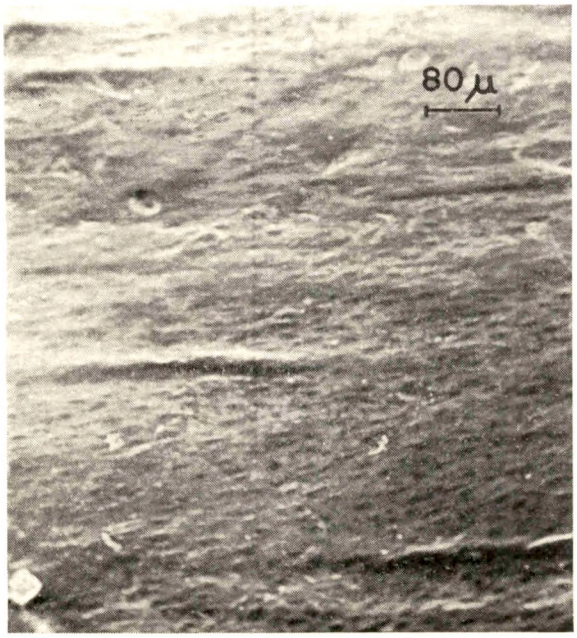


Fig. 1. Untreated surface of rat mandible.

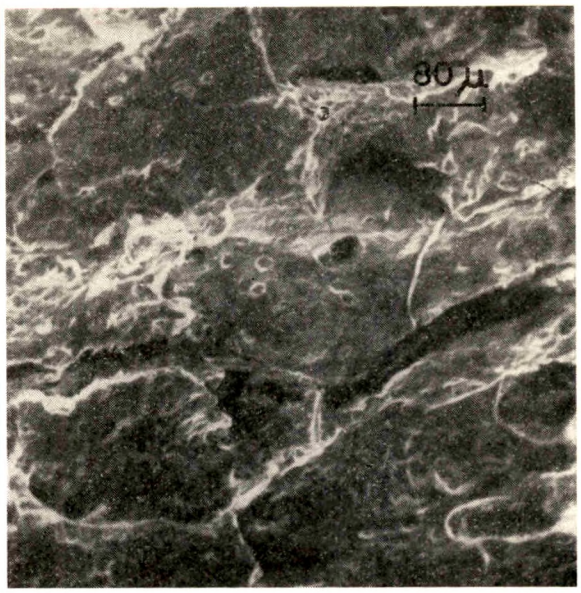


Fig. 2. Specimen of rat mandible incubated with 0·1 per cent collagenase for 6 h.

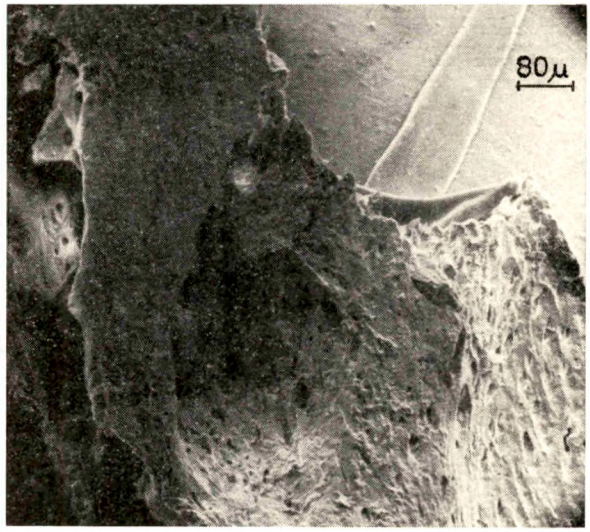


Fig. 3. Specimen of rat mandible incubated with 0·1 per cent collagenase and 0·01 per cent phosphoprotein phosphatase for 3 h.

After incubation all specimens were examined through a dissecting microscope and photographed. Selected specimens were then prepared for scanning electron microscopic study.

Fig. 1 shows an untreated control specimen of rat mandible as imaged by the scanning electron microscope. The surface is relatively smooth and intact, and there is no evidence of deformity. The surfaces of the mandibles exposed to buffer alone, water, trypsin or heat-inactivated phosphatase did not differ from those of untreated mandibles.

A typical specimen of bone exposed to collagenase for 6 h (Fig. 2) had a rough surface covered with numerous irregular holes, the length of which ranged from approximately 80 to 200 μm.

Fig. 3 is a representative scanning electron micrograph of a rat mandible exposed to a mixture of phosphatase

and collagenase for 3 h. There is extensive alteration of the mandibular surface topography with the formation of large craters, sometimes reaching several hundred microns across. Destruction was always more rapid when brought about with a mixture of phosphatase and collagenase than when either enzyme was used singly.

The scanning electron microscope has provided evidence that rapid destruction of bone can be accomplished *in vitro* by using enzymes. Similar enzymes are present in leucocytes and in the normal inflammatory process[5–7]. They can therefore be implicated in the mechanisms of bone destruction *in vivo*. Experiments are in progress to demonstrate enzymatic destruction of bone in the living animal in the hope of establishing a model for study of destructive bone diseases, such as periodontal disease.

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Bimodal Cochlear Response Curves in Rodents

LITTLE work has been done on hearing in rodents at high frequencies (over 30 kHz). Ralls[1] has shown sensitivity at frequencies up to 80 kHz in wild mice (*Mus musculus* and *Peromyscus*) and Crowley *et al.*[2] showed marked sensitivity to 40 kHz in the laboratory rat. Ultrasound emission has been shown by Anderson[3], Zippelius and Schleidt[4] and later by Noirot[5] and Sewell[6–8] to be widespread among rodents. In the light of this work on ultrasound production and the paucity of work on such aspects of hearing, I undertook an investigation of the cochlear microphonic response to these high frequencies.

Three species were used in the study: wild-caught wood mice (*Apodemus sylvaticus* Linnaeus), voles (*Clethrionomys glareolus* Schreber) and laboratory bred gerbils (*Meriones shawi* Rozet). Cochlear responses (CMs) were recorded from the round window membrane. Two techniques were used to elicit this response. The first was that of "pure tone" pulses of 1 ms duration, including 0·25 ms rise and fall times, delivered by an ionophone loudspeaker from 2 kHz to 100 kHz at rates of 1–20/s. The sound intensity of the pulse "plateau" was maintained constant at 60 dB sound pressure (level with respect to 2×10^{-4} μbar root mean square) during experiments. The second technique involved a high voltage pulse generator producing a spark across an air gap. The broad-band sound so produced had a dependable spectrum ±5 dB between 5 kHz and 100 kHz and was less than 60 dB SPL. Spark pulses were delivered at 200/s. Radiation artefact in both techniques could be recognized and distinguished from the results.

The recording equipment consisted of a 63 μm diameter silver wire electrode with a small terminal sphere formed by melting the tip. The latter was placed on or near the round window membrane after a lateral approach through the bulla. A stainless steel earth electrode was placed in the neck. A head-stage amplifier with a gain of 1,000 was used with a variable band-pass filter. The filter was narrowly tuned to the frequency being investigated when the ionophone was the stimulating apparatus, and the peak-to-peak amplitude of the cochlear response was measured from an oscilloscope screen. With the spark technique, the filter was set to a band-pass of 1 kHz–150 kHz and the CMs were recorded on tape to be displayed later on a sound spectrograph as a time-integrated spectrum. This was then replotted for comparison with the response curve obtained by the first technique.

Sound intensity produced by the ionophone was monitored with a B & K 0·25 inch (6·4 mm) microphone. The same apparatus was used to check the sound spectrum of the spark, so that slight, but consistent, corrections could be applied to the response curves from the cochlea. Experimental animals were injected with 'Nembutal', and the rectal temperature was maintained at 36°–38° C during experiments.

Cochlear response curves were obtained for fourteen individual *Apodemus*, eleven *Clethrionomys* and ten *Meriones* by both techniques. The most significant feature common to all three sets of results was the bimodal appearance of the curves obtained. In *Apodemus* peaks occurred in most animals at 17 kHz and 55 kHz with individual variation from 15 kHz to 20 kHz for the first peak and 50 kHz–65 kHz for the second. In *Clethrionomys*, for which specimen curves for one individual are shown in Fig. 1, the peaks appeared at 13 kHz and 50 kHz, with scatter over 10 kHz–17 kHz and 45 kHz–55 kHz, respectively, in different individuals. In *Meriones* the first peak was more variable, appearing at 4 kHz in some, as found by Finck and Sofouglu[9], but at 10 kHz in others. The second peak appeared fairly consistently at 40 kHz–45 kHz with some individual scatter over 35 kHz–50 kHz. In all forms there was a clear dip in sensitivity between the two peaks. There was also specific variation

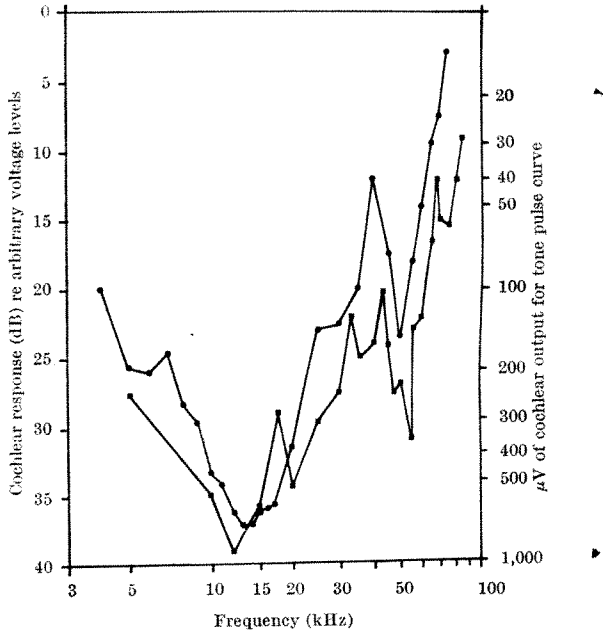


Fig. 1. Cochlear microphonic response curves for an individual *Clethrionomys*, obtained by two independent methods. The stimulating apparatus was: ●, ionophone; ■, spark.

in the height of the second peak, that of *Meriones* being considerably smaller than those of *Apodemus* and *Clethrionomys*.

The significance of these second peaks finds a possible explanation when the work of Sewell on ultrasound production in these species is considered. In all three cases the frequency of the second peak is closely related to the ultrasonic frequencies emitted by the animals, and the height of this peak appears to be inversely related to the intensity of the sounds produced.

Cries of young animals of the species studied have been used to stimulate the cochlea of adults of the same species. The cry has been recorded, simultaneously, as CMs from the cochlea, and with a B & K microphone. The cochlear response was shown to be strong in all cases, but especially when the adult showed a pronounced second peak in its response curve on later analysis.

While the CM response cannot be said to represent hearing at the conscious level[10], it is quite plausible that the marked sensitivity of the cochlea to specific high frequencies will facilitate hearing of these sounds. Sewell[11] has shown, by behavioural techniques, that *Apodemus* respond to the recorded cries of their young.

A bimodal response has also been shown from the colliculus of the bat *Plecotus*[12], where the frequencies of the two peaks of sensitivity correspond to the harmonic sound spectrum used in echolocation. A similar echolocatory connexion has been assigned to the sensitivity of the rat cochlea to 40 kHz (ref. 2), but it now seems more likely that this peak, like those of *Apodemus*, *Clethrionomys* and *Meriones*, could be related to the high frequency sounds emitted in social encounters.

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Initiation of Circadian Rhythms in Arrhythmic Churchyard Beetles (*Blaps mucronata*)

IN a recent article Brady[1] has drawn attention to the conflicting evidence on the physiological control of circadian rhythms in insects and concludes that the primary control is not hormonal but nervous. This conclusion is based in part on the failure of Roberts[2] and of Brady[3] himself to repeat Harker's[4] transplantation experiments in which the sub-oesophageal ganglia from rhythmic cockroaches elicited a rhythm in headless recipients. In Brady's work only two of forty-eight implanted animals subsequently became rhythmic. Similar results have been obtained by Fletcher[5] using the churchyard beetle (*Blaps mucronata*). We have subjected arrhythmic beetles to transplantation of organs from rhythmic donors, and sundry other treatments.

The beetles used in the experiments had been kept in culture chambers in constant light at 25° C for at least 2 weeks and mostly for much longer periods. Their locomotor activity was recorded in 6 inch diameter glass chambers with waxed floors. A cadmium sulphide photocell was triggered when the insect interrupted a beam of far red light provided by a 6·5 V bulb fitted with a far red filter. The photocell was connected by a high speed relay to a Rustrak 92 four-channel pen event recorder which marked on a moving paper trace. The number of times the beam was crossed in each hour was counted and the results plotted as a histogram. All recordings were made in a regime of constant light (intensity 40 lux). The rhythmic donors were maintained under conditions of alternating light and darkness, the light period extending from 0800 h to 2000 h. In these conditions *Blaps mucronata* shows a 24 h rhythm with peak activity from 2100–0400 h and little activity during the light phase[5].

The recording chambers were housed in unheated incubators but kept in a room at 25° C and 70 per cent RH. The light bulbs inside the incubators raised the temperature to 27°–28° C but each incubator remained constant to within ±1° C.

Table 1. THE RESULTS FOR TRANSPLANTATIONS OF SUB-OESOPHAGEAL GANGLIA AND BRAINS

Organs implanted	Result
Sub-oesophageal ganglion (12 transplants)	2 Died 3 No response 3 Level of activity dropped; no rhythm induced 4 Rhythm induced; peak in phase with operation
Brain (14 transplants)	4 Died 3 No response 3 Level of activity dropped; no rhythm induced 2 Rhythm induced; peak in phase with operation 2 Rhythm induced; peak in phase with donor

Two of the ten animals that survived the implantation of a brain from a rhythmic donor subsequently became rhythmic themselves, in phase with the activity of the donor. One of these results is shown in Fig. 1*a*. The proportion of implants inducing rhythmicity is similar to that found by Fletcher[5] and slightly higher than the results of Brady[3]. These workers induced rhythmicity by implanting sub-oesophageal ganglia but had no success with brains.

The low proportion of successful transplants may indicate that it is not the transplant itself that induces rhythmicity, but some side effect of the operation. The most likely factors are light and temperature changes. Lights used during transplant operations may be bright enough to initiate a rhythm. Even in headless recipients, as used by Brady[3], light acting directly on sensory cells of the body wall or on the central nervous system itself could be responsible, while thoracic and abdominal temperature receptors will, of course, still be present. A temperature cycle subsequent to the transplant operation has been suggested as the initiator of rhythmicity in headless cockroaches, but this could not be established experimentally[2].

We have tested the effects of single short-duration changes of temperature and illumination on arrhythmic animals in constant light (LL). 60 W lamps were used as a source of light and heat. Of ten animals heated (27° C + 10° C) for 60 min, eight became rhythmic. Eleven other animals were exposed to similar illumination but maintained for 60 min at 10° C below the temperature at which their activity had been recorded; of this group only three became rhythmic. The heat treatment produced a greater proportion of rhythmic animals than did transplants. Moreover, the activity induced is not usually in phase with the time of exposure to higher temperatures but 4–10 h later (Fig. 1*b*).

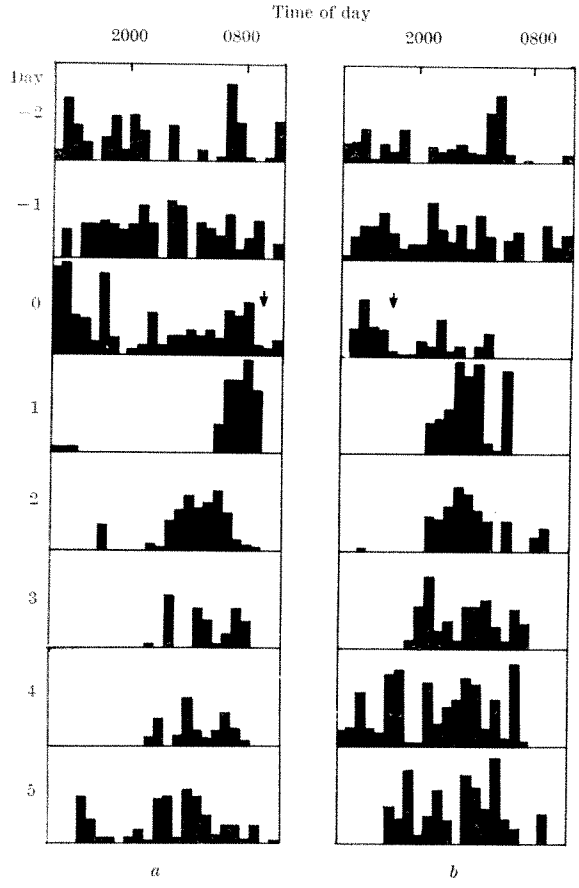


Fig. 1. The effect on the locomotor activity of an animal kept in constant light (LL) at 25° C of: *a*, the implantation of a brain from a rhythmic animal; *b*, heating to 35° C for 60 min. The time of treatment is shown by an arrow. The abscissa represents the number of crossings of the light beam in each hour. Full scale = 40 crossings.

Blaps is known to be sensitive to small changes of temperature: its rhythm will entrain to a diurnal temperature variation of ±1° C (ref. 6). Another treatment that induced rhythms was the application of hot wax to the thorax of an intact animal in simulation of the postoperative treatment given to a wound after a transplantation has been carried out (two out of three animals became slightly rhythmic 15–16 h after the treatment).

We have noticed that a few beetles removed from the culture chamber at 25° C and LL and placed in the experimental chamber in LL of similar light intensity became rhythmic and had to be kept for several days to allow the rhythm to fade. It now seems likely that the rise in temperature, rather than the small change in light intensity, induced a rhythm in these particular beetles.

Our conclusion is that a circadian rhythm may be induced in *Blaps mucronata* kept in constant light intensity by a variety of manipulations of which the most effective is a rise in temperature. An alternative explanation is that the animals have an underlying rhythmicity, as postulated by Harker[7] and Roberts[2], which the experimental treatment caused to become overt. Such an explanation seems unlikely for *Blaps* because in constant conditions the rhythm fades out progressively by an extension at the beginning and the end of the period of activity without any change in the general level of activity. It is difficult to imagine how a rhythm which disappears in such a fashion could still be running unobserved and be capable of resuscitation in its original form. The evidence for persistent latent rhythms is, in any case, insufficiently conclusive for such an explanation to be preferred to the simpler one that the experimental procedures we have described were responsible for inducing circadian rhythms. As the induced peak of activity comes some hours after the treatment it seems that the immediate effect is to depress activity. The physiological mechanisms underlying activity depression may be the key to much of the variability in our experiments and those of other workers.

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Haemoglobin in a Monogenean, *Oculotrema hippopotami*

Oculotrema hippopotami is a Polystomatid Monogenean that lives around the eye of the Hippopotamus. The anterior two-thirds of the body of mature worms is a dark red colour, while the posterior part, including the haptor, is almost colourless. Tests have shown that red colour is due to haemoglobin. Haemoglobin has been recorded in Turbellaria[1] and Digenea[2] among other invertebrates, but this is apparently the first record from a Monogenean. *O. hippopotami* feeds on epithelial cells and exudates. No blood is found in the gut caeca, so any haemoglobin that is detected must be of parasitic origin.

An initial positive test with benzidine indicated that the red pigment was probably haemoglobin. Direct observation of living *O. hippopotami* through a microspectroscope then showed 2 absorption bands similar to those of oxyhaemoglobin. After the worms were killed with 20 per cent NaOH and treated with pyridine and sodium thiosulphate, dark bands typical of pyridine haemochromogen were seen with the microspectroscope, and typical crystals developed.

For spectrophotometric analysis, living specimens of *O. hippopotami* were carried alive to Kampala on moist filter paper in Petri dishes, the Petri dishes being packed in vacuum jars. In the laboratory, the golden coloured eggs were removed from the uterus and ootype of the worms, and the worms were then deep-frozen at −13° C within 12 h after being collected from the hippopotamus.

Approximately fifty specimens of *O. hippopotami* were subsequently homogenized in 0·6 per cent saline, and the suspension was clarified by high speed centrifugation. Absorption spectra of the suspension were measured using a manually operated Unicam spectrophotometer. The following absorption maxima characteristic of oxyhaemoglobin were obtained: α band 587 nm, β band 548 nm, γ band 414 nm. Absorption was greater in the β band than in the α band, while the γ band could be measured only after diluting the sample 4 or 5-fold. The sample was then deoxygenated by the addition of a small quantity of sodium hydrosulphite. The α and β bands disappeared completely and were replaced by a single broad band, characteristic of deoxygenated haemoglobin, with a peak

at 560 nm, while the position of the γ band shifted to give a peak at 434 nm. It is concluded that the absorption spectra were those of haemoglobin and deoxygenated haemoglobin, and that any contaminants from the tissues of the worm were present in too small quantities to affect the absorption spectra.

The newly hatched larva of *O. hippopotami* has been described previously[3]. It is not pigmented, and small worms are also colourless. Immature worms in which the genitalia are developing are pale pink, with a concentration of pigment around the ootype. Mature worms retain the concentration of pigment around the ootype, but the anterior two-thirds of the body are a dark red colour. Histological sections were examined for the distribution of ferric iron by Perl's technique[4]. Although the staining reaction was weak, it showed that, apart from the concentration around the ootype, the main concentration of haemoglobin is probably just below the tegument, where a pale blue colour developed. Crompton and Smith[1] found that in the Turbellarian *Phaenocora unipunctata* ferric iron was concentrated around the pharynx but was also present just beneath the dorsal epidermis. They noted that respiratory pigments often occur in or near muscular tissues in which sustained or powerful contractions take place. In *O. hippopotami*, however, the haptor with its powerful suckers is virtually colourless. Stephenson[5], on the other hand, found that the haemoglobin of *Fasciola hepatica* was present in the region of the vitellaria and anterior uterine coils, and suggested that its function was to supply oxygen to these organs. The distribution of haemoglobin is similar in *O. hippopotami*, and it may similarly facilitate the transport of oxygen to tissues needing a good supply. Haemoglobin may also act as a store of oxygen, which might be necessary for *O. hippopotami* if the worm retreats under the eyelids of the hippopotamus to escape from drying or from excessively high temperatures.

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Altered Dihydrofolate Reductase associated with a Pyrimethamine-resistant *Plasmodium berghei berghei* produced in a Single Step

THE phenomenon and associated dangers of transferable drug resistance between enteric bacteria are now well established, but little information is available about the possibility of such transfers between eukaryotic cells. Evidence for the transfer *in vivo* of resistance to the widely used anti-malarial drug, pyrimethamine, from the rodent malarial parasite, *Plasmodium vinckei vinckei*, to another rodent malaria, *P. berghei berghei*, has recently been presented by Yoeli *et al.*[1]. They have coined the term "synpholia" to describe this occurrence. The method used involved the concomitant development of the two species in the mouse. This was followed first by the inoculation of blood from the mouse into hamsters to filter out the *P. v. vinckei* (they do not develop patent parasitaemias in this host), and second by the treatment of the hamsters with high doses of pyrimethamine to remove all the *P. b. berghei* except those that had acquired resistance to the drug. Ferone *et al.*[2] subsequently showed that this resistance to pyrimethamine in the strain of *P. b. berghei* was associated with changes in the amount and properties of the dihydrofolate reductase in the organism. We have found now that a pyrimethamine-resistant line of *P. b. berghei* containing a dihydrofolate reductase with these altered properties can be produced merely by treating hamsters or mice, infected by a sensitive strain, with high doses of pyrimethamine.

Patent infections of *P. b. berghei* (NK 65 strain) in twenty hamsters (40 g) and fifty male TFW-1 albino mice (20 g) were treated with doses of 200 mg/kg pyrimethamine base triturated in 0·5 per cent carboxymethylcellulose by subcutaneous injection on four consecutive days. Four days after completion of drug treatment, one hamster exhibited parasitaemia. In the 4 day suppressive test[3], the line derived from this infection was shown to be over 160 times less sensitive to pyrimethamine than the parent strain and this resistance remained stable even after twenty blood passages. The pre-two per cent bioassay technique[4] confirmed that the line was resistant to pyrimethamine. Infections were obtained in the mosquito *Anopheles stephensi* at 19° C but sporozoite inoculations of mice were unsuccessful.

Table 1. PROPERTIES OF PYRIMETHAMINE-SENSITIVE AND RESISTANT *P. b. berghei*

Strain of *P. b. berghei* (NK 65)	Percentage potency of parasites after 10 mg/kg doses of pyrimethamine*	Dihydrofolate reductase† V_{max} (mμmoles/min/mg protein)	K_m (× 10^6 M)	Concentration of pyrimethamine‡ (× 10^9 M) for 50 per cent inhibition
Sensitive	< 0·1	1·6	3·5	1·0
Resistant	100	5·0	30	30

* Estimated by the method of Warhurst and Folwell[4].
† 5,6,7,8-Tetrahydrofolate : NADP$^+$ oxidoreductase (EC 1.5.1.3).
‡ 2,4-Diamino-5-*p*-chlorophenyl-6-ethylpyrimidine.

Of the fifty mice treated with pyrimethamine, only one developed an infection which became patent 7 days after completion of drug treatment. This line had a pyrimethamine sensitivity similar to that of the resistant, hamster-derived line (Table 1) and was transmissible through *A. stephensi* at 19° C. Resistance was maintained after blood and mosquito passage.

Young and Burgess[5] have suggested from their work with *P. vivax* that resistance to pyrimethamine can develop in a single step. Our studies show that this can occur with *P. b. berghei*.

Batches of dihydrofolate reductase were prepared from our pyrimethamine sensitive strain of *P. b. berghei* and also from our resistant, mouse-derived line and their properties were compared. We have used the method of preparation of W. E. G. and P. I. T.[6], except that parasites were released from the red cells by lysis with saponin as described by Ferone *et al.*[7] rather than by lysis with anti-red cell serum. Enzyme was assayed as described elsewhere[6] except that 0·1 M Tris-HCl (*p*H 7·2) was used instead of potassium phosphate buffer. Properties compared were the specific activities of the preparations, affinities of the enzymes for the substrate, dihydrofolate (K_ms) and sensitivities of the enzymes to inhibition by pyrimethamine. The results (Table 1) indicated that resistance to pyrimethamine in our line of *P. b. berghei* was associated with an increase in the level of enzyme within the organism, a decrease in the enzyme's affinity for substrate and a decrease in its sensitivity to inhibition by pyrimethamine. Such changes are similar to those reported previously for other pyrimethamine-resistant lines of *P. b. berghei*[2,8].

We have produced a pyrimethamine-resistant line of *P. b. berghei* in a single step and have correlated this resistance with changes in the amount and properties of the dihydrofolate reductase in the organism. Our resistant line was produced by the method used for the control series in the demonstration by Yoeli *et al.* of drug resistance transfer among rodent *Plasmodia* and it contains

an altered dihydrofolate reductase similar to that present in their resistant *P. b. berghei* line. We do not know why a similarly resistant line did not arise in their control series, though a possible reason is that too small a number of animals was used. Our results do not disprove the claim that their pyrimethamine-resistant *P. b. berghei* arose by a transfer of genetic material between two malaria species. They indicate, however, that further experiments are necessary to demonstrate that greater numbers of resistant parasites appear after "sympholia" than after selection by drug treatment.

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Control of Moulting in an Insect

Moulting in insects is controlled by the prothoracic glands after they have been activated by the brain[1–6]. α-Ecdysone and 20-hydroxyecdysone stimulate moulting and are thought to be the principal moulting hormones[7–11]. It is common to assume that the prothoracic glands control moulting by synthesizing ecdysone, even though there is no direct evidence for this[12,13]. The experiments reported here show that the head and thorax of *Calpodes* larvae isolated with intact activated prothoracic glands do not moult; they can only do so if moulting hormone is injected or if at least part of the abdomen is present. This suggests that more than an activated prothoracic gland is needed for moulting.

Calpodes ethlius Stoll (Lepidoptera, Hesperiidae) has three phases of development in the last (fifth) larval stadium. Events in the fifth stadium can be timed accurately and the exact stage of development can be confirmed by physiological markers. There is a phase of growth from ecdysis up to 66 h by which time the brain is no longer needed to induce pupation; the prothoracic glands are activated, that is they can cause pupation without further influence from the head. After 66 h ligature around the neck to remove the head, removal of the brain or suboesophageal ganglia, or cutting the ventral nerve cord causes no delay in pupation[14,15]. Pupation is only blocked by ligature around the thorax which prevents the prothoracic glands from affecting the abdomen. These experiments rule out neuronal inhibition of prothoracic gland activation of the sort described in *Galleria*[16]. The second phase from 66 to 156 h is devoted to larval syntheses. At the end of this time the prothoracic glands are no longer needed and no operations are known to block the overt pupal syntheses leading to pupation at 192 h[17].

Because *Calpodes* has a long second phase, when the prothoracic glands are activated and necessary for pupation, it is possible to test whether they alone can induce pupation. Larvae in this phase were ligatured at one of the three positions shown in Fig. 1. Pupation was judged to have taken place if pupal cuticle could be seen after removal of the exuvium or in thick (1 μm) epoxy sections stained with methylene blue. Retraction of the epidermis from the cuticle is not sufficient indication of pupation; it occurs in isolated abdomens in which further development is blocked.

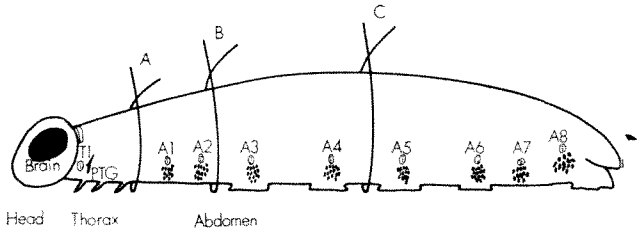


Fig. 1. Diagram of *Calpodes ethlius* showing the position of ligatures and the principal endocrine tissues, the brain and the prothoracic gland (PTG). Spiracles are labelled by region (T, thorax; A, abdomen) and by corresponding segment number. Ligature *A* between thorax and abdomen includes the brain, PTG, foregut and some fat body in the isolated anterior portion. Ligature *B* between the second and third abdominal segments adds to this anterior part some midgut, more fat body and four clusters of moult oenocytes (between thirty and forty oenocytes in a cluster). Ligature *C* between the fourth and fifth abdominal segments adds more midgut, still more fat body, four more clusters of oenocytes and the most anterior loops of the Malpighian tubules. The cells ventral to the abdominal spiracles are the oenocytes, postulated to be necessary in interacting with the prothoracic glands.

Most ligated anterior regions were viable for more than 2 days, many for 3–8 days after the operation. Ligatured abdomens were viable for 5 to 10 days. There was no pupation of either part in sixty-nine larvae ligatured between the thorax and abdomen (Fig. 1*A*). Twenty per cent of the isolated thoraxes were dark pink with a retracted epidermis. The pink pigment disappears at about 156 h in normal larvae going on to pupate. When the ligature was made between the second and third abdominal segments (Fig. 1*B*), one anterior portion out of twenty-eight had a partial new cuticle; no abdominal portions pupated. Ten out of twenty-three anterior portions, however, pupated when the ligature was between the fourth and fifth abdominal segments (Fig. 1*C*).

These experiments show that an activated prothoracic gland is not itself a sufficient stimulus for pupation in the isolated head and thorax. To test whether the failure of these anterior portions to pupate is a result of nutritional deficiency or generalized wounding, larvae were injected with moulting hormone 1 h after ligature. When 10 μg of 20-hydroxyecdysone (10 mg/1 ml. of 10 per cent EtOH; ecdysterone, Mann Research Labs; animals weighed between 0·9 and 1·3 g each) was injected into either anterior or posterior portions of larvae ligatured between the thorax and abdomen, pupation took place. When 5 μg of synthetic α-ecdysone (1·0 mg/0·1 ml. of 10 per cent EtOH) was injected in anterior portions ligatured in the same way pupation also took place. The new cuticle seen in material prepared for electron microscopy had a complete epicuticle and lamellate endocuticle. This normal pupation after the injection of hormone shows that the block to pupation in an isolated thorax is not due to an inability of the tissues to respond to ecdysones.

Neither hormonal[18] nor neuronal inhibition by the brain can explain the inability of the isolated thorax to pupate: when fifteen larvae in the second phase were ligatured between the head and thorax and between the thorax and abdomen, no thoraces pupated.

These experiments show that the prothoracic glands normally competent to cause pupation in the headless larva cannot induce pupation in an isolated thorax,

although the thoracic tissues themselves can respond to ecdysones. We presume that this is not a selective injury to the prothoracic gland, and that the failure of isolated thoraxes to pupate is caused by the absence of an essential interaction with a component present in the abdomen.

The ultrastructure of the activated prothoracic gland in *Calpodes* seems inconsistent with its independent stimulation of pupation. The newly activated prothoracic gland contains smooth endoplasmic reticulum confronting mitochondria and diverticula of the plasma membrane[19]. These structures are appropriate for steroid metabolism but for the most part the structures can be correlated with larval activities. Larvae decapitated at 66 h go on to pupate but have reduced larval syntheses and little structure appropriate for steroid metabolism in the prothoracic gland.

We postulate an additional step in the control of moulting—interaction of the prothoracic glands and some abdominal tissue. This would not be in conflict with the classical work showing control by the prothoracic gland, for earlier experiments have used whole abdomens or isolates with several abdominal segments to test the stimulatory effect of activated prothoracic glands on moulting[1–6].

α-Ecdysone is converted to 20-hydroxyecdysone in isolated abdomens[20]. Perhaps more than this hydroxylation can occur in the abdomen, for isolated abdomens are more sensitive to 20-hydroxyecdysone than isolated thoraces. When injections of 10 μg were given pupation occurred in eight out of ten abdomens but only in nine out of forty-three thoraces, even though the thorax is considerably smaller than the abdomen. The greater sensitivity of the abdomens to the injected hormone could be explained by stimulation of an abdominal site of ecdysone synthesis. Intermediates of ecdysone metabolism injected into diapausing pupae of the tobacco hornworm stimulate hormone synthesis from endogenous precursors[21].

Oenocytes, sometimes called abdominal endocrine glands[22,23], are the cells most probably interacting with the prothoracic glands in the control of moulting. Isolated pupal abdomens moulted after removal of gonads, digestive tract, Malpighian tubules and the entire central nervous system when an active brain and prothoracic glands were implanted[2]. The only significant abdominal tissues remaining in this experiment were oenocytes, fat body, muscle, trachea and epidermis. Oenocytes, found in all insects, have the appropriate ultrastructure, a cyclic nature of specialization and an unknown function[24]. Oenocytes have large amounts of smooth tubular endoplasmic reticulum which is typical of vertebrate steroid secreting cells. In *Calpodes*, the permanent oenocytes (seventh and eighth abdominal segments) maintain their ultrastructural specializations until about 156 h when they undergo massive autophagy[24]. The moult cycle oenocytes (first to sixth abdominal segments inclusive) increase dramatically in size and in development of endoplasmic reticulum at about 135 h (my unpublished work). Little specialized structure is evident in the prothoracic glands at this time although they are still needed for moulting (personal communication from M. Locke). The oenocytes concerned with the moulting cycle decrease in size and specialization at ecdysis. The development of these oenocytes is complementary to that of the prothoracic glands. Further evidence that the oenocytes are the abdominal tissue involved in the control of moulting comes from experiments on *Tenebrio*. Isolated larval abdomens go on to pupate and even to produce adults[25–26]. *Tenebrio* has exceptionally long cords of very large oenocytes along the length of the abdomen perhaps indicating that they are maintained in an activated state without the interaction of the prothoracic glands.

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Negative and Positive Behavioural Contrast in the Same Animals

The reactions of animals to situations that suddenly become more attractive or less attractive have long seemed potentially useful for the evaluation of drugs and other treatments assumed to affect emotional responsivity[1]. Crespi[2,3] reported that rats shifted to a larger (or smaller) amount of reward ran faster (or slower) to the goal in a runway than control rats who were accustomed to the larger (or smaller) amount. But it has usually been difficult to find reliable overshooting or "positive contrast" effects to an increase in reward magnitude, and even the more reliable negative contrast effect (undershooting to a decrease in reward magnitude) has proved troublesome when separate experimental and control groups are used[4]. Furthermore, the transient character of the effects, even when obtained, makes them inconvenient for the chronic testing of various treatments, such as antidepressant drugs or brain lesions. Finally, recent demonstrations of "behavioural contrast" have depended on somewhat complex multiple schedule situations, not necessarily related to that originally used by Crespi[5–7]. Here we report a simple technique for the repeated study of both positive and negative effects in the same animals, with results that are stable over weeks of daily testing.

Our subjects were pairs of groups of experimentally naive male albino rats (Tierfarm AG, Sisseln, Switzerland) maintained at 80 per cent of the body weight that they would have reached if feeding freely. The apparatus consists of two identical single lever Skinner boxes housed in sound-attenuated chambers. A force of 8 g was required to activate the levers.

The animals were gradually introduced during 12 days to a two-minute variable interval schedule, with one pellet per reward. Thereafter, small and large magnitudes

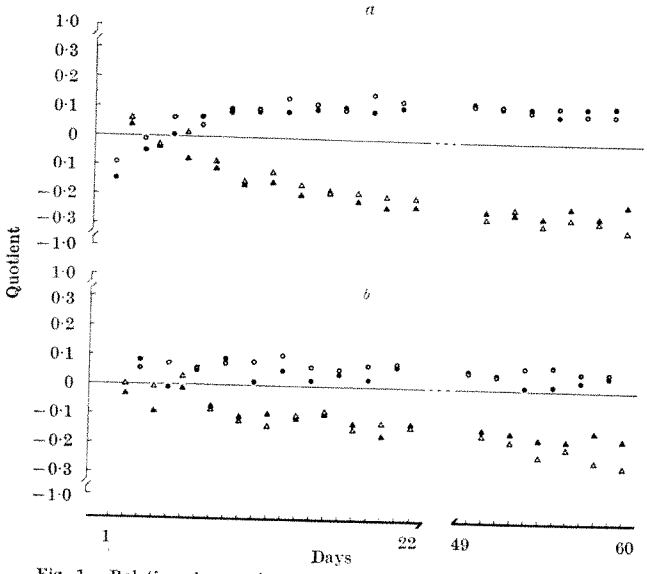


Fig. 1. Relative changes in response rate during intrusions in which reward magnitude was increased or decreased. *a*, Within sessions; *b*, between sessions (contrast). ○△, Strain 1; ●▲, strain 2; ○●, increased reward; △▲, decreased reward.

of reward (one and four pellets) were alternated daily for each group, with stimulus conditions associated with each magnitude also alternating appropriately. The differential stimuli were the standard illumination of the Skinner box (the house light) and a light on the front panel. The two stimuli associated with the reward magnitudes were varied from experiment to experiment, and on any given day one group was tested with the small reward and the other group with the large reward.

The reinforcement period was 4 s for the smaller reward and 16 s for the larger reward. The four pellets forming the larger reward were dispensed at 4 s intervals. During reinforcement the food tray was illuminated. Animals received the remaining portion of their daily food ration in their home cages. They were tested daily; each session lasts 63 min, excluding reward time.

After a few days of such alternating experience each group daily received two relatively brief intrusions of the contrasting reward condition, together with its relevant stimulus. An intrusion always started immediately after a reward, and last, 4·5 min. The temporal position of the intrusion periods was changed daily on a quasi-random basis. Thus, on any given day at the final stage one group received, as the principal reward, one pellet and there were two short periods with rewards of four pellets; for the other group this was reversed. Conditions were alternated daily.

The effects of the intrusions within each session were assessed by a quotient of the difference between the rates during and just before the intrusion divided by the sum of the two rates. Behavioural contrast was assessed by comparing each animal's rate on the intrusion conditions with its rate on the baseline conditions during the immediately preceding day (using the pre-intrusion period for this purpose.)

The results of one representative experiment are shown in Fig. 1, starting with the first day of intrusion training. (In this experiment two different strains of albino rats were used.) The intrusion experiment was continued for 60 days; during the last 12 days all animals received injections of NaCl as a control for subsequent drug trials.

The effects of intrusion within sessions are clear (Fig. 1*a*). Rates were consistently increased by the intrusions signalling larger rewards, and consistently decreased by smaller rewards. During days 11 to 22, when the results had become relatively stable, all thirty rats showed an average increase in rate to increased reward, and an average decrease in rate to decreased reward. Twenty-six days later during the final 12 days of the experiment, the same unanimity of results obtained, reflecting a remarkable stability in the conditioned increases and decreases in rates. Statistical analysis obviously is unnecessary.

Behavioural contrast results, based on between session comparisons, are shown in Fig. 1*b*. Negative contrast was easy to demonstrate: during days 11 to 22 all but one rat (out of thirty) showed an average undershooting of rate of response when they were switched from the larger to the smaller reward, as compared with their baseline rates for the smaller reward. Positive contrast effects for the same period were seen in fourteen out of fifteen rats in strain 1 ($P < 0{\cdot}001$) and eleven out of fifteen rats in strain 2 ($P < 0{\cdot}001$). About 26 days later, a similar pattern still obtained: all the strain 1 and thirteen out of fifteen strain 2 rats showed average positive contrast effects from days 48 to 60. A marginally significant greater positive contrast effect was seen in strain 1 over strain 2 at the beginning of the experiment (t test, $P < 0{\cdot}05$) but this was not significant by the end ($P < 0{\cdot}20$). With the two periods combined, however, the strain difference was significant ($P < 0{\cdot}01$).

In keeping with previous results, therefore, positive contrast is less marked than negative contrast, but the statistical reality of both phenomena seems unmistakable. The positive effect in our experiments cannot be explained by gradual trends in baseline rate, which in any event becomes stable after a few weeks[8]. The positive contrast effect could be masked by a "ceiling effect"[9]. Another possibility is that there are genuine individual differences in the tendencies of rats to show the positive effect, which might be amenable to genetic and/or biochemical analysis. There was a possible strain difference in the experiment described here. Finally, the precise values of experimental parameters are obviously important and could probably be made more nearly optimal. In pilot experiments we found that the control exercised by the intrusions was greatly reduced if: (*a*) the mean interval of the variable interval schedule was reduced from 2 min to 90 s, 60 s, or 30 s (with a corresponding reduction in the duration of the stimulus intrusion periods); (*b*) the magnitude of the basal reward was increased from one to two pellets, and (*c*) liquid reward (dilute milk) was used instead of food pellets. In all cases the positive shifts were more sensitive than the negative shifts to such variations.

The experiments clearly demonstrate that brief stimulus-associated changes in magnitude of reward can modulate continuing behaviour. The relative effect seen within sessions is highly stable for both directions of change. Stable positive and negative behavioural contrast effects, with animals serving as their own controls, are also readily produced.

We thank Mr T. Werren for technical assistance.

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Book Reviews

CHEMICAL MNEMOLOGY

Molecular Mechanisms in Memory and Learning

Edited by Georges Ungar. Pp. xiv+296. (Plenum: New York and London, 1970.) $15.00.

Molecular Approaches to Learning and Memory

Edited by William L. Byrne. Pp. xxix+358. (Academic: New York and London, April 1970.) 168*s*.

MOST neurophysiologists whose interests are not primarily chemical are aware that there has grown up since 1965 a substantial and largely bad literature on the induction of chemical changes in the brains of animals by training or conditioning procedures. The changes are detected in extracts of whole brains. Usually the extracts are examined by injecting them (sometimes after chemical pretreatment) into fresh animals, and observing the performance of these animals in training or conditioning schedules similar to those used to induce the chemical changes in the original animals. Some experimenters have examined the extracts by purely chemical means.

This new subject may be called chemical mnemology. I need not hesitate to call its literature "largely bad", because it is full, to a degree unprecedented in ordinary neurophysiology, of reports of failures to replicate published experiments. But among the mass of bad work, many outsiders have wondered whether there may not be some true observations that very severely upset orthodox neurophysiological thinking. What seems to be needed is a clear guide to the new and muddy subject, and outsiders looking at these two books for the first time will immediately want to know whether they provide such a guide.

It can be said at once that the book edited by Ungar is a better guide than that edited by Byrne. Neither book has any general cohesion, but the Ungar book, written by only nine authors, contains chapters (the first five of its eight) that are serious attempts to review aspects of the new subject. The Byrne book, with thirty-three authors, is a jumble of writings of various kinds. Many chapters are merely drafts of research papers; I hope that they will be well revised before they are submitted to journals. The better chapters of the Byrne book are comparable with the first five of the Ungar book, but they are shorter, mostly less critical, and in several cases marred by a strange facetiousness.

What no chapter of either book does is to concentrate on the central question of chemical mnemology: whether the changes in chemical composition produced when a memory is laid down are, at a molecular level, so finely related to the details of the memory that one might, in principle, discover what memories an animal has stored by studying the chemistry of an extract of its minced or homogenized brain.

If the answer to this central question of chemical mnemology were yes, the implications for neurophysiological thought would be revolutionary, and the new subject would be established as an immensely exciting one. Any disputes as to the soundness of the biochemical techniques used by the experimenters, and as to whether the relevant substances are nucleic acids or proteins, would be matters of minor detail.

In all the early papers on chemical mnemology, the central question was, roughly speaking, answered affirmatively, though it was often not stated as sharply as I have stated it above. Some of the experimental observations on which the affirmative answer was based seem to have been wrong, and some, even if right, obviously do not suffice to support so revolutionary a notion. One would expect a discussion of whether there remain any that do sustain an affirmative answer to be the core of any book published in 1970 on chemical mnemology. I can find no such discussion in the books edited by Ungar and Byrne.

Suppose that the answer to the central question is no; then what remains of chemical mnemology? It is not surprising or informative that any large difference of environment between two batches of rats taken from the same inbred strain should affect the chemical composition of their brains. On almost any theoretical notions one would expect such effects, and expect them to involve RNA, protein, lipids and other constituents. The finding that brain extracts, when injected into rats, affect behaviour was perhaps not altogether predictable. But it belongs only to toxicology, and does not clarify any neurophysiological problem, unless between the behavioural effects of a brain extract and the details of training or conditioning of the animal from which it was obtained there is a correlation so close as to support an affirmative answer to the central question.

Since Cajal, most neurophysiologists have thought it probable that memories are stored as changes in the conductivity of synapses. The difference between the old orthodoxy (which I support, but shall abandon if an affirmative answer to the central question is proved) and the view of the pioneers of chemical mnemology is that the old orthodoxy holds that the engrams of any two different memories differ as to which synapses have had their conductivity altered, and in no other important way. The change in conductivity of modifiable synapses may well involve change in the amounts of RNA and protein in them or near them. But if the rest of biochemistry is a fair guide, these synapses are likely to be built all to the same pattern, or at most to a handful of different patterns. The elucidation of their mechanism is an important problem, but it is not clear that the observations of chemical mnemologists up to now have contributed to solving it.

G. S. BRINDLEY

MIND DRUGS

Principles of Psychopharmacology

A Textbook for Physicians, Medical Students and Behavioural Scientists. Edited by W. G. Clark and J. del Giudice. Pp. xxvi+814. (Academic: London and New York, April 1970.) 182*s*.

"WHAT drug started it? Mandrake, morphine, mescaline or mushrooms? Maybe rauwolfia in India if not in Nigeria, teonanacatl in Mexico or the American Indians peyote? Perhaps alcohol, ibogaine, cannabis, cocaine, ololiuqui or yohimbine? Opinions vary as to drug, time and place. But if one defines psychopharmacology precisely as the utilization of drugs in restoring or maintaining mental health and for exploring the mind, then everyone is agreed: the drug is chlorpromazine, the time: 1952, and the place: Val-de-Grâce, the famed military hospital in Paris."

With such a start to the chapter by Caldwell on the history of psychopharmacology, one's hopes are raised that this book will not be yet another collection of disconnected articles written by research workers with little flair for communication. These hopes are fulfilled. The surprise at finding that the chapter on the pharmacology of chlorpromazine is only just over three pages long soon turns to admiration that the subject can be treated so concisely. Most of the fifty-five chapters are short and cover a specific topic in an authoritative manner. The achievement of this book far outweighs any errors which the expert may detect in the chapter

on his own speciality. Much of the credit must go to the editors, who have persuaded most of the seventy-one authors to write in a style that is clear and relatively free from the jargon so common in the clinic or laboratory. No budding psychiatrist need be put off by the size of the volume: he will find no better book to serve both as an introduction to the subject and as a standard reference work.

Beginning with a section on the history of psychopharmacology, the book is divided into ten sections; the subjects include basic anatomy and physiology, the biochemical mode of action of drugs, the pharmacological basis of therapy, methods of studying the actions of drugs in humans, and drug abuse. A valuable feature of this arrangement is that individual sections or chapters can be read independently. The cross-referencing and bibliographies allow the reader to go more deeply if he desires. Unfortunately the index is not quite up to the standard of the rest of the book; for example, under "dopamine" no reference is made to the excellent chapter on the extrapyramidal side effects of drugs, where this neurotransmitter features prominently.

By 1965 some fifty million people had taken chlorpromazine, and yet the fundamental mode of action of this drug is still not understood. The reader of this book will wonder whether there is too much emphasis at present on the theories which explain the action of drugs in terms of synaptic transmission in the central nervous system. Nevertheless, strong support for these theories was given by the discovery that reserpine depletes some nerve terminals of their transmitter (noradrenaline, dopamine or 5-hydroxytryptamine). If we remember, as Aghajanian points out in his chapter, that neurones containing monoamines represent less than 1 per cent of all brain neurones, we can be optimistic about the future of psychopharmacology. For the time being, "Clark and del Giudice" provides a balanced, clear and comprehensive introduction to existing knowledge.

A. D. SMITH

WORK FOR BIOLOGY TEACHERS

Nuffield Advanced Science

Biological Science. (Penguin: Harmondsworth, Middlesex, 1970. Published for the Nuffield Foundation.)

Maintenance of the Organism

A Laboratory Guide. Edited by C. F. Stoneman. Pp. xii + 158. 16*s*.

Organisms and Populations

A Laboratory Guide. Edited by John H. Gray. Pp. ix + 142. 16*s*.

Teachers' Guide

Vol. 1: Maintenance of the Organism, Organisms and Populations. Pp. xiv + 213. 25*s*.

Topic Reviews

Circulation. By W. H. Freeman. Pp. 43. 5*s*. Control of Breathing. By Margaret K. Sands. Pp. 17. 12*s*. Metabolism. By C. F. Stoneman. Pp. 35. 5*s*.

Key to Pond Organisms

By Alison L. Brown. Pp. 45. 6*s*.

THESE publications are those so far issued by the Nuffield A-Level Biological Science Project. The mass of printed material devised for the A-level trials has crystallized out into a modest set of interrelated books and it is only regrettable that all could not have become available simultaneously because together they form an integrated programme of work and, incomplete, the careful system of cross-reference and flexibility of treatment proposed by the promoters to some extent breaks down. Moreover, any review of present publications can only be an interim evaluation and might be considered premature.

The full set of materials will include, in addition to those reviewed here: for students, two more *Laboratory Guides* (*The Developing Organism* and *Control and Co-ordination in Organisms*), a *Study Guide* (*Evidence and Deduction in Biological Science*) and at least nine further *Topic Reviews*; for teachers, *Teachers' Guide 2*, complementary to the second two students' *Laboratory Guides*, *Teachers' Guide 3*, for the students' *Study Guide*, a *Laboratory Book* and *Projects in Biological Science*. A number of film loops will also be available.

The current *Laboratory Guides* are compact, clearly printed, systematic work books which fulfil their claim to provide a coherent treatment of a series of biological topics based on laboratory investigations. This enquiry approach leads students to gain information for themselves and, in doing so, to get some understanding of the processes whereby biological knowledge is acquired. Each set of investigations is preceded by an explanatory introduction and is followed by questions which give direction to the enquiry and by a bibliography which suggests supplementary reading and includes reference to the relevant *Topic Reviews*. In addition to this basic practical work, there is also optional "extension" work.

The *Students' Guides* are unusually and refreshingly explicit about certain points too often taken for granted, such as the interpretation of sections in relation to three dimensional structure and the detailed explanation of the successive stages in various technical procedures. The *Key to Pond Organisms* is simple and well illustrated by small line drawings. It has a generous bibliography and will obviously be of use as a ready means of identification in many ecological contexts. The *Topic Reviews* are concise and self-contained. They include much interesting information related to their particular subjects and have further bibliographies of their own. Although the standard of accuracy is high throughout, *Circulation*, page 7, paragraph 3, states that, "In a double circulation the left side of the heart always receives oxygenated blood". Presumably the writer refers to mammalian double circulation only and not to birds, although these are mentioned in a previous paragraph.

The *Teachers' Guide 1* is complementary to the two *Students' Laboratory Guides* with which it is linked by numerical cross-references. It distinguishes between students' elementary and main work and explains how the *Laboratory Guides* should be used. Each investigation is treated under five headings. (1) "Associated Materials", which refers to complementary work in the *Study Guide* and to appropriate visual aids; (2) "Principles", which gives an outline of the aims of the investigation; (3) "Teaching Procedure", which suggests approaches, gives links with work in the *Laboratory Guide* and pinpoints possible difficulties; (4) "Practical Problems of Teaching"; and (5) "Questions", which delineates expected answers to the questions posed in the *Students' Laboratory Guide* and suggests possible further discussion.

It is justly claimed that these materials do not represent a rigid syllabus. They could be used in a variety of ways related to the different circumstances found in schools, and the varied abilities, backgrounds and aspirations of students. "A flexible approach has been adopted for presenting biological science as an interesting and important subject related to the potential biologist and non-biologist alike." A teacher with access to the complete set of materials should certainly be able to "develop that balance between the theoretical and practical best suited to his own students". All the same, I suspect that this programme could be just as much of a strait-jacket as the traditional external syllabus and the work makes stringent demands on teacher and student. Still, the priorities are right, investigation is paramount and biology really could come to life as a relevant, exciting and absorbing pursuit for individual study at a level appropriate to the previous experience and future prospects of the sixth-former concerned. The onus is on the teacher.

JOYCE VINNICOMBE

IMMUNE SYSTEMS

Factors Regulating the Immune Response

Report of a WHO Scientific Group. (World Health Organization Technical Report Series, No. 448.) Pp. 88. (WHO: Geneva, 1970.) 10*s*; $1.75; Sw.fr.5.

A PANEL of wise men was assembled by the World Health Organization in Geneva on September 1–6, 1969. This report is a distilled version of their deliberations. It is easily the bargain of the year for immunologists. The presentation is somewhat didactic, but this is probably an advantage; to have dotted all the i's and crossed all the t's would have extended the booklet tediously.

The first five chapters define the immunological apparatus and the basic variables in immune responses. Current theories of cellular immunology are enunciated concisely and in a readable manner. The next two chapters are concerned with intrinsic regulatory factors in primary and secondary immune responses. A chapter follows which is largely devoted to the effect that extrinsic regulators may have on the course of immunity. The last chapter is grandly named "How Does the Entire Immune System Become and Remain Functional ?". In a way this chapter heading epitomizes the comprehensiveness of the book. It is a most remarkable production which should be required reading for all those interested in current developments in immunology.

It is perhaps sad that a booklet of only eighty-eight pages takes a year to produce and it is unfortunate that points of fact in the text may not easily be traced (except by the expert who knows, anyway) to specific entries in the abbreviated reference lists which relate to each subject. These are minor flaws and the members and secretariat of the group of scientists involved are to be congratulated on a first-class piece of work.

A. J. S. DAVIES

VETERINARIAN IMMUNOLOGY

Veterinary Immunology

By W. J. Herbert. Pp. xi + 356 + 22 plates. (Blackwell (Scientific): Oxford and Edinburgh, 1970.) 85*s*.

IMMUNOLOGY transcends the boundaries of scientific disciplines gaining in stature as it draws upon the resources of each. It has emerged from its historical association with microbial infections, from the days when immunity was first recognized, to become an all-embracing science attracting followers from most sections of the scientific community. Therein lies its strength and dynamism and the explanation for its recent dramatic transformation.

For these reasons it is desirable that textbooks of immunology should be written specifically for definitive groups of students and others, whose need for knowledge of the subject was once thought to be subsidiary or peripheral to their chief objectives. Such books should be designed to introduce these large numbers of undergraduates and graduates to a fuller understanding of fundamental and updated immunological concepts. Hopefully then, individual interest, enthusiasm and participation may follow, and gaps in our understanding of many diverse aspects of the subject be filled. One such specific group is the veterinary profession many of whose members have been actively participating in the development of immunology during its emergence as a subject.

In this book Dr Herbert has succeeded in producing a text that is well orientated to the requirements of veterinary undergraduates and practising veterinary surgeons, as well as being of benefit to those biologists interested in immunological responses of the various species of domesticated animals. Fundamental physiological mechanisms, theories of antibody production, descriptions of diagnostic serological tests, and physicochemical techniques for characterization of immunoglobulins are necessarily similar whether one is dealing with humans or animals. There is, nevertheless, considerable diverse data recorded in this book on the various species of domesticated animal, and sufficient examples of clinical disease states, the diagnosis and prevention of which are dependent on immunological methods, to make it an extremely valuable aid to the student of veterinary science and medicine. Good veterinary examples of allergy and atopy are perhaps rather lacking, but this is no fault of the author; indeed, he makes specific reference to these gaps in our knowledge of veterinary allergic conditions.

The diagrams, figures and photographs are clearly reproduced and well chosen to illustrate the text. They introduce the reader, in a concise and clear manner, to the subject, giving him a sound grasp of the various concepts of antigen–antibody reactions. Details of molecular configurations, spatial arrangement and steric hindrance are wisely left for the enquiring reader to consult texts of immunochemistry; indeed, such readers are provided with suggestions for further reading. Some errors creep into most textbooks and this book is no exception. In a rapidly expanding subject it is, however, difficult to be precise and the author does well to maintain clarity in an eminently instructive book with wide appeal to students of veterinary and biological science alike.

D. ALLAN

FISH OF COLD CLIMES

Biology of Coregonid Fishes

Edited by C. C. Lindsey and C. S. Woods. (Proceedings of an International Symposium held in Winnipeg, Canada, August 25–29, 1969.) Pp. 560. (University of Manitoba: Winnipeg, 1970.) $8.

THE coregonid fishes are a dominant group in cold freshwaters of the northern hemisphere. They are distributed across the northern Eurasian and North American land masses and limited at the southern extreme of their range to mountainous regions, as in the British Isles and the Alps. Especially in inland areas they are valuable commercially. Perhaps because of their wide distribution and fisheries value they have attracted the attention of many workers, yet they still remain a difficult and elusive group to ichthyologists.

This volume comprises thirty-five papers read to an International Symposium on Biology of Coregonid Fishes held in 1969. It has been reproduced photographically from typewritten contributions, with the result that it was available only eleven months after the symposium meetings and at a remarkably low price. The editors, Dr C. C. Lindsey and Dr C. S. Woods, are to be congratulated on this considerable achievement.

The coregonid fishes thrive in regions which have been repeatedly and recently disrupted by glaciation, and they have acquired variable body forms and plastic life styles that challenge conventional classification. It is understandable therefore that most of this volume is devoted to studies bearing on the classification or systematics of the group. The remaining papers cover a variety of subjects including parasitology, ecological aspects, reproductive biology and fisheries topics.

Coregonid systematics are certainly involved, but with recent advances in their study, particularly the use of protein properties, a clearer picture is emerging. Given such a plastic group, with populations often living in isolation in lakes, with the introduction of exotic forms, and introgression within lakes also taking place, descriptive systematics has become very complex. Populations have been described as species, or subspecies, until nobody could see the wood for the trees. Present trends, however, are towards systematic rationalization, with many fewer species, and the recognition that phenotypic variants do not require names.

This is nowhere more apparent than in northern Europe where Berg and Pravdin had named numerous forms from Karelia, although Himberg in a comprehensive review of the zoogeography of the North European coregonids in this volume reduced them to two only, one of which, *Coregonus lavaretus*, is represented by three "cryptic species". Himberg further considers that Europe was populated following the last glacial epoch by two ancestral forms which at that time were ecologically distinct, and have since given rise to many phenotypic populations, and hybridized extensively in isolated waters.

A contribution which I found particularly interesting is that discussing the origin and distribution of *Coregonus* in the British Isles by Peter S. Maitland. There has been a similar confusion of nomenclature over the British populations, some fifteen in number, traditionally recognized as belonging to three species and eight subspecies found in lakes in Scotland, the Lake District, North Wales and Ireland. Maitland suggests that all could have originated from two (or three) sympatric species in a single post-glacial water body in the site of the present Irish Sea. The connexion between this western water mass and the central European one implied by Himberg's hypothesis has yet to be established. In the light of advances in taxonomy of the European forms it is disappointing to find Maitland still advocating the retention of Regan's scientific names for the British coregonids. This nomenclature has confused the relationships and affinities of these populations in the past, and was based on what by present day standards would be regarded as inadequate samples. Modern studies[1] show that the British freshwater forms are referrable to the two species widespread in Europe, *Coregonus lavaretus* and *C. albula*.

ALWYNE WHEELER

[1] Wheeler, A., *Fishes of the British Isles and North West Europe* (Macmillan, London, 1969).

SIEVE PLATES AND TUBES

The Phloem

By Katherine Esau. (Encyclopaedia of Plant Anatomy. Second edition. Histology: Vol. 5, part 2.) Pp. ix+505. (Gebrüder Borntraeger: Berlin and Stuttgart, 1969.) DM 188.

IT was almost inevitable, once the editors had decided to include a volume on the phloem in the "Handbuch der Pflanzenanatomie", that the task should be entrusted to Professor Katherine Esau, for she has established for herself a world reputation, not only as a plant anatomist, but specifically as a student of the phloem. This splendid volume is the outcome of three and a half years of work, interspersed, no doubt, with research of the same original and stimulating up-to-the-minute quality which one has come to expect from her, and which shows no signs of falling off in either quality or quantity.

Professor Esau happily relates her treatment, on the one hand, to the place of the phloem in the life of the plant, and on the other to the historical development of our knowledge of the subject. Both these emphases are evidence of her breadth of sympathy, and both are important in these days of narrow technology-orientated specialization, when knowledge of the natural world is too often valued merely in terms of the use we can make of it in the interests of physical existence. The historical note finds expression in a recall of the original occasions on which significant new ideas were put forward: "sieve plate", "slime", "nacré walls" all come alive in the words of their originators. This is a welcome feature.

In the systematic development of the subject, the sieve element naturally comes first in this book, and is given a thorough treatment (about one hundred pages). The electron microscope evidence is discussed fully as far as the summer of 1968. Esau herself has contributed very largely to this; and although, to single out one crucial point, she inclines to the view that the functioning sieve plate pores are "open", she is nevertheless very fair to the opposite view. "There is hardly a feature in the sieve element that does not arouse some controversy," she remarks, with justice. Sieve tube physiology is not neglected.

Sections on parenchymatic elements and sclerenchymatic elements follow (about thirty pages), that on the companion cells being particularly full. Then, moving from histology to anatomy, there is a section on the distribution of the phloem in the plant body (twenty pages) and on its development as a mature system, the latter naturally lengthy (eighty pages) and including references to experimental work. Professor Esau has a well known interest in the phloic transmission of virus diseases. There is a section of ten pages on this, followed by a much longer section (100 pages) on comparative studies, which ranges from the larger brown algae and mosses to present-day angiosperms and the fossil record. This leads naturally to the discussion of phylogenetic trends, to which Esau devotes ten pages. Here, as with comparative studies, she acknowledges a great dearth of information.

Professor Esau concludes her great work with twenty pages attempting to relate structure to function. Here, few final conclusions are at present possible; but, as a physiologist, I was struck by the comprehensiveness of the author's grasp of a subject not her primary interest.

Needless to say, there is a large selection of first-rate figures and plates, about one hundred in the text and fifty in a terminal collection. These include many electron micrographs. There is a bibliography of more than a thousand references, and three indexes (author, plant and subject). The printing and paper are of high quality. This is clearly a book which every library must have, and which many researchers would wish to be able to afford. It will long remain a classic. D. C. SPANNER

PETROLOGICAL TOPICS

Mechanism of Igneous Intrusion

Edited by G. Newall and N. Rast. (*Geological Journal* Special Issue No. 2.) (Proceedings of a Symposium held in the University of Liverpool, January 9–11, 1969, under the auspices of Liverpool Geological Society.) Pp. 380. (Gallery: Liverpool, 1970.) 140*s*.

PROVIDED the reader of this book does not expect a comprehensive, authoritative or even fully relevant series of articles on the subject of the mechanism of igneous intrusion, he will not be disappointed, for, judged as an array of petrological topics, both descriptive and theoretical, representative of current research and thinking, this is a useful and stimulating publication. It has the merit of bringing together ideas and conclusions stemming from radically different approaches; all of these must be reconciled in the formulation of coherent genetic theory. An attempt at such a synthesis is the subject of the final chapter by N. Rast, which owes much to records of the discussion at the symposium as well as to notions of his own. This, indeed, is an aspect of symposium reports, namely, the opportunity to express ideas in invited contributions that would doubtfully find place in the literature of standard journals. Correspondingly, however, the reader himself must recognize the need to exercise a critical appraisal of the contents.

Some of the contributions are essentially reviews. The first article, on igneous activity in orogenic cycles by E. K. Ustiyev, is appropriately of this nature and introduces the subject of the symposium on the broadest scale in terms of Earth history. A very relevant review, by W. S. Pitcher, is concerned with an assessment of the

geometry of well-studied granite plutons in their various structural settings.

More specific descriptive articles are those by J. W. Aucott, on the Galway granite, which analyses the tectonic and intrusive history of the mass, and by B. E. Leake, a most instructive account of the mode of dislocation and disruption of basic and ultrabasic intrusions by Caledonian deformation in Connemara. The remarkable intrusions within the volcanic superstructures of Réunion, described by G. J. Upton and W. J. Wadsworth, add significantly to our knowledge of the relations between "plutonic" and effusive formations in central volcanoes; these have become increasingly well documented in recent years, but by their diversity have given rise to somewhat conflicting data on the question of the nature of "magma reservoirs" to central volcanoes. Geophysical methods are currently providing an important source of information even if they rarely allow of unequivocal interpretation; in this respect the paper by M. H. P. Bott and others on the crustal structure beneath the granites of south-west England has the additional merit of leading to a geologically acceptable model.

Ring structures are represented in contributions by A. C. Dunham and C. J. Stillman, and papers by D. Bridgwater and K. Coe and by B. F. Windley are concerned with basic dykes; the latter is of interest in describing dykes of dolerite composition, which have a garnet-amphibolite primary mineralogy.

Among the physico-chemical and geochemical contributions are papers by B. G. Jamieson, D. K. Bailey, P. G. Harris and others which relate to processes of magma generation and to considerations of thermal conditions on ascent and emplacement; if they do no more than cover familiar ground, their themes have a place in the context of the volume. W. S. Fyfe introduces some naïve thoughts on granites; thus he says, more happily in lecture than in print, "Once a melt forms, it may rise. There is no 'room' problem . . .". Fortunately, it is possible to turn to the thoughtful and ingeniously contrived experiments on intrusion mechanisms by H. Ramberg, for these have been inspired by an appreciation of geological problems, and the conclusions are correspondingly critical in their appraisal both of the usefulness and the limitations of the models, while J. L. Roberts in an extremely well-considered study evaluates the factors involved in magmatic emplacement in the environment of brittle rocks. J. W. Elder, arguing from slender and doubtful assumptions, gained a vantage point from which he could comment on every other contribution without any feasible rejoinder. S. A. F. Murrell in the first part of his paper ably summarizes earlier work of his own on rock mechanics, but follows this with an unappreciative review of global tectonics and vulcanism; the significance of the contribution as a whole is obscure and its value doubtful.

The list of titles is completed with the articles by H. Tazieff on ignimbrites and by J. M. Ade-Hall and E. A. Lawley on opaque minerals in lavas and dykes. Both are important contributions on their particular topics, but their relevance to the subject of the book is even less obvious than are several others.

B. C. KING

PLUTONIUM CHEMISTRY

The Chemistry of Plutonium

By J. M. Cleveland. Pp. xxii + 653. (Gordon and Breach: New York and London, July 1970.) $23.40; 195*s*.

THIS very useful book is divided into four parts; a short general introduction is followed by seven chapters on the solution chemistry of plutonium, including a very complete (107 pp.) coverage of solvent extraction systems. The third section, nine chapters on the compounds of plutonium, provides a very comprehensive account of this aspect of plutonium chemistry and the final part on chemical processing is just as complete.

From the data which have been collated and critically examined it is evident that by far the greater part of the work, in the 30 years since the discovery of the element, is related either to the process chemistry or to nuclear fuel applications, as one would expect for an element of such importance in the field of nuclear energy. For example, 1,118 references are cited, covering published (and some classified) material to the beginning of 1969, and no less than 533 of these deal with the chemistry of the complexes in aqueous solution, the related methods of solvent extraction, the separation of plutonium from reactor fuels and the chemistry of the halides, which are also of importance in metal production and halide separation processes.

Related to these aspects of plutonium chemistry, one would have expected there to be a great deal of published work on the thermodynamics of such systems, but it is evident from Dr Cleveland's careful assessments of the reported data that there are more conflicting estimates of thermodynamic quantities in this field than there are reliable measurements.

One minor criticism of this valuable assembly of data is the omission of any information on the molecular structure of plutonium compounds; admittedly very few complete X-ray structure analyses of plutonium compounds have been recorded, but tables of the known X-ray crystal data are provided in the book and it would have been useful to have stereochemical information as well.

The book is very attractively produced, with exceptionally few typographical errors, all of which are trivial. One which might possibly mislead occurs on p. 434 (line 33), where "concentrations N_3 of Pu (IV) and H_4NO" should be "concentrations of Pu (IV) and NH_4NO_3"; there are also two Tables 2.1 (pp. 12 and 20). These are only very minor points, and the book is warmly recommended to any chemist interested in the chemistry of the actinides; indeed, it is a book which no chemist working with plutonium, whether in a research laboratory or in a production plant, can afford to be without.

K. W. BAGNALL

COLLISION RESEARCH

Atomic Collision Phenomena in Solids

Edited by D. W. Palmer, M. W. Thompson and P. D. Townsend. Pp. xxvi + 678. (North-Holland: Amsterdam and London; American Elsevier: New York, 1970.) Hfl. 100; 233*s*; $28.

THIS book presents the proceedings of an international conference held at the University of Sussex in September 1969. A valuable aspect of the holding of specialist conferences is said to lie in the opportunities provided for informal and fruitful contacts between workers with shared interests; perhaps this spirit is evoked for those who could not be present by the inclusion of a list of conference participants and a group photograph. But in the more formal activities, with liberal use of visual aids, the density at which information is transmitted can daunt even the most alert and assiduous note-taker, so that a printed record is invaluable for later reference as well as for disseminating the proceedings to a wider audience. The contributors and editors are to be congratulated on the quality and on the speed with which this record has been produced.

The book consists of more than fifty papers, some available in abstract only, which range from short reports to papers which are more nearly review articles. The

extensive and recent references given with some of the longer papers is a helpful feature. The editors warn in their preface that the conference, and hence by implication this book, was not intended to be educational in character. The book is intended as a tool for the active worker in the field. Although authoritative and up to date, some of the papers are rather esoteric and make hard reading—this is certainly not the book for the beginner.

The principal sections of the book cover electron channelling, atomic collision theory, radiation damage, sputtering, inelastic atomic collisions, ion channelling, electron excitation and photon emission, surface scattering of ions, and ion penetration profiles. There is a fair balance between presentation of theory and of experimental results. The sputtering of KI by exposure to ultraviolet radiation is interesting because of the support which the results lend to current theories of the mechanism of the formation of colour centres by irradiation of the alkali halides. The description of the direct observation of heavy ion collision cascades by field ion microscopy is very convincing. The papers on the channelling of electrons—important in the transmission electron microscopy of thin crystals—emphasize the need for a proper wave mechanical treatment of the interaction of the electron beam with the Bloch waves. There have been further refinements in the calculation of interaction potentials; Robinson has calculated these using the method of Firsov and starting from the simple scattering cross-section introduced by Lindhard. All in all, this book gives a picture of a flourishing and active field of study.

T. P. P. HALL

SPINS IN SOLIDS

Spin Temperature and Nuclear Magnetic Resonance in Solids

By Maurice Goldman. (International Series of Monographs on Physics.) Pp. ix+246. (Oxford University: London; Clarendon: Oxford, July 1970.) 85*s*.

THE aim of this book is to present a unified description of the high temperature approximation to spin temperature theory. Its chief application is to nuclear spin systems in solids. Although the treatment throughout is theoretical, appropriate experimental information is discussed whenever possible.

The concept of temperature in spin systems is introduced in the first chapter and modifications applicable to nuclear magnetic resonance are described. This chapter is followed by a study of the properties of nuclear spin systems which are isolated from the crystal lattice but subject to both sudden and adiabatic changes in the Hamiltonian. The effects on the spin temperature of a finite spin-lattice interaction are introduced. The two following chapters describe in detail the Provotorov theory of saturation which is applicable to cases when the probabilities of radio-frequency induced transitions are comparable with those for transitions induced by spin-lattice coupling. This theory is basic to much of nuclear magnetic resonance. The effects of spin-spin interactions on the spin temperature concept are then discussed. Finally, a description is given of dynamic polarization methods, which is particularly appropriate to the "solid effect" of thermal mining in low fields. The appendix contains some of the more important results found useful in spin-temperature theory.

This monograph will be of value to both the experimentalist and theoretician interested in the properties of interacting spin systems and particularly to research workers in nuclear magnetic resonance. The material is presented in a clear and logical way and the subject matter is very carefully arranged and indexed. The text may be regarded as complementary to the book by Professor A. Abragam on the *Principles of Nuclear Magnetism* (1961) and could prove an equally valuable addition to the literature of physics.

C. A. BATES

FOR TRANSLATORS OF RUSSIAN

Russian–English Scientific and Technical Dictionary

By M. H. T. Alford and V. L. Alford. Vol. 1: Pp. xxvi+1–764. Vol. 2: Pp. 765–1422. (Pergamon: Oxford, London and New York, October 1970.) 240*s*; $32.

ACCORDING to the dustjacket, this dictionary, containing "over 100,000 entries drawn from 94 different disciplines, technologies and other professional subjects", is specially intended for "people with weak or moderate knowledge of Russian grammar" and will be "invaluable to scientists learning the language". To facilitate its use by the latter, it is provided with a preliminary chapter, "Instructions for Learning Large Vocabularies", based on work carried out in the University of Essex and supported by the Office for Scientific and Technical Information, for developing new methods of learning to read foreign languages. New methods of vocabulary learning are presented, based on methods of identification of stimulus and remembering the stimulated response, and a procedure for learning large vocabularies by means of special prompt cards. Valuable as these methods may be to the language student, it is open to doubt whether this emphasis on those beginning Russian contributes much of value to this dictionary. A book of this size and price will surely be purchased chiefly by libraries, and by already competent translators of Russian wishing to extend and modernize their vocabularies, rather than by beginners, who would be more suited initially with one of the existing smaller dictionaries limited to their own field of study.

Used in practical translation, the potential use of this book is greatly decreased by this emphasis on the student. Some years back, L. M. Milne-Thompson, in his *Russian-English Mathematical Dictionary* (University of Wisconsin, 1962), introduced the practice of equipping each entry with a reference to the basic root of the word: "*primechanie* remark, note (*met* mark), *prodolzhenie* continuation, extension (*dolg* long)" which, although a valuable aid to learning, did not impede the fluent Russian speaker from using the dictionary. In this new work, however, each word is dissected into a complicated morpheme structure (prefix-root-suffix-inflexion) in which the elements are separated by raised points: *pri·mech·ani·e, pro·dolzh·eni·e*, which proves wearisome to the eye and considerably slows down the process of word-finding.

With regard to the content of the dictionary, it is, as claimed, extremely comprehensive (including such fringes of technology as "*korset* corset (garment)", "*kosilka* a mowing machine" and "*mast* colour of animal hair, suit at cards"). There are, however, a number of misprints, some of which (for example, "Lynds" for Lyrids) are not corrected in the errata. Russian versions of Latin-script proper names, (often a stumbling block) are given, though not always cross-referenced (thus the *Fabri-Pero* (Fabry and Perot) interferometer is listed under *Fabri*, but there is no cross-reference for Perot under *Pero*).

Apart from these (admittedly minor) defects, the vocabulary contained in this dictionary will be an invaluable aid to anybody regularly involved in the reading or translation of scientific papers in Russian. It is, however, somewhat too large and unwieldy a vocabulary for the beginner (even with the use of learning aids), and it is a pity therefore that the emphasis in format on the student (who will be less likely to use it) detracts from its undoubted value to those already proficient in the language.

VERA RICH

Correspondence

Scientific Censorship

SIR,—Recently we submitted a paper to a leading chemical journal in Czechoslovakia—*Collection of Czechoslovak Chemical Communications*. The whole work described in our paper was performed at the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, between 1967 and 1969. We felt it our obvious duty to publish our paper in the journal where most of our previous papers had appeared. Moreover, our work was done exclusively on Czechoslovak territory and completely funded by the Czechoslovak Academy of Sciences.

We have been informed that in view of the publishing policy adopted by the present Czechoslovak authorities, which prohibits Czechoslovak authors who are currently living abroad without the approval of the Czechoslovak government from publishing in the Czechoslovak journal, our paper cannot be accepted for publication.

We would like to point out that the submitted paper deals exclusively with problems of the natural sciences without any relation to the present political situation in Czechoslovakia. The decision of the Czechoslovak authorities can hardly be labelled otherwise than as a discrimination of scientific work.

Yours faithfully,

JIRI ZEMLICKA

Michigan Cancer Foundation,
4811 John R Street,
Detroit, Michigan 48201.

STANISLAV CHLADEK

Department of Biochemistry and Biophysics,
Texas A and M University,
College Station,
Texas 77843.

Medvedev's Complaint

SIR,—Bikerman (*Nature*, **228**, 297; 1970) has offered a completely misleading analogy between the refusal of the National Science Foundation to supply travel funds for his trip to France, and the refusal of the Soviet government to permit Zhorez (that is *not* Jaurès) Medvedev to attend a Ciba Symposium. In Medvedev's case, authorization to leave the country was adamantly denied, although travel funds were actually available. Presumably Bikerman, as a naturalized US citizen, would have had no trouble in obtaining a visa to enter France.

Bikerman says that the excerpt from Medvedev's book strikes him as subtle communist propaganda. Since Medvedev, for publishing a book exposing Lysenkoism and advocating international cooperation in science has (i) lost his job, (ii) been prevented from moving to another district to look for a new position, (iii) been hauled off forcibly to a "psychiatric" institution, the way of a subtle propagandist would seem to be a steep and thorny road.

Yours faithfully,

THOMAS H. JUKES

University of California,
Berkeley, California 94720.

Definition of Intelligence

SIR,—H. A. Fatmi and R. W. Young (*Nature*, **228**, 97; 1970) propose a definition of intelligence for use in drawing distinctions between machine and human behaviour. They base their definition on the ability of the system to perceive order in a situation previously considered to be disordered.

While this definition is valid within extremely narrow constraints, it has serious limitations in defining the difference between men and machines. In the first place it excludes many aspects of intelligence which are of the greatest interest from a human point of view, namely, the relation between set theory on the one hand and the ability of intelligent systems not only to perceive but also to generate and choose new structures and instruction sets and ascribe meaning to both internal states and input sets.

A single machine with a finite phrase-structure grammar would not be able to realize the extended definition of intelligence given above. An extension of Fatmi and Young's definition allows us to include the missing elements on the human side of the equation.

> "Intelligence is that faculty, of mind, by which order is perceived in a situation previously considered to be disordered, and whereby similar structures can be generated, recognized and semantically defined without the necessity of complete *a priori* specification."

This revised definition permits the inclusion of linguistic competence, as defined by Chomsky, as an aspect of intelligent behaviour, which it certainly is. In the trivial case, we know that the child produces valid grammatical sentences in the absence of a complete instruction set. On a deeper level we also know that the set of valid grammatical statements is an infinite set and that the instruction set for a natural language cannot be completely specified. By Fatmi and Young's argument all linguistic behaviour is only apparently intelligent or, in the trivial case, we would have to assume that the same sentence uttered by a child or a parent or teacher would be sometimes a sign of intelligent and sometimes a sign of intelligence-like behaviour.

My own work leads me to the view that attempts to relate simplicistic finite set theory and automata to faculties of mind and the mechanisms of the brain inevitably leads to such difficulties. I suggest that the brain should be seen as a transfinite automaton, which cannot be defined by restricted set theory (ZF), but only by extended set theory including the all important "axiom of choice"[1-3].

Yours faithfully,

GORDON HYDE

11 The Close,
Dunmow, Essex, CM6 1EW.

[1] Lofgren, L., *Explicability of Sets and Transfinite Automata in Automata Theory* (edit. by Caianello, E. R.) (Academic Press, 1966).
[2] Cohen, P. J., *Set Theory and the Continuum Hypothesis* (W. A. Benjamin, New York, 1966).
[3] Cohen, P. J., and Hersh, R., *Sci. Amer.*, **216**, 107 (1967).

Decimal Lakh

SIR,—As the day of formal and wide acceptance of the decimal system draws closer, may I make a public request in favour of an Indian unit ?

It is Laksha, written in English as Lakh, standing for 100,000. Surely, a lakh is a better way of saying one hundred thousand.

Yours faithfully,

S. ROY

Department of Mechanical Engineering,
University of Aston,
Birmingham 4.

Anomalous Water

SIR,—K. S. Chua (*Nature*, **227**, 834; 1970) has proposed certain structures to account for the density of anomalous water (1·4 g cm^{-3}) with a resulting oxygen-oxygen bond length of 2·4 Å. We add that the work of J. C. Speakman *et al.* (University of Glasgow) also reveals shared O-H-O bonds of similar lengths for certain crystalline acid salts.

Although unmentioned in the article, however, it turns out that the first of Chua's structures simply reduces to a scaled down (2·4/2·76) version of the well known ice structure (but with hydrogens shared between the oxygens). The second is the similar equivalent of the ice Ic structure —a low temperature polymorph of ice.

Can one really believe that these particular crystal structures as such could demonstrate the properties of liquid anomalous water?

Yours faithfully,

IAN CHERRY
PAUL BARNES
JOHN FULLMAN

Department of Crystallography,
Birkbeck College,
Malet Street, London WC1.

Obituary

Dr K. Biswas

DR KILIPANDA BISWAS, the Indian botanist famous for his study of the flora of India and Burma, died on December 29 last year at the age of seventy.

Early in his career he was superintendent to a number of botanical gardens in India, including the Royal Botanic Gardens of Calcutta and the Indian Botanic Gardens. For this work, he was awarded the Elliott gold medal and prize in 1928 and 1936, and the coronation medal in 1937. He came to Europe in 1936 and worked in the Herbarium of the Royal Botanic Gardens of Kew and Edinburgh and in the British Museum. In 1937 he was awarded a DSc at the University of Edinburgh for his valuable contributions to the knowledge of the Indian flora, and in 1938 he was elected a fellow of the Royal Society of Edinburgh.

In 1954 he was appointed director of the Botanical Survey of India and in the following year he became director of medicinal plants, a post he held until his retirement. During this time, his task was to develop the large scale cultivation of ipecac. His success here was important to the economy of India and earned him the Barclay memorial medal for the best research work in biological science in Asia in 1961.

After 1964, he continued as honorary lecturer in botany at the University of Calcutta and as a member of various organizations in India, Britain and UNESCO.

He had been suffering from a heart disease and chronic uraemia for one and a half years. He leaves his wife, two sons and a daughter.

Announcements

University News

Professor C. Rimington, formerly of University College Hospital Medical School, London, will join the Department of General Medicine, **University of Capetown,** for about four months as Royal Society Leverhulme visiting professor.

Dr D. B. Moffatt has been appointed to a personal chair in the Department of Anatomy, **University College, Cardiff.**

Dr R. F. Churchhouse, head of the Programming Group of the SRC Atlas Computer Laboratory, Oxford, has been appointed director of the Computing Centre and professor of computing mathematics.

Dr Elmar Seitler, assistant chief of biophysics in the US Armed Forces Institute of Pathology, Washington DC, has been appointed professor in the Departments of Physics and Biophysics, **University of Chicago.**

Appointments

Dr Ruth M. Davis, director of the Lister Hill National Center for Biomedical Communications, has been appointed director of the Center for Computer Sciences and Technology of the **US National Bureau of Standards.**

Sir Edward Bullard, University of Cambridge, **Professor A. D. Bradshaw,** University of Liverpool, and **Dr C. E. Lucas,** Department of Agriculture and Fisheries for Scotland, have all been appointed members of the **Natural Environment Research Council.**

The following have been elected officers of the **International Astronautical Federation:** president, **Professor A. Jaumotte** (Belgium), vice-presidents, **Dr G. E. Mueller** (USA), **Mr H. G. S. Murthy** (India), **Professor L. G. Napolitano** (Italy) and **Professor L. I. Sedov** (USSR).

An **Academic Advisory Committee** has been appointed to the **Cranfield Institute of Technology** to monitor and advise on academic standards. Members of the committee are: **Lord Caldecote,** Delta Metal Company (chairman); **Mr H. F. R. Catherwood,** National Economic Development Council; **Professor A. R. Collar; Sir George White,** University of Bristol; **Dr H. M. Finniston,** British Steel Corporation; **Dr F. R. Jones,** Mullard Limited; **Sir Morien Morgan,** Royal Aircraft Establishment; **Mrs M. K. McQuillan,** Imperial Metal Industries (Kynoch) Limited; **Professor S. C. Redshaw,** University of Birmingham.

Miscellaneous

Professor Brinley Thomas, University College, Cardiff, has been awarded a fellowship by the **US National Science Foundation.**

The **International Meteorological Organization prize** for 1970 has been awarded posthumously to **Professor R. Scherhag** who was director of the Institute for Meteorology and Geophysics at the Free University of Berlin.

Dr Richard D. O'Brien, director of the Division of Biological Sciences at Cornell University, has won the **1970 International Award for Research in Pesticide Chemistry,** sponsored by Burdick and Jackson Laboratories Inc., Muskegon, Michigan.

The 1970 **Edward B. Rosa award** of the US National Bureau of Standards has been won by **Mr P. R. Achenbach,** chief of the Sensory Environment Branch of the NBS Building Research Division. **Dr Robert P. Madden,** chief of the Far Ultraviolet Physics Section in the NBS Optical Physics Division, will receive the 1970 **Samuel Wesley Stratton award** of the NBS. Each award consists of a bronze plaque and a $1,500 honorarium.

Nominations are invited from academies or scientific societies concerned with zoology for membership of the **International Commission on Zoological Nomenclature** which has seven vacancies arising from the resignation or retirement of Commissioners from Brazil, Germany, Japan, the Netherlands, the UK and the USA. Candidates may be of any nationality and should have a distinguished record of work in any branch of zoology. The Commission is particularly interested in recruiting members concerned with herpetology, parasitology, marine biology, Protista and Coelenterata. Nominations should be sent to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London SW7, before December 31.

ERRATUM. The title of the article by R. G. Crounse, A. J. Bollet and S. Owens which appeared in *Nature* for October 31 (**228**, 465; 1970) should have read "Quantitative Tissue Assay of Human Malnutrition using Scalp Hair Roots".

ERRATUM. In the article entitled "Synthetic DNA-RNA Hybrids and RNA-RNA Duplexes as Templates for the Polymerases of the Oncogenic RNA Viruses" by S. Spiegelman *et al.* (*Nature*, **228**, 430; 1970), the title of Table 1 should read "RESPONSES OF ONCOGENIC VIRAL DNA POLYMERASES TO ADDED DNA AND A SYNTHETIC RNA-DNA HYBRID". Table 4 should read as follows:

Template	Incorporation (pmoles/5 min/10 g viral protein)			
dA·rU	dT	(0·8)	dA	(<0·1)
dC·rG	dG	(141·0)	dC	(1·9)
dC·rI	dG	(44·5)	dC	(1·9)
dG·rC	dC	(1·2)	dG	(13·3)
dI·rC	dC	(1·3)	dG	(104·6)
dT·rA	dA	(0·9)	dT	(10·3)

ERRATUM. In the article by J. N. Mehrishi entitled "Cell Surface Charge Increase by both Tumour Inhibitory and Tumour Growth Promoting Polyanions" (*Nature*, **228**, 364; 1971), references 14 to 46 were unfortunately printed on page 375 of the same issue of *Nature*.

International Meetings

January 18–22, 1971, **Chemical Oceanography** (Gordon Research Conference), Santa Barbara (Dr Alexander M. Cruickshank, director, Gordon Research Conferences, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881, USA).

January 18–22, 1971, **Electrochemistry** (Gordon Research Conference), Santa Barbara (Dr Alexander M. Cruickshank, director, Gordon Research Conferences, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881, USA).

January 25–29, 1971, **Polymers** (Gordon Research Conference), Santa Barbara (Dr Alexander M. Cruickshank, director, Gordon Research Conferences, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881, USA).

March 29–31, 1971, **Non-Ionizing Radiation Safety,** Cincinnati (Laser Laboratory, Children's Hospital Research Foundation, Cincinnati, Ohio 45229, USA).

British Diary

Monday, November 9

Batteries mW to kW (6.30 p.m.) Mr C. J. Bruce, Institution of Electrical Engineers, London Graduate and Students Section, at Savoy Place, London WC2.

How Does the Introduction of SI Units Affect the Mechanical Engineer? (6 p.m., discussion) Institution of Mechanical Engineers, Thermodynamics and Fluid Mechanics Group, at 1 Birdcage Walk, London SW1.

Proofs: Positive and Presumptive (5.30 p.m.) Mr B. Gower, British Society for the Philosophy of Science, in the Joint Staff Common Room, University College London, Gower Street, London WC1.

Semiconductor Gamma Camera System (6 p.m.) Mr E. Moss and Mr W. Gore, Institution of Electronic and Radio Engineers, Joint IEE/IERE Medical and Biological Electronics Group, at 9 Bedford Square, London WC1.

Training Engineers to Use Computers (7.30 p.m.) Mr W. S. Meadows, Institution of Electrical Engineers, at Mander College, Bedford.

Tuesday, November 10

Adrenocortical Function in Psychiatric Disorder (5.30 p.m.) Professor J. L. Gibbons, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Sixth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Anthraquinones as Analytical Reagents (5.15 p.m.) Mr M. A. Leonard, Society for Analytical Chemistry, jointly with the Leeds University Union Chemical Society, at the University, Leeds.

Changes and the Future in Electrical Engineering (2 p.m. and 7 p.m.) Mr S. Z. de Ferranti, Institution of Electrical Engineers, at Usher Hall, Edinburgh (Faraday Lecture).

Clearing the Air (6 p.m.) Mr F. E. Ireland, Institution of Chemical Engineers, at the Renold Building, UMIST, Manchester.

Construction of the Monmouth Tunnels in Soft Rock (5.30 p.m.) Mr R. R. Parry and Mr G. D. Thornton, Institution of Civil Engineers, at Great George Street, London SW1.

Laboratory Automation (three-day conference) Institution of Electronic and Radio Engineers, jointly with IEE and other Institutions, at the Middlesex Hospital Medical School, Mortimer Street, London W1.

Noise and Vibration in Motor Vehicles (7.30 p.m.) Dr D. Saunders, Society of Environmental Engineers, at the University of Birmingham.

Present and Future Problems of Operation of British Grid System (7.30 p.m.) Mr J. I. Bird, Institution of Electrical Engineers, at the University of Reading.

Project Evaluation and Technological Forecasting in Agriculture (10 a.m.) Society of Chemical Industry, Agriculture Group, jointly with the Management Group, at 14 Belgrave Square, London SW1.

Social Implications of Automation (6 p.m. discussion) Institution of Mechanical Engineers, jointly with IEE and the Institute of Measurement and Control, at 1 Birdcage Walk, London SW1.

The Seasonal Reproductive Changes in the Red Deer Stag, Mr G. A. Lincoln; **Studies on Caddis Flies,** Dr M. I. Crichton; **Monuments in Mangroves—a Study of Bird Life on a Small Island Off the Coast of Florida** (film) (5 p.m.) Zoological Society of London, at the Zoological Gardens, Regent's Park, London NW1.

Training of Microanalysts for Industrial Laboratories (6.30 p.m. discussion meeting) Society for Analytical Chemistry, Education and Training Group, at Imperial College, London SW7.

Wednesday, November 11

Analytical Applications of Lasers (6 p.m.) Mr P. J. Hendra, Society for Analytical Chemistry, Midlands Section, at the University, Birmingham.

Applications of Thermal Analysis (7.30 p.m. discussion meeting) Society for Analytical Chemistry, Thermal Analysis Group, at the Leicester Lounge, 44 Glasshouse Street, London W1.

Conservation and the Electrical Industry in the 70s (9.30 a.m. colloquium) Institution of Electrical Engineers, at Savoy Place, London WC2.

Discussion Meeting (two days) Geological Society of London, Volcanic Studies Group and the Royal Society, at the Royal Society, 6 Carlton House Terrace, London SW1.

Economic Comparisons in Planning for Electricity Supply (6.30 p.m.) Mr T. E. Norris, Institution of Electrical Engineers, at the Great White Horse Hotel, Ipswich.

Jacques Besson's "Livre des Instruments Mathematiques et Méchaniques" (1 p.m.) Dr A. G. Keller, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Stirling Cycle Engine (6 p.m.) Dr A. Organ, Society of Environmental Engineers, in the Mechanical Engineering Department, Imperial College, Exhibition Road, London SW7.

The Biological Assays of the British Pharmacopoeia (6.30 p.m.) Mr V. J. Birkinshaw, Society for Analytical Chemistry, North East Section, at the School of Pharmacy, Sunderland Polytechnic.

The Development of Inventions (6 p.m.) Mr T. A. Coombs, Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

The Human Pharmacology of Sedatives and Hypnotics (5.30 p.m.) Dr M. H. Lader, University of London, at the Institute of Psychiatry, Maudsley Hospital, Denmark Hill, London SE5.

The Large Scale Production of Unexpected and Unexplained Laboratory Results (2 p.m.) Professor T. P. Whitehead, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

The Optical Variables in White Paint Films, Mr D. F. Tunstall; **Uniform Radical Tolerance Method of Colour Difference Assessment,** Mr J. S. Mudd (3 p.m.) Colour Group (Great Britain), in the Mechanical Engineering Department, Imperial College, London SW7.

The Significance of Glass Transition Temperatures of Paint Films (7 p.m.) Mr J. L. Prosser, Oil and Colour Chemists' Association, at the Borough Polytechnic, Borough Road, London SE1.

Volcanism and the Structure of the Earth (two-day discussion meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

Thursday, November 12

Addition Copolymers of the Vinyl Esters of Branched Chain Fatty Acids for Cure with Isocyanates (6.30 p.m.) Mr P. de Carpentier, Oil and Colour Chemists' Association, at the Royal Turks Head Hotel, Grey Street, Newcastle upon Tyne.

Aircraft Pavement Design (9.30 a.m. symposium) Institution of Civil Engineers, at Great George Street, London SW1.

Applications of Silicas and Silicates in the Surface Coating Industry (6 p.m.) Mr L. Muller-Fokken, Oil and Colour Chemists' Association, at the St Enoch Hotel, Glasgow.

Electronic Aids in Medicine (6.30 p.m.) Mr J. L. Gedye, Institution of Electrical Engineers, at the University of Essex, Colchester.

Fresh Observations on Calcium and Phosphorus Metabolism in Chronic Renal Failure (5.30 p.m.) Professor H. E. de Wardener, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Seventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Handkerchief Perfumes (7.30 p.m. demonstration meeting), British Society of Perfumers, at the Tropical Products Institute, 127 Clerkenwell Road, London EC1.

Hybrid Engineer (6.15 p.m.) Mr W. G. Evans and Mr C. S. Gledhill, Institution of Electrical Engineers, at Sumpner Building, University of Aston, Birmingham.

Thermal Analysis in Mineralogy (3 p.m.) Mr F. W. Wilburn, Mr H. G. Midgley and Mr W. B. Jepson, Society for Analytical Chemistry, Thermal Analysis Group, at the Wellcome Building, Euston Road, London NW1.

What Management Expects from Electronic Engineers and What the Young Graduate Expects from Management (6 p.m. discussion) Institution of Electronic and Radio Engineers, at 8–9 Bedford Square, London WC1.

Friday, November 13

Cosmic Ray Electrons (4.30 p.m.) Professor H. C. van de Hulst, Royal Astronomical Society, in the Scientific Societies Lecture Theatre, 23 Savile Row, London W1. (George Darwin Lecture; Chairman: Sir Bernard Lovell.)

Photoaddition of Amines to Unsaturated Compounds (1 p.m.) Dr Silva de Brito Costa, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

The Plastics Revolution (9 p.m.) Professor J. C. Robb, Royal Institution, at 21 Albemarle Street, London W1.

Water in Industry (6.30 p.m. discussion) Society for Analytical Chemistry, Western Section, at the George Hotel, Chepstow.

X-ray and Gamma-ray Astronomy (2 p.m. discussion; Chairman: Dr K. A. Pounds) Royal Astronomical Society, at Burlington House, Piccadilly, London W1.

Monday, November 16

Developments in the Science of Wood Preservation (10.30 a.m. symposium) Society of Chemical Industry, Pesticides Group, at 14 Belgrave Square, London SW1.

Inflation and Eruption of Kilauea Volcano, Hawaii, 1965-68 (5.30 p.m.) Dr R. S. Fiske, University of London, in the Chemistry Lecture Theatre, King's College, Strand, London WC2.

Space Technology and the Future (6.30 p.m.) Mr G. K. C. Pardoe, Institution of Electrical Engineers, at the University, Liverpool.

The Origins of Money (5.30 p.m.) Mr P. Grierson, University of London, at Senate House, London WC1.

The University's View of Industry (6 p.m.) Professor J. W. Linnett, FRS, Royal Society of Arts, at John Adam Street, London WC2. (Cantor Lecture.)

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Reports and Publications

(not included in the monthly Books Supplement)

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The Edinburgh School of Agriculture. Calendar 1970/1971. Pp. 71. (Edinburgh: The Edinburgh School of Agriculture, 1970.) [289

Agricultural Research Council. Report of the Institute for Research on Animal Diseases for 1969. Pp. 56. (Compton, near Newbury: ARC Institute for Research on Animal Diseases, 1970.) [289

Fabian Tract 403: Labour and Inflation. By Thomas Balogh. Pp. 64. (London: Fabian Society, 1970.) 8*s* (40*p*). [289

Tweed River Purification Board. Sixteenth Annual Report for the year ended 31st December, 1969. Pp. 39. (Newton St. Boswells: County Offices, Tweed River Purification Board, 1970.) (289

Department of Education and Science. Animals (Restriction of Importation) Act, 1964. Report of the Advisory Committee year ending 31st December 1969. Pp. 32. (London: Department of Education and Science, 1970.) [289

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Extension of the Chester Beatty Research Institute Serial Abridged Life Tables to Cover the years 1961–1965 and a Provisional Estimate for 1966–1970. By R. A. M. Case and Christine Coghill. (Supplement to The Chester Beatty Research Institute Serial Abridged Life Tables, England and Wales, 1841–1960. Compiled by R. A. M. Case Christine Coghill, Joyce L. Harley and Joan T. Pearson. (London: The Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, 1970.) [210

Other Countries

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A Propos du Mécanisme Terrestre. Par Georges Dubourdieu. Pp. 70. La Ceinture du Pacifique et les Synchronismes de son Activité Sismique. Par Georges Dubourdieu. Pp. 55. (92-Meudon, France: Collège de France, Laboratoire de Géologie, Avenue Marvelin-Berthelot, 1969 et 1970.) [249

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What Next at CERN?

EUROPEAN members of the European organization for nuclear research (CERN) are once more screwing up their courage for a decision about the future of the project to build a 300 GeV proton accelerator (see page 597). In the past four years, this proposal has served powerfully to challenge a great many of the conventional assumptions about European collaboration in science. In spite of the success with which the first of the two storage rings at Geneva has been commissioned, and in spite of the ingenuity with which Dr J. B. Adams and his colleagues have been able to modify the original proposal for a very much larger accelerator, it remains unsure just what the outcome will be. Theoretically, the next meeting of the CERN council should be in a position to decide what happens to the project.

The first need is for a reappraisal of the part played by high energy physics in European science. To say this is not to question in any important way the success with which CERN has provided a focus for a great deal of research in physics and also a model of good behaviour in international relations but, rather, to suggest that the time has come for a more thorough look at the way in which high energy physics is carried out not merely at the collaborative centre at Geneva but in Europe as a whole. And for all its virtues, it is only proper to acknowledge that CERN itself has often been too presumptuous in its belief that Europe owes high energy physics a living.

When the scheme for building a machine with an energy of about 300 GeV was first proposed in 1964, CERN did its cause a good deal of damage by asking not merely that European countries should build the large machine on a collaborative basis but also that they should provide a broader base for European high energy physics by building more national facilities as well. In reality, it would have been more tactful and probably more realistic as well to acknowledge that many if not all of the governments then asked to contribute would be more inclined to do so if they could feel that collaboration would help to reduce the cost and not increase it. More recently, in May 1967, when the European committee on future accelerators produced its detailed proposals, this lesson had been learned but the organization was still over-rigidly committed to the minutiae of its proposals. The argument was that flexibility would be interpreted by unwilling governments as irresolution about the merits of the project as a whole. The fact that CERN has since then been able to modify its supposedly final proposals on at least two occasions suggests not merely that it would have been wise to be more flexible at the beginning (see *Nature*, **214**, 1283; 1967) but, to outsiders perhaps, that the organization's pronouncements are less credible than they might be. When only six of the twelve potential members of the 300 GeV project declared their willingness to contribute, for example, the organization was able to lop off from the original bill some 15 per cent of the cost.

More recently, since 1969, CERN has been working on a design in which flexibility is everything. One proposal would make it possible to increase the energy by adding to or improving the magnets around a ring 3 km in diameter from 250 GeV at the beginning to 1,300 GeV some years later. Another more modest version of this proposal, based on a ring 2 km across, would allow the operating energy to be increased from 300 GeV to 800 GeV. One of the advantages of this second version is that the new machine could be built near the present laboratory at Geneva, using the existing proton synchrotron as a source of protons. In real terms, the cost of the first stage of this preferred design is merely two thirds of that quoted originally for a 300 GeV machine. The paradox is that if CERN had been prepared in 1967 to admit the possibility of substantial cost reduction, it might well have found more than six European governments prepared to help foot the bill.

As things have turned out, much of the enthusiasm for high energy physics which made the outlook for the 300 GeV accelerator seem bright in the mid-sixties has now tended to evaporate. The French Government, for example, was then considering a project since abandoned for a 40 GeV machine. In the past four years, it is clear that there will be a British contribution to the 300 GeV machine only if there is a more rapid decline than originally planned in the operation of the existing laboratories at Harwell and Daresbury. But in these circumstances, is it wise that the building of the 300 GeV machine should be considered independently of the plans being made by separate governments for the continued operation of the machines which exist already? Might it not be preferable to devise some means by which European collaboration in high energy physics is not centred on a single machine or even a group of machines such as could emerge on the outskirts of Geneva, but, rather, diffused throughout high energy physics in Europe? One possibility, not to be dismissed on the grounds of sheer novelty, would be to make arrangements for allowing individuals with projects of some virtue to be able to carry these out at whichever of the high energy physics laboratories in Europe seem to be the most appropriate. To do this would require nothing less than a research foundation for European high energy physics, drawing financial support from member

nations not merely for the operation of the machines which exist at present but also, with luck, for the building of the 300 GeV accelerator as well.

But is there time to think of negotiating such a complicated arrangement at this late stage? This is what the enthusiasts will say. The truth is, however, that ever since the publication of the Swann report which formed the basis for the British Government's decision not to participate in the 300 GeV project, it has been clear that the continued financing of high energy physics could not be accommodated within the ordinary framework of support for basic scientific research. In Britain, the fear is that high energy physics can only continue to exist at the expense of other more worthwhile activities. Elsewhere the sheer cost of what is now proposed seems to be a sufficient disincentive, while other nations in Europe are understandably disinclined to participate either because they have recently acquired reasonable facilities of their own or because they attach low priority to the work proposed.

There is no cause for the high energy physicists to complain about this state of affairs—instead, they must learn to live with it. On the long view, the 300 GeV machine would be an asset to European science but it would be even more valuable if it could be built within a framework giving expression to the belief that physicists in particular are fond of quoting that branches of physics like these are already thoroughly international.

Warm and Cheerful

THE British Government has put itself into an awkward box on fuel policy and there is no assurance that the Conservative Cabinet will be any better than its Labour predecessor in finding a way out. A part of the difficulty seems to be that of forecasting the demand for energy a few years ahead. By now, the record of failure in this field is as long as any arm. In the mid-fifties, people were anxious that Britain would be brought suddenly to a halt by a lack of fuel, and miners were exhorted to dig more coal. Then, in 1955, the government pinned its colours to the mast of nuclear power, and miners were exhorted to find jobs outside the coalfields. Since then, the prevailing view has swung radically between the promise of shortage of coal and the threat of glut. The most recent development in Britain is that a shortfall in the output of nuclear power from nuclear fuelled power stations has been aggravated by a decision some years ago that there should be a reduction in the rate of capital investment in electricity generating plant and by a wastage of miners from the coalfields at such a rate that the miners now think themselves in a position in which they can successfully launch the first substantial strike since 1926.

The fluctuations in the available supply of energy which seem to create difficulties are, it must be remembered, exceedingly small. The fuel economy is still so precariously balanced that a few million tons of coal either way, or the delay in the commissioning of a single nuclear power station, can tip the balance substantially towards surplus or shortage. Governments have a natural tendency to hope to manage their affairs with such precision that failures of this magnitude are rare or even non-existent but that is a great conceit—prudent management would suggest that fluctuations of a few per cent, equivalent to ten million tons of coal a year, in the fuel budget ought to be expected. In short, if the new government is anxious to devise a fuel policy to replace that of its predecessor, it ought to legislate for some system under which the substitution of one fuel for another is comparatively straightforward.

What should this device be? For the best part of a decade, the British Government has done its best to make sure that the Central Electricity Generating Board will be discouraged from replacing indigenous coal by imported oil by means of a levy on fuel oil imported into Britain. This tax of ten per cent has been sufficient not merely to reduce the volume of oil imports but, alas, also to persuade the electricity generating board that taking out an option to burn fuel oil in British power stations would be an expensive luxury. By the same test, the electricity generating board has been discouraged from providing the excess capacity necessary to insure against unexpected developments. It is, however, idealistic to expect that newly commissioned innovations such as nuclear power stations will function perfectly in the first year of their service, and it is wrong to suppose that there is something wrong with the process of innovation if these devices fail to work perfectly the first time round. The moral should be, on the practical level, that the time has come to abolish artificial restraints on the British fuel economy and, in particular, that restraint on the use of fuel oil embodied in the present tax. In the long run, however, the British Government must sanction at least enough capital expenditure to ensure that there is enough give and take in the fuel economy for the most advantageous mixture of fuel to be that chosen by the market. It will be a lesson to us all to see whether a government which has flaunted its respect for market forces in the past few weeks is prepared to let these market considerations be acted out when a little extra capital investment may thereby be necessary.

Telling It Like It Is

ONE of the problems of contemporary biography is that it is bound to read a little like obituary. Certainly the article on Dr Francis Crick in the current issue of *Daedalus* (**99**, 938) by Dr Robert Olby is not merely an intriguing preview of the full length manuscript on which Dr Olby has been working for several years but also a device that almost succeeds in fixing Crick in amber, or at least in aspic. What Dr Olby and the

other contributors to a symposium on "The Making of Modern Science" have done is to attempt to provide an account of current or recent events in the development of science. What Professor Gerald Holton, the editor of this issue of *Daedalus*, and his collaborators have been looking for is a way of writing about the way in which science is done that lies somewhere in between the reverential history and Watson's *The Double Helix*. Their objective was partly to record important events, partly to provide an insight into the authors of them and partly to throw light on the workings of the institutions which provide refuge and sustenance for scientists and other kinds of scholars.

How well is it possible to succeed? Most of this issue of *Daedalus* is as readable as any editor could wish. Professor Erik Erikson, to be sure, has more to say about Freud than about himself. Professor Talcott Parsons is more revealing of the sources from which sociologists draw their strength. The hard scientists are, however, more explicit in what they have to say and everybody seems to be preoccupied with molecular biology. Professor Curt Stern marvels at the way in which the abstract concept of the gene has been made more subversive by being made more tangible. Professor Gunther Stent takes the view that now that biology has been made a branch of physical chemistry, only the nervous system remains a serious challenge—the kind of fallacy on which many heads have already

Nature plus TWO

As already announced, *Nature* will appear on Monday, Wednesday and Friday each week in 1971. The intention is that this reorganization of the pattern of publication should enable readers of all kinds to find their way more easily to the items which concern them most directly. The new development is not meant as an extra demand on the time of busy scientists but, rather, as an attempt to help them to decide what not to read.

To this end, the Monday edition of *Nature* will be called *Nature Physical Science* and will carry original research which seems likely to be predominantly of interest to specialists in the physical sciences together with comments on research, news and book reviews which are similarly restricted in interest to the physical sciences. In the same way, the Wednesday issue of *Nature* will be called *Nature New Biology* and will be so composed that the material it contains is unlikely to interest people working in the physical sciences.

The Friday issue of *Nature* will as now contain original research articles in all fields of science together with news, comment and book reviews. One advantage of the new development is that it will provide scope for increasing the space devoted to the brief reviews of important papers appearing elsewhere in the literature ("News and Views") and review articles of general interest. The Friday issue of *Nature* will be designed in such a way that those reading it will also be provided with information about much of the content of the editions appearing on Monday and Wednesday in the following week. In short, the Friday edition of *Nature* should provide not merely a record of important developments in the whole of science but a guide to matters of more specialized interest elsewhere in the journal.

Under the new arrangement, the pages in the three weekly issues of *Nature* will be numbered consecutively. In order that the increased volume of publication should not lead to inconveniently large bound volumes, there will be six complete volumes in 1971. Copies of the Monday and Wednesday editions will be supplied only to subscribers to the Friday edition. Unexpired portions of subscriptions at January 1971 will be fulfilled by the supply of issues of the Friday edition. The subscription rates for 1971, applicable at least until March 1, 1971, are as follows:

Annual subscription to:

Nature	£14
Nature + *Nature Physical Science*	£24
Nature + *Nature New Biology*	£24

For subscribers taking all three editions there will be a special rate of £29.50. The cover price of each journal will be 5*s*.

For North American subscribers the journal is sent by airfreight. The annual subscription rates are:

	US	Canada
Nature	$48	$52
Nature + *Nature Physical Science*	$83	$90
Nature + *Nature New Biology*	$83	$90

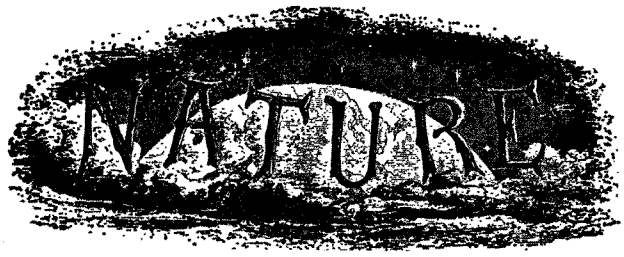
For subscribers taking all three editions there will be a special rate of $108.00 (US) or $116.00 (Canada).

Existing subscribers will be notified by letter in the next few days of these new rates.

been broken. Professor Linus Pauling is more anecdotal than Dr Olby, but what he has to say throws very little light on the reason why his own contributions to molecular biology came bubbling to the top. Dr R. R. Wilson of the Batavia accelerator is perhaps the most amusing of the contributors, for he has the chance to reflect on the irony that he was once a campaigner against large laboratories and is now the originator of one of the largest of them all.

What is to be made of all this? *Daedalus* has demonstrated that scientific biography is as intriguing as anybody could ask. This symposium makes a good read. At the same time, however, it is also clear that autobiographical writing is by no means sufficient to provide an illuminating record of the present or the recent past. On the whole, those contributors who write about themselves tend to dodge the awkward questions. What they have to say is invaluable, for nobody else could say it, but what they have to say is also incomplete. For what it is worth, this special issue of *Daedalus* has demonstrated the need for a contemporary understanding of the mechanisms of science and also, by its inevitable defects, for some method of making this record at once more incisive and more objective.

100 Years Ago



The Electric Telegraph and Earthquakes

THE use of the electric telegraph for recording earthquakes is not so new as the *Echo* (quoted in your last number, p. 35) supposes. Dr. Hector, Director of the Geological Survey, has, ever since the last great convulsions, systematically used the telegraph for this purpose in New Zealand, where indeed it forms an important element in the admirable system he has inaugurated for recording shocks throughout the islands. In his last letter he writes as follows:—"Not long ago, one operator asked another 200 miles distant 'Did you feel that?' and got the answer, 'No. What? Yes; there it is,' all in a breath, so to speak!"

Dr. Hector, writing in August last, goes on to say, "I have called attention to the coincidence of the aurora in both hemispheres on April 5, and can't help thinking that when our observations are sufficiently extended, we shall find many phenomena that are looked upon as local to be general. Could not NATURE give us a column recording such phenomena as auroras, earthquakes, tornados, &c., experienced in all parts of the world, somewhat in the form of an almanac? At the present one has to rummage over all sorts of periodicals, and after all find the matter most imperfectly recorded. We shall soon have a capital earthquake register here, since I induced the Government to cause the observers to report every shock they felt in the colony. Their number and coincidence is very remarkable, and I shall publish the results as soon as I have collected a sufficient number. I feel a great want of a good table eusmometer which should be cheap enough to distribute to all telegraph stations." Perhaps some of your readers can inform Dr. Hector where he can procure this great desideratum. JOS. D. HOOKER

From Nature, **3**, 47, *November* 17, 1870.

OLD WORLD

EUROPEAN SPACE

More Confusion in Brussels

THE European Space Conference in Brussels last week turned out to be a series of misunderstandings and misrepresentations. The outcome of the conference is, on the face of things, another wide difference of opinion between the member countries, this time over participation in the US post Apollo programme. France, West Germany and Belgium have decided to go ahead independently with a more detailed study of NASA's proposal for Europe to take a 10 per cent share in the space shuttle and tug programme. But Britain, Sweden, Italy and Norway have declined to participate.

Debate on the post Apollo programme centred around the report made to the conference by its chairman, Mr Theo Lefevre, the Belgian research minister, of his visit to Washington to hear NASA's proposals. The confusion started with conflicting ideas of what the NASA proposals entail. On the one hand, Mr Lefevre maintained that the United States would only make launchers available for European satellites—as it has done in the past—if Europe decides to participate in the post Apollo programme. But on the other hand, the British delegation, briefed by Lord Bessborough who was present at the Washington meetings, believed that no such provision was made. The full story turned out to be somewhere in between these two extremes—it seems that if Europe does not participate in the post Apollo programme, the United States will consider launching European satellites case by case, but a blanket agreement would be given if Europe decides to accept NASA's invitation.

It was on this point, however, that much of the debate hinged, because Britain stuck to its well worn theme that it would be foolish for Europe to develop its own launch capability while it can buy American launch facilities. This, of course, is the argument which Britain used for pulling out of ELDO when that organization decided to go ahead with development of Europa III.

Confusion also arose over a proposal by Mr Lefevre that $35 million should be spent on a more detailed study of the NASA offer. But it was later made clear that the money would be spent on development of Europa III. The British delegation found such a proposal unacceptable, in spite of the argument that development of Europa III would be useful if Europe decided to take part in the space tug programme, and Britain decided not to participate in the study.

The upshot of the debate about participation in the post Apollo programme is that only France, West Germany and Belgium decided to go ahead with the study, and to put up the $35 million between them, in the hope that other countries may join in later. Britain, however, seems to have made up its mind to stay out. Mr Frederick Corfield, Minister of Aviation Supply, said in London last week, for example, that participation in the project would probably cost Britain alone about £100 million over ten years. Given the government's pledge to reduce public expenditure, this entry fee is too high and, in any case, Mr Corfield pointed out that the full financial implications of the

post Apollo programme are so vague that the offer of participation must be turned down. He did, however, say that "If the Europeans can come up with a firm project, balancing costs with benefits, we could reconsider".

CHEMISTRY

Merging Chemists Beware

THE Chemical Society and the Royal Institute of Chemistry will be legally merged by January 1972 if the arrangements, now well advanced, go ahead as planned. The merger of several of the principal British chemical bodies was first talked of in 1967, when the councils of the Chemical Society, the Royal Institute of Chemistry and the Society of Chemical Industry agreed to start negotiations on a possible amalgamation. Sir James Taylor produced a working document in June 1968 which suggested an equitable method of effecting the merger. The three bodies later held referenda and an overwhelming majority of the members of the Chemical Society and the institute voted in favour of the amalgamation. Only 68 per cent of the membership of the Society of Chemical Industry felt the same way, however, and their council withdrew from the negotiations on the grounds that it favoured a looser confederation. This prospect did not appeal to the other two bodies, however, who looked forward to the increased impact which a unified body of about 40,000 chemists would have in matters of mutual interest. The Society of Chemical Industry, the smallest and least financially secure of the three, was obviously worried about negotiating from a position of relative weakness.

Sir James Taylor's proposals were to accommodate other interested bodies on equal terms within the Chemical Society and to create an overall amalgamation between the society and the institute. To date, the Faraday Society and the Society of Analytical Chemistry have agreed in principle to become incorporated into the Chemical Society as the Faraday and Analytical Divisions. The president of the Faraday Society, Professor G. Gee, believes that its status as a multidisciplinary society of considerable international standing will be enhanced by closer integration with the main body of British chemists and that this integration will not be to the detriment of, for example, its members who are physicists. The Society of Chemical Industry also has a neat slot, into which it could fit if it so chose, in the shape of the existing industrial division.

The linkup of the Chemical Society, with its new offshoots, and the institute is a more complex affair and it is to be hoped that it will cause less friction both now and in the long run than has been generated during the negotiations of the Institute of Physics and the Physical Society for a Royal Charter. The situation is, however, somewhat different because both the society and the institute already have charters. The plan is that they should continue to operate under their respective charters, both because older charters are much wider in scope and freer in their provisions and because of the enormous expense of renegotiating a single one. The added advantage of this arrangement is that the existence of the Chemical Society within the new framework is assured for future years by the retention of its charter. Both bodies plan to use the same buildings as at present but there will inevitably be staff reorganization because of the centralization of financial control and the shift of most of the publishing onus to the Chemical Society.

The chief restrictions which are to be written into the merger agreement reflect the different purposes of the society and the institute. The society is essentially a learned society whose membership has always been open to all with an interest in chemistry, as is the case with the now almost defunct Physical Society. The institute is, however, the chemists' professional body and as such must require its members to be suitably qualified in chemistry, either by a university degree or through its own graduateship examination. It is proposed that the two councils should govern the new body jointly and that matters should be voted on by all members at a general meeting, except, of course, those items which are professional in nature and can only be decided by the members of the institute.

Dr Timofeev-Resovskii

THE recent appointment of Dr Nikolai V. Timofeev-Resovskii to a consultancy in the Moscow Institute of Space Biology and Medicine (see *Nature*, **228**, 4; 1970) does not, unfortunately, signify an end to his harassment by the Soviet State. A special meeting of the Moscow Society of Naturalists was recently called to mark his 70th birthday, but official pressure to cancel the meeting was brought to bear on the society and it had no option but to give in.

Dr Timofeev-Resovskii, who was forced to retire last year from the Obninsk Institute of Radiology, is now in hospital recovering from an abdominal operation. But it seems that the cancellation of the jubilee meeting had nothing to do with his illness, because he asked for a long-standing intestinal complaint to be investigated after he heard that the meeting had been cancelled.

HIGH ENERGY PHYSICS

First Spin for Storage Ring

AT long last, the first of the two intersecting storage rings at CERN has been commissioned and, by all accounts, a shudder of excitement has spread through the establishment. One witness of the occasion on November 3, when a beam of protons at an energy of 15 GeV was fed into the anticlockwise ring, said afterwards that the experience had been "totally remarkable". What seems most of all to have impressed the people concerned is the great stability of the protons in the storage ring. On the very first injection, a proton current of 5 mA was allowed to circulate for twenty minutes and tests carried out suggested that the lifetime of this beam would have been fifty-six minutes. More densely packed beams of protons will, of course, be less stable, but the stacking procedure which allows successive pulses of protons to be stored in the ring has so far been used to accumulate a current of 1·5 A—a much more intense beam than that provided by the proton synchrotron at CERN.

The team at CERN is also pleased about the accuracy with which the performance of the storage rings had been predicted. The oscillatory characteristics of the beam seem, for example, to have been predicted accurately. The efficiency of stacking is close to what calculations had predicted while the efficiency of injection seems to have been virtually ideal. It is also remarkable that the first of the two storage rings which will ultimately be used for arranging for collisions between protons travelling in opposite directions should have been tried out some weeks before the date on which it was due to be commissioned. A spokesman at CERN also made much of the fact that the cost of the project as now nearly completed is well within the budget.

Financial probity is no doubt one of the virtues of which CERN will make much in the weeks ahead, when member states will once more be screwing up their courage to decide whether or not to go ahead with the 300 GeV proton accelerator as now modified in design. More may be known about the status of this project when the council of CERN meets in Geneva on December 17 and 18. In the meantime, it seems clear that the final decision will depend very much on what the British delegation has to say. Since the visit of Mrs Margaret Thatcher and Sir Brian Flowers in the summer, it has been assumed that the British Government's decision not to contribute to the new project was at least up for reconsideration. The Italian Government has let it be known that it will not itself agree to belong to the new project if there is no British contribution, which only goes to strengthen the belief that the future of the 300 GeV accelerator will be determined by Mrs Thatcher.

NUCLEAR REACTORS

Export Hopes Revived

THE announcement this week that the Nuclear Power Group (NPG) of Great Britain is among the last four contenders for the contract for Australia's first nuclear power station has revived hopes that the steam generating heavy water reactor (SGHWR) may yet break into the export market for which it is designed. Canadian, West German and United States consortia are also still in the running for the Jervis Bay station in New South Wales, but it seems likely that the final decision really lies between the British and Canadian designs. The Australian government has specified that the reactor chosen must be able to run on the natural uranium recently found in the Northern Territory. Although only the Canadian design fulfils this requirement, the British package includes granting Australia access to European gas centrifuge technology, for the slight enrichment required for the NPG reactor. Such an agreement would be subject to international safety precautions.

The only SGHWR reactor in operation in the UK is the 100 MW station at Winfrith in Dorset. Designed to the specifications needed for a 300 MW reactor, this has always been seen by the NPG as an export proposition rather than a competitor on the home market with the more sophisticated and expensive advanced gas cooled reactors. Early in its development an SGHWR reactor was offered to Finland, who chose a Russian design instead. More recently, the Greek government approached Britain with an offer for one of these reactors which included other trading deals (notably in tobacco) in one package. This offer has so far proved unacceptable, but it seems that Greece is still interested in the reactor and that it is the associated trading arrangements which have blocked progress.

The outcome of the Australian decision may be important because similar stations will certainly be required by other Australian states in due course. With reports that Brazil and South Africa will also be asking for tenders for nuclear reactors in the near future, success in Australia could open up the southern hemisphere and Latin America to the NPG, which is ready to press its case as soon as requests for tenders are made.

FRENCH SPACE CENTRE

Engineers back to Work

ENGINEERS and technicians at the site of the Conseil National d'Etudes Spatiales at Bretigny returned to work last week after a stoppage of five weeks, the longest in the history of the French Space Agency with the exception of that during the national crisis in May 1968. Although the dispute was ostensibly about salary, arising out of the failure of the CNES to implement an agreement with the unions reached last April, it became clear during the course of the strike that a major concern of the engineers and technicians was the threat of the transfer of personnel to the new CNES site at Toulouse.

There seems to have been some confusion about the amount of work apportioned to the new site. The only major projects already earmarked for Toulouse are the testing of the Franco-German satellite, Symphonie, and the construction of a meteorological satellite called Météosat. The 250 engineers and technicians on strike had been asked to decide by March 1971 whether they would be willing to accept a transfer to Toulouse, but it seems that there had been some doubt about the role of the new site, or at least about the rate at which work would be shifted to Toulouse.

The agreement reached between the unions and the CNES last week includes an indemnity from transfer to Toulouse and an assurance that the restructuring of the space centre will not mean redundancies at the CNES. One result is that it now seems improbable that many of the engineers and technicians will accept a transfer to Toulouse with the French space programme in such a state of uncertainty. It is thought likely that a large number will prefer to move into the aerospace industry following the lead of the hundred or so men who have already made the move during the past eighteen months.

SCIENTIFIC INSTRUMENTS

Astrolabes at Greenwich

A GLIMPSE back to the days when scientific instruments were also works of art is given by the permanent exhibition of astrolabes and other models of the heavens in the Bliss Gallery of Flamsteed House at the Old Royal Observatory, Greenwich. The collection is second only to that in the Museum of the History of Science, Oxford, and has been accumulated since about 1935.

The planispheric astrolabe, a sort of circular astronomical slide rule, is the most common type. As a



Islamic astrolabe made in 1707–8, now part of the Greenwich collection.

mathematical model of the heavens, instruments like these can be used to compute the positions of heavenly bodies at any time, and, conversely, to determine the time from the altitudes of the Sun and stars. The origin of the astrolabe may well be Greek (a forerunner was possibly invented by Hipparchus in 150 BC) but it was certainly in regular use from the 4th century AD to the 17th century.

The National Maritime Museum is planning to commemorate the tercentenary of the foundation of the Greenwich Observatory in 1675 by a special exhibition to be mounted in the main part of the Observatory building, the Queen's House. Some of the telescopes used by John Flamsteed—appointed "astronomical observator" by royal warrant in 1675—are also expected to be re-erected for the occasion.

DIABETES

Drug Fears Dismissed

THE nervousness with which the American Food and Drug Administration regards the anti-diabetic drug tolbutamide is clearly not shared by British health authorities. Although doctors in the United States were warned earlier this month by the US Department of Health, Education and Welfare not to prescribe tolbutamide or any of the related sulphonylureas unless there was no alternative, the British Diabetic Association and the Scowen Committee on the Safety of Drugs agreed unanimously last week that there is little substance to the American fears.

Tolbutamide is the best known of a group of compounds which have been used for more than a decade in the treatment of some forms of diabetes mellitus. The drug is now used by about one third of the 500,000 British diabetics, and by about the same proportion of American sufferers. It is administered orally, in contrast to the classical treatment for diabetes of insulin injections, but because it works by stimulating the production of insulin by the pancreas, it can only be used for those diabetics in whom the pancreas is still functional.

American anxiety over tolbutamide is based on a study carried out by the University Group Diabetes Program (UGDP) which indicated that the risk of cardiovascular disease is greatly increased in patients treated with the drug. In dismissing this conclusion, a spokesman for the British Diabetic Association emphasized that the American evidence had been unconvincing. The patients chosen for the study were not a typical group, other possible causes of cardiovascular disease and death had not been taken into account, and no comparison with the normal death rate from cardiovascular disease had been taken into account. Furthermore, the UGDP results were in direct conflict with two careful European studies which suggested that patients derived positive benefit from taking tolbutamide, but which induced no adverse cardiovascular effects. The British attitude, therefore, is that there are no grounds for recommending changes in treatment policy for diabetics.

While everyone holding recent tragedies like thalidomide in mind will agree that there is no room for laxity in drug supervision and control, it seems strange that the American Diabetic Association—which seems to have turned about face in the last few months—the Council on Drugs of the American Medical Association and the Food and Drug Administration should be so eager to jump onto this particular bandwagon on the basis of such unsatisfactory evidence.

WAR

Slaves and Prophets

WAR is a pathological condition of human society and as such it must be treated not by blind application of politics but by scientific methods based on an understanding of human biological needs. This is the claim of the Medical Association for Prevention of War, an association formed in 1951 to promote the objective study of war from the standpoint of medical and biological sciences. The first volume of the association's proceedings has just been published (available from the association, 50*s*); it contains a number of short papers contributed by physicians, psychologists and others during the past five years under the chairmanship of Professor Lionel Penrose.

Interspersed among analyses of human aggression and the psychology of warfare by, for example, Felix Brown and Ismond Rosen, there are individual views on the ethical problems now facing doctors. There are also a few papers on racial conflict, and Malcom Potts talks about family planning. But there is surprisingly little discussion of one of the association's chief problems—the direct involvement of medicine in the development of biological and chemical weapons. Victor W. Sidel and Robert M. Goldwyn presented a paper in 1966 (abstracted from their paper in another journal) that classifies chemical and biological weapons, and in which they say that there has been remarkably little debate, particularly among physicians, on the advisability of developing, stockpiling and using such

weapons. The association itself seems to have added little since then.

Various trumpet calls have, of course, been sounded at intervals throughout the proceedings. In a recent paper, for example, Elisabeth Shoenberg underlines the hypocrisy of the medical code of ethics and the change in the doctors' moral position brought about by chemical, biological and atomic warfare. Doctors, she says, as medical philosophers bound by an acknowledged code of ethics, must opt out of chemical and biological warfare research. They must also start planning for peace—as citizens with a special responsibility, Dr Shoenberg suggests, they must educate and inform, and they must change from slave technicians to prophets.

POLLUTION

Valuing the Environment

LITTLE new thinking emerged from the Liberal Party's conference on "The Pollution of Our Environment" which took place last weekend. But it was a welcome change to hear views expressed in concrete terms, rather than the woolly subjectivity which marks so much of the present concern about the environment.

Two speakers in particular, Dr D. H. Sharp, general secretary of the Institute of Chemical Engineers, and T. C. Sinclair of the Science Policy Research Unit at the University of Sussex, emphasized the importance of equating the costs of anti-pollution measures with the value of the benefits. They pointed out that it is all too often overlooked that pollution control programmes are very subject to diminishing returns. Gross pollution can usually be dealt with cheaply and easily, but the cost escalates sharply if further refinement is required. The real conservation problem seems to be deciding where to draw the line.

An example cited by Dr Sharp is the cost of treating liquid sewage. Water can be purified by a factor of 15 for about 1*s* 3*d* per 1,000 gallons, but to reduce contamination by a further factor of 2 costs more than four times as much.

Some of these problems are clearly food for governmental thought. Sulphur dioxide is a major atmospheric pollutant, but sulphur can be completely removed from crude oil at a cost of $177 a ton, in comparison with the world price per ton for sulphur of $49. It is also now possible to recover sulphur from the sulphur dioxide in stack gases at a much more attractive price. In such a case, Dr Sharp believed that only the government can take a view broad enough to equate the cost of recovering sulphur with the cost of importing it, and to balance these costs against the expense of eliminating sulphur dioxide as a pollution hazard.

Mr Sinclair thought that it will soon be necessary to develop a formula which can be used to compute the value of free natural amenities such as clear air and water so that these could be compared with the costs of material products. The cost, for example, of air pollution in damage to health and fabric has been estimated at about £400,000,000 a year. Against this, the cost of building a single stack 425 foot high to disperse gaseous effluents is about £200,000. If the stack was twice as tall, the concentration of effluent reaching the ground would be halved, but the cost of construction would then be £1,250,000, and the danger to low-flying aircraft would have to be taken into consideration.

Parliament in Britain

Thames Flood Barrier

THE government has decided that the Thames flood barrier should be constructed at Silverton in Woolwich Reach. Announcing this decision, Mr Peter Walker, Secretary of State for the Environment, said that the Greater London Council is being asked to press ahead with detailed site investigation and that detailed design work will begin as soon as a decision can be taken on the type of barrier. The government, he said, considers the need to construct a movable barrier to be urgent.

The decision to site the barrier at Silverton was attacked, however, by Mr James Wellbeloved, who said in an adjournment debate that a more suitable site would be downstream from the proposed housing development at Thamesmead. If the barrier is placed at Silverton, Mr Wellbeloved suggested, some 60,000 people would be at risk if the banks at Thamesmead collapsed.

But Mr Eldon Griffiths, Under Secretary for the Environment, pointed out that the Silverton site was chosen on the basis of cost, ease and speed of construction, availability of land and on safety to shipping. (Written answers, November 4, and debate, November 9.)

Nancekuke

MR IAN GILMOUR, Under Secretary, Department of Defence, gave details of the Registar General's report comparing the incidence of death among those who have worked at the Chemical Defence Establishment outstation at Nancekuke with figures for Britain as a whole. He said that the report shows there is no evidence that workers at Nancekuke run an increased risk of death from any group of causes. Deaths from all causes, and in particular deaths from respiratory diseases among workers at Nancekuke, are slightly less than would be expected from figures for the rest of England and Wales. (Written answers, November 2.)

Smoke Control

MR PETER WALKER, Secretary of State for the Environment, said that 139 smoke control orders have been suspended in London, and another seven have been partially suspended. The suspensions affect 14 London boroughs, and Mr Walker said that they would apply to 40 per cent of the total number of premises usually covered by the orders in each individual borough. These measures have been taken because of the shortage of smokeless fuels. (Written answers, November 4.)

Dutch Elm Disease

THE Ministry of Agriculture cannot recommend any satisfactory chemical method of controlling the spread of dutch elm disease in Britain. Mr Anthony Stodart, Parliamentary Secretary, Ministry of Agriculture, said that a conference has been held to publicize measures for controlling the disease, but at present no satisfactory chemical control method is available. Mr Stodart also said that he does not believe legislation to be necessary for controlling the disease, but the position is being kept under review. (Written answers, November 2.)

NEW WORLD

Bringing Hippocrates to the People

by our Washington Correspondent

MEDICAL educators have so far had little but praise for a recent report which calls for a 50 per cent increase in the annual production of doctors, for two years to be knocked off the present eight year training course and for an immediate doubling of federal expenditures on medical education. The report*, prepared by the Carnegie Commission on Higher Education, gives American health services a sharp nudge towards the European model of socialized medicine. Its underlying theme is that the great advances in medical knowledge made over the last two decades are not reaching the people for whom they were developed.

Released at a meeting of the Association of American Medical Colleges on October 27, the report has been compared with, and takes as its deliberate point of departure, the Flexner report of 1910, which set the pattern of medical education in the United States for the subsequent six decades. The Flexner report, also commissioned by the Carnegie Foundation, established the emphasis of medical training in biomedical research and brought about the close connexion of medical schools with universities. The new report in no way repudiates the emphasis on research—and indeed provides mechanisms for establishing the volume of research at its present level—but it criticizes the Flexner model for ignoring the delivery of health care outside the medical school and its own hospital. Another major criticism is that the Flexner system sets science in the medical school apart from science on the general campus, with a resulting duplication of effort.

Despite the fact that it is calling for radical changes in a conservative profession, the new report seems likely to be welcomed by everybody except the Administration, which is called upon to foot the bill. The Association of American Medical Colleges has endorsed the report in principle, and the medical education director of the American Medical Association has termed it "consistent with the AMA's feelings and beliefs". Dr Robert Q. Marston, director of the National Institutes of Health, said last week the report is a "very timely and uesful document which clearly displays the results of a lot of serious discussion. We are now doing a critical analysis of it". Although many deans of medical schools may be apprehensive of tampering with a system that has produced an outstanding product, there seems to be a wide measure of support for one of the Carnegie Commission's most radical suggestions, that of reducing the length of training by two years. But how fast the Administration moves to implement what is, in its own way, an inflammatory document, may depend on the extent to which the public and the Congress takes up the Commission's suggestions. At a time when the Administration is seeking hard to reduce expenditure, the Carnegie Commission calls for the federal government, which in the last financial year devoted some $275 million to the support of medical education, to spend almost double that amount in 1971–1972. Adding to the Administration's probable inertia is the recent accession of Mr Elliot Richardson as Secretary of Health, Education and Welfare—he may not yet be ready to embark on a radical restructuring of this part of his domain. For these reasons the initiative in pushing the Commission's reforms is likely to come from Congress.

The immediate focus of the Carnegie Commission's concern is the present critical shortage of all levels of medical personnel. Estimates vary as to the size of the deficit but Dr Robert Egeberg, Assistant Secretary of Health, Education and Welfare, has put it at 50,000 physicians, a couple of hundred thousand nurses and 150,000 technicians. Whatever figures are taken, the fact that 46,000 graduates of foreign medical schools practise in the United States, amounting to 15 per cent of all physicians, is undeniable evidence that the native supply is insufficient. So too is the long working week of the typical doctor—60 hours was the median in 1968.

The chief bottleneck in the system is a shortage of places at medical schools, which habitually turn away half their applicants. Simple, old fashioned prejudice has also helped: only 6 per cent of all physicians are women (compared with 20 to 30 per cent in some European countries) and less than 2 per cent are black. An equally formidable barrier to talent is the cost of medical education (tuition charges at present range from $750 to $2,000 a year) which has put the ambition of a medical career largely beyond the reach of students from poor families; nearly two thirds of medical students in 1967 reported that they came from families with incomes of $10,000 or more.

The Carnegie Commission believes that an attainable and desirable goal is to increase the production of physicians by 50 per cent before the end of the decade. If the intake into medical schools can be raised from the 10,800 who entered this year to 16,400 by 1978, and assuming the brain drain infuses some 13,000 more foreign doctors between 1968 and 1977, it should be possible to raise the number of physicians per 100,000 of population from 147 to 161 before the end of the decade.

The extra 5,600 places in medical schools can be achieved by reducing from four years to three the course between the BA and MD degree, by increasing the average class size in medical schools to at least 100, and by establishing 9 new medical schools in addition

*_Higher Education and the Nation's Health_. The Carnegie Commission on Higher Education. McGraw-Hill, October 1970. $2.95.

to the 107 existing schools and the 27 already under development.

The switch from a four-year to three-year MD is to be effected by straightforward revision of the curriculum, by cancelling much of the summer vacation, and by demanding higher standards of students on entry to the MD course. The Commission also follows the American Medical Association in approving the elimination of the internship year. With these two changes the production cycle for doctors would be reduced from eight years to six.

Another major change recommended in the structure of the medical education is to shift instruction in the basic sciences out of the medical schools and on to the main university campuses. The courses should be arranged so as to increase the student's options, allowing him after basic training in the "health-related sciences" to pursue a variety of health-related professions as well as medicine and dentistry. The Commission also calls for serious attention to be paid to students' wishes, in particular the demands for more flexible admission standards and for greater student participation in deciding what is to be taught.

To improve on delivery of medical services, the Carnegie Commission envisages a two-tier hierarchy comprised of campus-based medical schools, or "university health science centres", and regionally based "area health education centres". Describing the role of the health science centres, the Commission says that their education and research programmes must be broadened to include concern with improving the delivery of health care and with innovations such as group practice and prepaid health plans. This will require closer cooperation with social science departments on the main university campus as well as with organizations and individuals in local communities. Each university health science centre should be capable of handling the most complex medical problems but should also serve as the coordinating hub and reservoir of expertise for a system of institutions ranging from area health centres to rural clinics and group practice organizations.

The role of the area health education centres is to be satellites of the university health science centres. Their education programmes would be developed and supervised by the health science faculty to which they were affiliated, and they in turn would provide assistance to community health care facilities, including the private practitioner. The chief functions of the health education centres would be to maintain a community hospital serving smaller communities in the surrounding area, to conduct education programmes and to provide clinical instruction for candidates from the university health science centre.

Turning to the financial support of medical education, the Commission argues the case for increased involvement by the federal government in underwriting the necessary improvements. This should not be done at the expense of federal funds for research, although the practice of funnelling federal funds for education through research grants to medical schools should cease and support of the education function should be made separate and direct. "In the years since World War II," the Commission notes, "the federal government has assumed major responsibility for providing funds for biomedical research. The Commission believes that this support should continue, but the time has now come for an equally vigorous effort to expand the education of health manpower and to stimulate major changes designed to relate the future education of health manpower to probable changes in the delivery of health care." In a proposal that may catch the attention of scientists in other fields, the Carnegie Commission suggests that federal research funds provided to university health science centres should be pegged at their present percentage of the GNP (0·042 per cent) though with changes in appropriations being based on a moving average of the total GNP so as to avoid abrupt shifts in funding. Between 10 and 25 per cent of the research grants to any university should be in the form of institutional grants rather than grants for specific research projects.

To make more equal the opportunity of entering the medical profession, a means-tested system of student grants is recommended whereby a maximum grant of $4,000 a year would be accorded to medical and dental students. The federal government should, in addition to research funds, provide cost-of-instruction grants to health science centres. These grants would be made conditional on the institution concerned abolishing admission requirements that either favour residents of the state or discriminate against minority groups.

Federal government would also be required to provide construction grants for new buildings and funds for the continuing education of physicians who, such is the pace of advances in knowledge, "must remain students throughout their professional careers or face partial obsolescence in five to ten years". In sum, the Carnegie Commissioners are asking the federal government to double its present level of support for medical education to $547 million in 1971–72, rising to $728 million by the end of the decade.

The Carnegie report has come at a time when health services in the United States are facing not one but several different crises. In addition to the shortage of doctors and other medical workers, medical schools in common with other academic institutions have suffered an abrupt decline in income, particularly that from private sources. More than half of the country's 107 medical schools have become eligible for special distress grants from the federal government. The cost of medical treatment increasingly concerns a public which is coming to regard health care as a right. The national expenditure on health care now amounts to $60,300 million, or nearly 7 per cent of the GNP, but little more than half of this great burden is met by insurance schemes—the Medicare and Medicaid programmes were meeting 36 per cent of the total in 1968–69, while private insurance schemes, despite the fact that about four fifths of the population had some insurance protection, were covering only 22 per cent.

In the light of these deficiencies there is less surprise in the Carnegie Commissioners' verdict that "although the best medical care in this country is as good as any in the world, many Americans receive inferior care, and some health needs go entirely untreated".

Accompanying the rising expectation of health care has been a shift in public attitudes towards doctors. According to a Louis Harris poll published this week, 78 per cent of Americans have a high personal regard for their family doctor but many nurture a long list of grudges against the medical profession in general. A majority (63 per cent) believe that doctors try to see so many patients they don't give enough time to anyone; 62 per cent agreed to the assertion that doctors

have jumped their fees to take advantage of the Medicare and Medicaid programmes, and half of those polled thought justified the statement that "the patient who gets sick on Wednesday afternoon or Sunday could die before getting a doctor off the golf course". Whatever the significance of these sentiments, the issues raised by the Carnegie Commission report are unlikely to be forgotten when Congress reassembles next spring.

Carnegie Commission

Summary of Chief Recommendations

- THE number of students entering medical school should be increased by half over the next eight years, from 10,800 admitted this year to 16,400 in 1978.
- Universities should develop plans for accelerating premedical and medical education. The Commission favours particularly reducing from four years to three the time after the BA degree to obtain the MD, and abolishing the internship year, thus reducing the total period to become a practising doctor from eight years to six.
- All university health science centres should give serious consideration to curriculum reforms. Admission policies should be made more flexible and their programmes more responsive to the expressed needs of students.
- More women and members of minority groups should be trained as doctors.
- The federal government should award means-tested grants of up to $4,000 to students from low income families training to be doctors or medical associates or assistants, as well as cost-of-instruction subsidies to university health science centres for each medical student enrolled.
- Federal support of research at university health science centres should be maintained at its present percentage of GNP (0·042 per cent). Federal allocations should cover the total cost of research projects. Between 10 and 25 per cent of the research grants to any university health science centre should take the form of institutional grants rather than grants for specific research projects. Funds should be made available to support research on methods of improving medical education and delivery of health care as well as for biomedical research.
- National requirements should be set for the periodic re-examination and recertification of all physicians and dentists by specialty and other appropriate bodies.

A National Health Manpower Commission should be appointed to study changing patterns of medical education, health care delivery and the use of health manpower, as well as the feasibility of setting national licensing requirements for all types of medical worker.

- Universities should plan for medical schools to expand, to offer a broader curriculum, and to integrate their instruction in the biomedical and social sciences more closely with that given by the relevant university departments.

POLITICS

Addio Daddario

by our Washington Correspondent

MR EMILIO Q. DADDARIO, six times elected Congressman, chairman of the House Subcommittee on Science, Research and Development, most conspicuous of science's few friends in either chamber of Congress, was defeated last week in his campaign for the governorship of his home state of Connecticut. Since the Nixon Administration is unlikely to cross party lines to offer him a job in government, Mr Daddario's experience of science policy making seems likely to lie fallow for some time. He will probably return to the successful law practice he ran in Hartford, Connecticut.

With the loss of the governorship that a few months ago seemed almost assured, Mr Daddario's political career seems set for a long recess. He is unlikely to run for governor again, and his safe seat as representative for the first district of Connecticut has been won by a Democrat, who by etiquette will have the party's support if he chooses to stand again. Mr Daddario's earliest chance of re-entering Congress will probably be when the Senate seat of Democrat Abraham Ribicoff comes up for re-election in 1974, should the present incumbent not wish to try for another term.

Mr Daddario's defeat is the more the pity in that it is commonly supposed he would have preferred to run for the Senate seat that was up for re-election this year, but was persuaded by the Democratic party machine in Connecticut to stand instead for the governorship, which disposes of more patronage. The House Subcommittee on Science, Research and Development, whose first chairman he became in 1963, now passes into the hands of Representative John W. Davis of Georgia. Mr Davis inherits the subcommittee along with much unfinished business, notably the proposals to set up an Office of Technology Assessment and the structure to be known as the National Institute of Research and Advanced Studies. There is also Mr Daddario's newest and grandest design, the inquiry into national science policy, which asks the Administration to consider again the whole machinery of science policy making, starting with its own creature, the Office of Science and Technology.

Even if all the projects that issued from Mr Daddario's subcommittee had met with the legislative success they deserved, a more significant achievement may prove to be one whose full fruits are not yet apparent—the way in which Mr Daddario laid down the basis for intelligent and informed discussion in Congress and beyond of how science should be done and what part government should play in it.

NASA

Astronomers' Favourite Satellite

by our Astronomy Correspondent

EXPERIMENTS and experimenters have now been picked for the High Energy Astronomical Observatory (HEAO), the satellite that astronomers would most like to see in orbit. Although the project is being given the highest priority by the scientific community (see *Nature*, **228**, 204; 1970), it has not yet become an approved NASA programme. The project will be considered during a series of congressional hearings this

winter. The reason for the excitement about HEAO is that it will be a platform for the study of cosmic X-rays and gamma rays that up to now has been carried out from temporary platforms on rockets and balloons, with a few notable exceptions. Now that there is practically incontrovertible evidence from the rocket flights that X-ray sources come and go, there is an urgent need for the continuous monitoring that can only be carried out from a satellite. The same goes for gamma ray observations, at last becoming respectable, and detectors on satellites away from the influence of the atmosphere are the only way of identifying the isotropic gamma ray background that was hinted at from the OSO 3 measurements.

Twelve experiments have been chosen out of fifty-five submitted for the two satellites planned in the HEAO programme. The first satellite intended for launch in 1974 is being designed chiefly for a sky survey, but according to NASA it should also provide crude spectral measurements. In the X-ray field the chief piece of equipment is a large area detector sensitive from 100 eV to 100 keV from Dr H. Friedman's group at the Naval Research Laboratory which will provide an X-ray map of the sky. To fix the positions of the sources found in Dr Friedman's survey to one arc second there will be a low energy experiment fitted with modulation collimators and sensitive from 900 eV to 20 keV (Dr H. V. D. Bradt of MIT and Dr H. Gursky of American Science and Engineering), and a similar experiment for the higher energy sources from Dr W. H. G. Lewin's group at MIT, sensitive from 13 to 150 keV. To provide extra sensitivity for soft X-rays there is an experiment from Dr R. Novick's group at Columbia University to map weak sources in the range 500 eV to 1·5 keV. Extending the measurements to higher energies than those covered by the NRL detector there will be a gamma ray detector from Dr L. E. Peterson's group (University of California at San Diego) sensitive to high energy X-rays and low energy gamma rays (300 keV to 10 MeV). There will also be two cosmic ray experiments, one to study the elemental abundances of heavy and superheavy nuclei (Dr M. H. Israel of Washington University at St Louis, Dr E. C. Stone of Caltech, and Dr C. J. Waddington of the University of Minnesota), and the second to measure the charge composition and spectra of cosmic rays with energies between 10 BeV and 100 GeV (Dr J. F. Ormes, Goddard Space Flight Center).

The second HEAO is dependent on the successful working of the first in that it will carry equipment to measure in detail the spectra of sources turned up by the sky surveys. It should also provide more information about the background radiations that possibly play an important and so far not understood part in the energetics of the universe. X-ray spectra will be provided by a high resolution Bragg spectrometer (Dr A. B. C. Walker of Aerospace Corporation and Dr J. R. P. Angel of Columbia University), and spectra covering the transitional region between X-rays and gamma rays, 60 keV to 10 MeV, from an experiment from Dr A. S. Jacobson's group at JPL. An unusual experiment supplied by Dr L. W. Alvarez (University of California at Berkeley) will, it is hoped, measure the momentum of cosmic ray particles from the curvature of their paths in the magnetic field of a superconducting magnet. There will be two experiments on the background radiation. Dr E. A. Boldt of the Goddard Space Flight Center and Dr G. P. Garmire of Caltech will be observing the X-ray background from 200 eV to 60 keV, and Dr C. E. Fichtel of Goddard and Dr R. Hofstadter of Stanford University are hoping to learn more about the background radiations observed from OSO 3 by means of an equipment sensitive to high energy gamma rays.

X-ray and gamma ray astronomy in the US will also be encouraged by a series of small astronomy satellites now planned, with the first launch this winter, and the first two satellites will be devoted to X-ray and gamma ray sky surveys.

The gamma ray telescope in SAS-B, for example, will have twenty-five times the sensitivity and ten to fifteen times the angular resolution of the gamma ray equipment on OSO 3. HEAO is still at the design and definition stage, however, which is expected to take between ten and twelve months, after which money will have to be appropriated if the project is to go ahead.

PEACEFUL ATOMS

Plowshare Ploughed Again

by our Washington Correspondent

WITH the hearings for next year's budget of the Atomic Energy Commission now at a close, it is beginning to look as if the Plowshare programme for the development of peaceful uses for nuclear explosions is once again going to lose funds. Up to the current financial year, the Plowshare programme, which has its most vivid expression in a scheme to use nuclear bombs to excavate a new Panama canal, has been receiving $12 million to $14 million each year. But in transmitting the current budget to congress in January this year President Nixon requested virtually no funds for the excavation aspects of the programme, but continued the funding for what are called the underground engineering aspects. Thus the Plowshare budget was cut from an estimated $14·5 million for the fiscal year 1970 to a presidential request of $8 million, which was then further trimmed by Congress to $7·5 million. It is now thought that President Nixon will be taking the same action this year with the 1972 budget which comes into action on July 1, 1971. What this means is that once again there will not be enough money in the Plowshare programme to carry out tests of the excavative ability of nuclear explosives.

The distinction between the two aspects of the programme arises partly because in excavative explosions there is the danger of radioactivity escaping from the state carrying out the explosion, prohibited by the test ban treaty. In underground explosions such as the gasbuggy series there should be no release of radioactivity. That there is likely to be a continuation of the standstill on the more ambitious parts of the plowshare programme will not come amiss to those who doubt the wisdom of using nuclear explosions for earth-shifting projects in the first place.

FUNDING CRISIS

Science Throttled, but Can Live

by our Washington Correspondent

TOTAL expenditures on academic science in the United States have increased by 15 per cent over the last two years, but the growth has not been enough to cover

Table 1. PER CENT CHANGES IN MANPOWER, BY FIELD, SPRING 1968–69 AND 1969–70

Selected science departments, by field	Faculty		Post-doctorates		Graduate students	
	1968 to 1969	1969 to 1970	1968 to 1969	1969 to 1970	1968 to 1969	1969 to 1970
All selected science departments	6·5	3·5	6·5	4·0	−0·5	0·5
Chemistry	6·0	4·5	1·5	5·5	−1·5	−0·5
Physics	8·0	2·5	7·5	2·0	1·5	−2·0
Mathematics	5·0	3·0	−4·0	16·0	−4·5	−2·0
Electrical engineering	7·0	3·0	21·0	21·0	−4·0	2·5
Chemical engineering	3·0	6·0	14·5	−3·5	1·5	−2·0
Biochemistry	5·5	5·5	4·5	5·0	0·0	−4·0
Biological sciences	9·5	4·0	19·0	1·0	2·5	2·0
Microbiology	4·5	2·0	6·0	−1·0	0·0	−2·0
Physiology	5·5	5·0	9·5	−2·0	−1·0	3·5
Sociology	6·5	5·0	*	*	6·0	3·0
Economics	8·5	4·5	*	*	−2·0	6·0
Psychology	5·5	2·5	22·0	−9·5	3·0	3·5

* Per cent not shown because base number less than 25.
Note: Figures rounded to nearest half per cent.

higher costs and increases in enrolment. This is evident from a survey by the National Science Foundation. Many of the 86 institutions polled reported that graduate programmes and research had been impaired and the morale of students and staff lowered by the changes in federal funding.

Preliminary results of the survey—Impact of Changes in Federal Science Funding Patterns on Academic Institutions, National Science Foundation, October 15, 1970—which took the form of a questionnaire mailed this spring to 100 institutions granting science doctorates, indicate the total funds for academic research increased by 7·0 per cent between financial years 1968 and 1969, and by 8·5 between 1969 and 1970. But in terms of constant dollars the increase on the two occasions was near to 5 per cent, and during the same period college enrolments rose by 15 per cent.

Table 2. APPROPRIATENESS OF DIVISION OF RESEARCH FUNDS BETWEEN JUNIOR* AND SENIOR† STAFF

Selected science departments, by field	Per cent departments reporting inadequate division of funds for junior staff		
	FY 1968	FY 1969	FY 1970
All selected science departments	25·0	20·5	21·5
Chemistry	34·5	27·0	27·0
Physics	29·0	23·5	24·5
Mathematics	24·5	23·5	27·5
Electrical engineering	25·5	15·0	9·0
Chemical engineering	18·5	21·0	18·5
Biochemistry	31·0	NA	38·0
Biological sciences	15·0	29·0	23·0
Microbiology	16·5	19·5	26·5
Physiology	16·5	22·0	23·0
Sociology	31·5	15·0	18·5
Economics	22·0	16·5	9·5
Psychology	16·0	12·0	15·0

* Seven years or less from the PhD.
† More than seven years from PhD.
Note: FY 1968 data may not be strictly comparable with FY 1969 and FY 1970 because of a change in the format of the wording on the survey question. Figures rounded to the nearest half per cent.

The most tangible effect of tighter resources has been a deceleration in the addition of faculty members and postdoctorates, whilst in many departments there has been a net reduction in graduate students (see Table 1). Except for chemical engineering and biochemistry, all departments showed smaller growths in their science faculty between 1969 and 1970 than between 1968 and 1969. There have been wide variations in the number of postdoctorates produced in different specialties and institutions, but the data "do not in any event support anecdotal reports of widespread increases in postdoctorates". The largest of recent declines in graduate students has been suffered by mathematics departments, who report a total drop of twenty per cent over the last two years in students supported by federal funds.

The NSF survey has found no evidence to support stories that new doctorates are being starved of their fair share of appropriate research funds. The proportion of departmental heads reporting inadequate division of funds for junior staff has in fact declined since two years ago (see Table 2). Institutions asked to identify the departments most seriously affected by the cutback in federal funds cited their physical science departments most often (44 of the 86 institutions) followed by life science departments (31 institutions) and chemistry departments (19 institutions).

ELECTIONS

The Environment Lobby Wins

by our Washington Correspondent

BOTH Republicans and Democrats are putting the best face on last week's indecisive election results, but it is humbler organizations such as the environmental lobbying groups whose cheers really come from the heart. The environment has been a strong issue in many campaigns and the two national lobbying groups most active on environmental issues, the League of Conservation Voters and Environmental Action, are well pleased with how their candidates fared. Another lobbying interest supported by scientists for arms control and disarmament received less joy from the way the votes fell; of the nine Senate candidates supported by the Council for a Livable World, only four were successful, and the chairman of the Senate Disarmament subcommittee, Senator Albert Gore of Tennessee, was defeated.

The League of Conservation Voters reports that sixteen of the twenty candidates it endorsed were re-elected. The organization is particularly pleased with the defeat of Governor Samuelson of Idaho, whose policies on environment protection are said to have given excessive favour to industrial interests. The League also rejoices over the defeat in a Maryland Democratic primary of Representative George Fallon, a 13-term Congressman who had been chairman of the House Public Works Committee and was one of the originators of the Highway Trust Fund. A satisfactory score is also recorded by Environmental Action. Seven of the twelve Congressmen it singled out for bad voting records on environmental matters went down to defeat and six of the "dirty dozen" were replaced by candidates thought to be favourable to the group's aims.

The two groups are both of recent standing. Environmental Action is an outgrowth of the activities of

last April 22, designated Earth Day, and the League of Conservation Voters, the political arm of Friends of the Earth, has been in action for slightly over a year. The league, which contributed a total of $45,000 to its candidates, was founded by Marion Edey, a former legislative assistant to Representative Wolff of New York. Its interests are no narrower than "preservation of the ecosystem" but the particular issues it is now pursuing are the curtailment of the supersonic transport and the "development of alternative power sources" (alternative, in particular, to nuclear power). Environmental Action has chosen as its three main issues for the immediate future clean air legislation, with particular respect to automobile exhaust standards, the SST, and occupational health and safety. A member of the group said this week it was hopeful the new Congress would be "more environmentally conscious" than the last.

The new Congress is less decidedly favourable to advocates of arms control although Thomas A. Halsted, director of the Council for a Livable World, describes the net effects of the changes in the Senate as fairly neutral. The council, founded in 1962 to halt the arms race and eliminate war, operates by arranging seminars for Senators on matters such as the anti-ballistic missile system and by supporting candidates of its choice in Senatorial elections. In last week's elections five of the council's nine favoured candidates were defeated and one of them, Senator Vance Hartke of Indiana, scraped in only after a recount. But the council, which raised some $300,000 in support of its candidates, is sanguine about the losses. "In the past our batting average has been better, but we took a few long shots", Halsted said this week. Several candidates were prime targets of the White House campaign, including in particular Senator Gore, whose defeat is the most serious loss. A member of Senator Fulbright's Foreign Relations Committee and chairman of its Disarmament Subcommittee, Gore has been one of the chief opponents of the ABM system in the Senate debates this year and last. His successor as chairman of the Disarmament Subcommittee has yet to be announced.

POLLUTION

Lawyer for Environment

by our Washington Correspondent

An assistant attorney-general of the Justice Department, Mr William D. Ruckelshaus, is to be the first head of the Environmental Protection Agency, President Nixon announced last week. The agency, which will become operative on December 2, will have the care of 5,700 employees and an estimated budget of $1,400 million. The agency will be entrusted with the task of enforcing present and future legislation against all forms of pollution. It draws together some fifteen programmes and bureaux at present scattered round various departments, of which the most important are the Federal Water Quality Administration, now in the Department of the Interior, and the National Air Pollution Control Administration and the Bureau of Solid Wastes, now part of the Department of Health, Education and Welfare. The agency will also inherit the pesticide control programmes operated by the Department of Agriculture and some part of the AEC's responsibility for control of radiation.

Like Mr Russell Train, the chairman of the Council on Environmental Quality, Mr Ruckelshaus's background is that of a government lawyer. Whereas Mr Train specialized in tax law, Mr Ruckelshaus's interests have lain appropriately in the control of pollution. After a law degree at Harvard, he became assistant attorney-general with the Indiana State Health Department where he prosecuted violators of water pollution and other health laws. In 1963 he drafted Indiana's air pollution control law and from 1966 to 1969 he was majority leader in the Indiana State legislature. Two years ago he was the Republican candidate for Senator but was defeated by Senator Birch Bayh. Mr Ruckelshaus becomes head of the Environmental Protection Agency at the age of 38.

Ecology Rampant

"We are frequently told that Lake Erie is dead—when the total fish catch is rising, not falling. Lake Washington was on its way to becoming as dead as Erie, so it was said, and its reversal is viewed as a great triumph. And it surely was a triumph of political organization. But the polluted lake was great for salmon fishing, and now the water is so pure it has difficulty supporting the salmon. Air pollution is always unpleasant and a lengthy inversion can undoubtedly be dangerous—but automotive emissions are controllable and I cannot help but wonder whether this problem is trivial compared to the carnage we suffer on the highways to which we seem to have become inured, with intoxicated youth the most frequent offenders. Bumper stickers decry DDT across the land demanding its total ban rather than regulated usage—but the next generation of pesticides has killed or blinded dozens of people this year—and no American is known to have been injured by DDT. . . . I have no wish to minimize the seriousness of our environmental problems. They are huge and in need of large-scale, urgent attention. But is it not time to stop frightening the American people and instead demand the very large systematic programme necessary to acquire the data which would permit quantitative evaluation of the risks versus the benefits in all those areas where man's intervention has already or yet may degrade the environment? We need to know far more about pesticide usage, food additives, drugs, industrial practices, radiation hazards, atmospheric phenomena and the alleged fragility of ecosystems than we do today if we are to make sound judgments and establish public policy. What puzzles me is the apathy of college students toward our vast arsenal of nuclear weapons—the infrequency of demands for a massive systematic approach to the attainment of a stable, enduring, just peace. Or why our students do not become angry about the desperate state of our rural poor." From a speech given by Dr Philip Handler, president of the National Academy of Sciences, at the Sesquicentennial of the University of Indiana, October 13.

NEWS AND VIEWS

Are Ribosomes Artefacts?

ALTHOUGH it has been known almost since the very beginnings of molecular biology that the functional unit which translates messengers is the 70S ribosome, the question of whether 70S ribosomes exist free in the cell remains as perplexing as ever. Initiation of protein synthesis requires formation of a complex between messenger RNA and a 30S ribosomal subunit; the 50S subunit only subsequently joins the complex to create the 70S ribosome which then sets off to translate the messenger. Because ribosomal subunits undergo many rounds of protein synthesis, a ribosome must dissociate into its two subunits after completing synthesis of one protein but before starting synthesis of another. But just when ribosomal subunits exchange their partners is one of the most vexed issues of protein synthesis.

When *Escherichia coli* cells are lysed in the presence of sodium ions, only polysomes and free subunits are found; this suggests that ribosomal subunits dissociate at termination of protein synthesis, not to reassociate until initiation of the next protein, so that cells contain virtually no 70S ribosomes free of messengers. But when potassium ions are used instead of sodium, free 70S ribosomes are present as well as the polysomes and subunits. This would result if ribosomes are released from messengers into a pool of free 70S ribosomes and only subsequently dissociate to maintain the pools of 30S and 50S subunits.

Deciding which of these distributions represents the situation *in vivo* and which is an artefact is complicated by the finding that ribosomes do not dissociate spontaneously into subunits (whether at or after protein termination) but seem to do so only when a protein factor complexes with the 30S subunit. This dissociation factor is itself released from the 30S subunit on initiation of the next round of protein synthesis. On page 534 of the last issue of *Nature*, Kaempfer has exacerbated the issue by proposing that the ribosome subunit cycle is yet more complicated.

The need for cyclic dissociation and reassociation of ribosomal subunits in an *in vitro* system has already been shown by dilution experiments. In principle, an extract of polysomes derived from bacteria grown in heavy isotopes is transferred into an excess of polysomes from bacteria grown in normal medium. The source of the ribosomes can be identified by their sedimentation behaviour. After the dilution, their density distribution shows a progressive replacement of heavy ribosomes by hybrids which contain one heavy subunit and one light subunit. This implies that dissociation and reassociation must be taking place.

Further details of the cycle have now been found by changing the conditions of the dilution. When the reassociation of ribosomal subunits but not the completion of nascent polypeptide chains is prevented, ribosomes should accumulate in the extract in whatever form they are released from messengers. This proved to be subunits and not 70S agglomerates.

But using sub-optimum conditions for protein synthesis (limiting initiation by a low availability of free subunits), free 70S ribosomes accumulate in the extract. These could represent ribosomes which have run off polysomes but cannot, for some reason, dissociate into subunits as usual. If this were so, placing an extract of heavy polysomes in an excess of light polysomes should produce only all heavy or all light ribosomes. But, instead, all the 70S ribosomes proved to be hybrid; this indicates that they must derive from the association of free subunits after release from messengers. Their behaviour shows some significant differences compared with ribosomes derived by breakdown of polysomes; they sediment a little more slowly and can be dissociated into subunits by ribonuclease. And they dissociate more easily in the presence of sodium ions.

What role do these ribosomes play in the translation cycle? Kaempfer suggests that free 70S ribosomes do not exist during rapid protein synthesis; ribosomes are released from messengers as subunits and reassociate only at the next initiation. But when protein synthesis slows down, the free subunits accumulate and then associate to yield 70S ribosomes. These ribosomes comprise a dead end pool and do not participate in protein synthesis, which takes place by a preferential recycling of a different set of subunits. When the rate of protein synthesis increases again, the storage pool of inactive ribosomes is mobilized to return to subunits, perhaps by mediation of a protein factor.

How does this cycle fit in with what is known about the action of the dissociation factor? This is difficult to decide because these ribosomes and subunits have

not been assayed for the presence or absence of the factor. Indeed, a major weakness in the theories of those workers who indulge in arguments about which ionic conditions represent the true distribution of ribosomes and which an artefact, is their apparent reluctance to examine these ribosomes for the presence or absence of dissociation factor. The more is discovered about protein synthesis, the more it seems that no aspect is so negligible as to be left to the vagaries of spontaneous action. Specific protein factors mediate each step of protein synthesis, and it is unlikely indeed that the association and dissociation of ribosomal subunits is any exception. It is doubtful whether the significance of free 70S ribosomes can be resolved until the dissociation factor has been properly characterized and its participation in the ribosome subunit cycle completely assayed.

TUMOUR IMMUNOLOGY

Foetal Antigens and Tumour Cells

from our Cell Biology Correspondent

CELL surface chemistry has become one of the great rallying cries of cancer research. Currently the changes in the cell surface which accompany the transformation of cultivated cells by tumour viruses and which can be detected by agglutination tests with plant agglutinins are exciting great interest (see *Nature*, **228**, 502, 512; 1970). For the exposure of the agglutinin sites or some concomitant change in the cell surface may well play an integral part in the establishment and/or maintenance of neoplastic transformation. And, of course, those changes which result in new antigenicities play a crucial part in the interaction between tumour cells and their hosts.

One of the most intriguing discoveries in this field, made by Gold and Friedman in 1953, is that tumour antigens from human adenocarcinoma cells cross-react with foetal antigens found on gut, liver and pancreatic cells only during the second to sixth months of foetal life. These observations, and similar observations made subsequently by other groups, which indicate that transformation results in the exposure and/or synthesis anew of foetal antigens, have now found an interesting parallel *in vitro*. Baranska, Koldovsky and Koprowski (*Proc. US Nat. Acad. Sci.*, **67**, 193; 1970) have obtained a serum from guinea-pigs immunized with unfertilized mouse eggs which, in the presence of complement, is cytotoxic for allogeneic and syngeneic mouse eggs. This serum is also toxic for two lines of mouse cells transformed by SV40, but it is not toxic for the corresponding untransformed cells. Neither is it toxic for rat eggs, a variety of tissues of adult mice and the cells of tumours induced in mice by methylcholanthrene. Baranska *et al.* clinch their argument that the SV40 transformed cells carry an antigen present on unfertilized eggs by showing that after the serum is absorbed with SV40-transformed cells it is no longer toxic for mouse eggs. The chief question now, of course, is whether this antigen is present in a cryptic state in all mouse cells and is unmasked by transformation or whether transformation derepresses the synthesis of a foetal antigen.

Huebner and nine co-workers (*ibid.*, 366) have also detected an antigen in tissues of foetal mice which is not present in young adult mice but reappears with increasing age. They found the group-specific antigen of the murine leukaemia and sarcoma viruses in embryos of all the strains of mice tested even though the mice, in adult life, may be free of infectious virus and subject to only a low incidence of leukaemia. Perhaps the most interesting observations are those relating to wild mice trapped in various parts of the United States. Apparently most wild mice have the group-specific antigen at some stage in their foetal life but less than 2 per cent of young adult animals have the antigen in their spleen or liver cells. In older mice, however, especially those bearing chemically induced sarcomas, the antigen can be detected. On the face of things it seems that most mice carry the genes which specify the group-specific antigens of the murine leukaemia and sarcoma viruses. There is a precedent for the occurrence of the genes of RNA tumour viruses in apparently uninfected animals; some strains of chickens carry the group-specific antigens of the avian RNA tumour viruses and the genes for these antigens are inherited as a dominant trait in a simple Mendelian fashion. Needless to say, Huebner *et al.* interpret all these observations in terms of their oncogene hypothesis; they believe that the genes for the RNA tumour viruses are part of the genome of animals and that these genes may have a role in normal embryogenesis as well as specifying the viral genomes and acting as determinants of cancer.

Happily, even tumour virology has its lighter sides, and the headline writers of the popular dailies might learn a lesson or two from the current issue of the *Proceedings* (*ibid.*, 37). "DNA and Gene Therapy" is after all by any standards a riveting headline even if what follows, "Uncoating of Polyoma Pseudovirus in Mouse Embryo Cells", belies it. All Osterman and his colleagues have shown is that polyoma pseudovirions, pieces of host DNA enclosed in polyoma virus capsids, are uncoated in mouse embryo cells. What they so pretentiously herald, apart from a series of their own articles, is the dawn of "the use of DNA for gene therapy in man" and what they are well on the way to discovering is the road to ridicule if not damnation.

TRANSFER RNA

Looking for Sites

from our Molecular Biology Correspondent

TRANSFER RNA is a molecule which embodies almost all the characteristics of an enzyme, with, moreover, the ability to engage in a great variety of recognition and binding processes. The delineation of its active sites has occupied many laboratories. One facet of its action that has received relatively little attention so far is its interaction with protein synthesis factors. Chen and Ofengand (*Biochem. Biophys. Res. Commun.*, **41**, 190; 1970) now report the results of the first attempt to identify the site of the interaction of yeast phenylalanyl-tRNA with the factor T_u and its associated GTP, which constitutes the first step in the peptide synthesis cycle on the ribosome. It is established that the reaction requires that the bound amino-acid on the tRNA be intact, and also apparently that the RNA be in its native conformation. Chen and Ofengand have found that after oxidative cleavage of the ribose ring with periodate, followed by reduction of the

product to a diol, by means of borohydride, the resulting molecule can still be charged with its amino-acid. The modified phenylalanyl-tRNA was unable, however, to attach to the T_u-GTP complex, neither could any binding to ribosomes and poly U be detected; that is to say, recognition of the T_u-independent peptidyl-tRNA site (P-site) on the ribosome was lost. It seems then that the intact 3′-terminus is a requirement for the T_u-GTP interaction, which is a function common to all tRNAs, but not for the aminoacylation reaction.

The exploration of essential and inessential parts of the chain by excision of nucleotides has been pursued especially by groups in Russia and in Japan. The latest study by Chuguev, Axelrod and Bayev (*ibid.*, 108) deals with yeast valine tRNA, which, like a number of other species, can be split in two by nuclease hydrolysis of a single bond in the central loop. The halves can be separated and recombined with retention of activity towards aminoacyl synthetase, as well as the enzyme which attaches the terminal CpCpA sequence, when this has been removed. By the use of alkaline phosphatase and periodate oxidation, Chuguev *et al.* have progressively nibbled away nucleotides at the 3′-end of the 5′-half of the chain. Five residues, including the pseudouracil, were eliminated in this way, and when the resulting fragment was recombined with the intact 3′-half substantial activity in respect both of amino-acid uptake and the CpCpA joining reaction was found to have survived. Thus sites for two activities do not involve the central loop directly, nor is this required to maintain a conformation which is active in these respects.

Thiebe and Zachau (*Biochim. Biophys. Acta*, **217**, 294; 1970) have tested the acceptor activity of yeast phenylalanine tRNA after chemical modification. The RNA contains a unique base of unknown structure, Y, next to the anticodon. It is labile to acid, and this forms the basis of its chemical excision from the chain. The product has acceptor activity in the endogenous system, but requires a higher magnesium concentration to be charged at an appreciable rate. Moreover, towards *E. coli* synthetase it is completely inactive, by contrast with the native yeast phenylalanine tRNA. The indications are that the removal of the Y-base brings about a conformational destabilization, an inference which is supported by a comparison of the optical properties of the native and modified species.

It may be noted that Cheng and McAllister (*ibid.*, 217) give evidence of specific protection of two ribosomal proteins in *E. coli* towards iodoacetamide by bound aminoacyl-tRNA and poly U. This raises the question of recognition sites on the tRNA for ribosomal proteins, which perhaps serve to register it on the ribosome. Sho, Daneo-Moore and Shockman (*Biochemistry*, **9**, 3372; 1970) have also reported the existence of a ribosomal protein, present in the CsCl-treated core of the 50S particle, which, when isolated, is found to inhibit the poly U directed binding of phenylalanyl-tRNA to the ribosome.

TUMOUR VIROLOGY

The Paris Fashions

from a Correspondent

THE success of the Second Lepetit International Colloquium, held in Paris from November 6–8, must have established these annual meetings, sponsored by the Italian pharmaceutical company Lepetit, as a regular feature of the scientific calendar. And these meetings seem, especially to those who were invited, an enlightened way of using up advertising budgets. The subject discussed this year—the biology of tumour viruses—has had an almost continuous airing at more than half a dozen international meetings held since May; the fact that, in spite of this, new and exciting results were reported in Paris speaks much for the extraordinary progress that is being made and Lepetit's good luck.

The meeting started badly with the first two sessions dominated by poor speakers presenting dull data. Only Basilico (New York University), who reported preliminary results which suggest that transforming wild-type polyoma genomes may be rescued from mouse–hamster hybrid cells by superinfection with the ts-a mutant of polyoma virus, and Darnell (Columbia University), who reported that some of the RNA molecules in the nuclei of SV-3T3 cells contain cellular and SV40 sequences, relieved the tedium.

It was the second day's proceedings, devoted to the RNA tumour viruses, which made the trip to Paris worthwhile. Schäfer (Tübingen) reported the separation of two group-specific antigens in feline leukaemia virus and a third interspecific group antigen which is also present in mouse, rat and hamster sarcoma and leukaemia viruses and perhaps in bovine leukotic cells, human breast cancer and leukaemia cells in culture. This suggests, of course, that these human cancers might be caused by a virus of the mammalian leukaemia and sarcoma virus assemblage. Aaronson (NIH) reported that murine sarcoma virus can transform BALB/c 3T3 cells without the help of a murine leukaemia virus. Interestingly enough, although the transformed cells do not liberate murine sarcoma viruses these can be rescued by superinfection with a leukaemia virus but the transformed cells do not contain detectable group specific antigens of the sarcoma virus.

These two speakers were followed by Hanafusa (New York Public Health Research Institute). His work on Rous sarcoma virus is legendary and he did not disappoint. He first reported the identification of the transmissible factor which renders COFAL negative cells effectively COFAL positive as an avian leukaemia virus of subgroup O. Apparently when Rous sarcoma virus of this subgroup infects COFAL positive cells it rescues avian leukaemia virus which, integrated into the host chromosome and inherited in a Mendelian fashion, seems to be the COFAL factor. He went on to provide the most direct evidence yet available that the RNA and DNA dependent polymerase activities in RNA tumour viruses play a part in transformation. Certain cells transformed by Rous sarcoma virus liberate progeny virus that is uninfectious and cannot transform even when it is fused into susceptible cells with inactivated Sendai virus. RSVα(O), as this progeny virus is called, lacks both polymerase activities. When cells producing RSVα(O) are superinfected with an avian leukaemia virus the progeny include sarcoma virus particles which can transform cells. But the cells transformed by this virus once again produce sarcoma virus progeny which are absolute defectives for infectivity and transformation. These data suggest that the superinfecting leukaemia virus provides RSVα(O) with the two polymerase activities;

the virus particles can then transform cells but, because they cannot specify the polymerases, the progeny from the transformed cells are defective. Clearly if this interpretation is correct the RNA and DNA dependent polymerase activities are essential for the establishment of transformation but not its maintenance. The temperature sensitive Rous sarcoma viruses isolated by Martin (Berkeley), which can transform cells at 36° C but not at 41° C, on the other hand prove that the continuous production of some other viral gene product is required for maintenance of transformation.

Duesberg (Berkeley) has shown that the small pieces of DNA made by the polymerase activities in Rous sarcoma virus include at least 90 per cent of the sequences in the viral DNA. He also reported that the 60–70S RNA in avian sarcoma viruses can, after denaturation, be resolved into two classes of subunits, the *a* and *b* subunits. The RNA of the avian leukaemia viruses lack the *a* subunits as does a non-transforming mutant of Rous sarcoma virus described by Vogt (Washington). He suggested the *a* subunit of the viral RNA genomes may contain the genes required for the transformation of chick embryo fibroblasts. Temin (Wisconsin) described his protovirus hypothesis; he suggests that the two polymerase activities may be present in somatic cells and might, together with RNA molecules, have a role in the intercellular transfer of genetic information and gene amplification in such processes as embryogenesis, antibody production and memory.

Perhaps the highlight of the meeting was yet to come. Spiegelman (Columbia), Gallo (NIH) and Green (St Louis) all reported detecting either RNA or RNA · DNA hybrid dependent polymerase activities in extracts of human leukaemic white blood cells. Spiegelman has now detected this activity in nine out of nine cases but never in controls, and after chemotherapy the white blood cell count of these patients returns to near normal and the activities can no longer be detected. His group have solubilized the hybrid dependent polymerase activity present in the leukaemic cells of one patient and find, as with the cruder extracts from the other patients, it prefers poly dT · poly rA to any other template tested to date. In an impassioned plea for cooperation rather than competition Spiegelman said "the prize here is to help sick kids get over leukaemia" and not, to paraphrase his words, a weekend in Stockholm.

The detection of polymerase activities in leukaemic cells obviously suggests that human leukaemia viruses may exist, but, as Todaro (NIH) noted, these activities are also present in visna virus and primate syncytium forming viruses. They are not specific to the RNA tumour viruses and that should warn against over hasty conclusions.

The final day was devoted to mutants of the DNA and RNA tumour viruses and to changes in the cell surface of transformed cells which result in the exposure of agglutinin receptors. Burger (Princeton) described the experiments he reported in *Nature* last week (**228**, 512; 1970). The only cloud on that horizon is that there is a surprising lack of information about these sites on cells transformed by RNA tumour viruses. If the exposure of the sites is crucial for maintaining the transformed cell phenotype they should be exposed on cells transformed by the RNA viruses. It is high time someone looked in detail at such cells.

POLLUTION

Danger in the Air

THE relaxing of smoke control orders in Britain this winter will provide some interesting, if not entirely welcome, work for the staff of the Medical Research Council's Air Pollution Research Unit. The shortage of smokeless fuel will give them the opportunity to discover whether the health of bronchitic patients declines, after a steady improvement which has been evident since the implementation of the Clean Air Act of 1955.

One of the epidemiological studies which visitors inspected when the unit put its work on show earlier this week began in the winter of 1955–6. A group of patients in London, who were susceptible to bronchitis, began to record their daily state of health. At this time bronchitis clearly worsened rapidly when the concentrations of smoke and sulphur dioxide in the air increased suddenly. The situation was much the same in the winter of 1959–60, but five years later there was far less correlation of illness with changes in air pollution. Even when the concentrations of smoke and sulphur dioxide in the air reached 1,000 μl./m^3 patients did not always record a worsening of their conditions. And by 1969–70 there was very little change in the health of patients when the amount of atmospheric pollution increased.

This apparent decline in sensitivity to air pollution during fifteen years could well be a consequence of the drastic reduction in the concentrations of smoke and sulphur dioxide in the London air, although Mr R. E. Waller, the unit's epidemiologist, points out that improved methods of treatment could have been partly responsible for the improvement in the health of the patients. In any case, he says, it is too early to say whether the acute effects of air pollution in London have been eliminated. The signs are encouraging, however.

The epidemiology of lung cancer is another of Mr Waller's interests, but it is becoming increasingly apparent that cigarette smoking is a far more significant cause of this disease than air pollution. Investigations have started recently into the effects of polluted air and smoking on the function of the lungs. The basis for this work is the maximum rate at which a subject can exhale air—the peak flow rate—which is a measure of the state of health of the lungs. The peak flow rate is less for lungs which are infected, or diseased in any other way, than for a healthy set of lungs. Members of the staff of the unit have been measuring their peak flow rate daily for five years and have found a steady increase in the value recorded.

Smaller scale investigations in progress include chemical analysis of various pollutants, including asbestos, which is no longer confined to industrial atmospheres. Various constituents have been identified, including paraffinic hydrocarbons and 3 : 4-benzpyrene, but their biological significance has yet to be assessed. Work also continues on bacteriological aspects of air pollution. Particulate pollutants from London air turned out to stimulate the growth of *Haemophilus influenzae*, which is recovered from patients with chronic bronchitis. The pollutants also inhibited the growth of various bacteria found in the respiratory tract. So far it seems that the best inhibitors are obtained by evaporating aqueous filtrates of the pollutants, while the best stimulators come from the fractions extracted with

organic solvents. The precise mechanism will no doubt be worked out later. They do not look for sudden breakthroughs at the Air Pollution Research Unit.

INTERSTELLAR DUST

Ultraviolet Observations a Problem

by our Astronomy Correspondent

A NEW observation of Barnard's Loop Nebula in the constellation Orion, reported in *Science*, has turned up another piece in the jigsaw which one day will reveal the nature of the interstellar dust (**170**, 527; 1970). The new data refer to the far ultraviolet part of the spectrum, which, according to recent observations of stars that are partially obscured by dust clouds, is removed by the dust to at least the same extent as the near ultraviolet, if not more. Either absorption or scattering of the starlight by the dust must be responsible. In the near ultraviolet, the more accessible part of the spectrum, scattering seems to be important. Barnard's Loop Nebula has been detected at these wavelengths, by an ultraviolet camera carried on a Gemini flight in 1966, presumably because the dust in the nebula is scattering ultraviolet radiation from the hot stars in Orion toward the Earth. It might be expected that the nebula could be detected in the far ultraviolet by the same scattering process, but rather surprisingly this has turned out not to be the case.

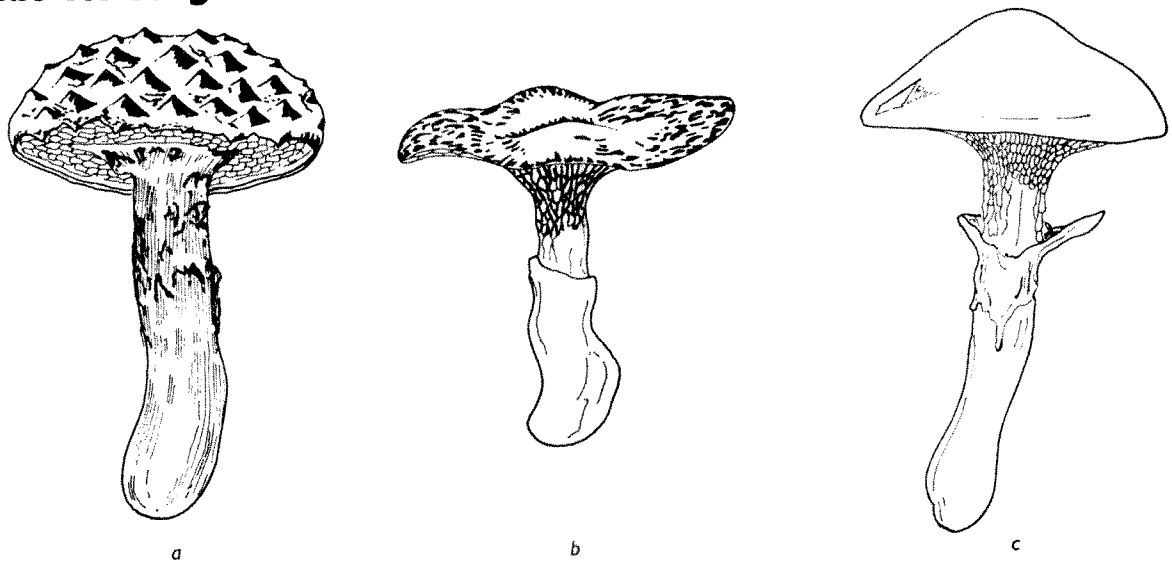
In the *Science* article, three photographs of Orion which ought to have shown Barnard's Loop Nebula are discussed by Richard C. Henry and by George R. Carruthers, who first reported observations of interstellar molecular hydrogen. Using a special camera developed at the Naval Research Laboratory and to be carried on one of the later Apollo missions, assuming there are no more cancellations, photographs of Orion and the Monoceros constellation were obtained in the wavelength band 2100–1230 Å in the far ultraviolet. This stops short of the Lyman α emission at 1216 Å which could easily have fogged the photographs. Although a conservative estimate makes their camera at least as sensitive as that which recorded Barnard's Loop Nebula in the near ultraviolet, Henry and Carruthers found no sign of the nebula on their photographs.

What this implies about the material of the nebula is by no means certain, but at least it shows that the far ultraviolet is not being scattered toward the Earth like the longer wavelengths. There are no strong reasons for supposing that the stellar radiation falls off toward the far ultraviolet to account for the non-detection of the nebula at 2100–1230 Å. Either absorption by the dust is coming into play at these wavelengths, or the radiation is not being scattered through sufficiently large angles for significant amounts of it to be directed at the Earth. Either way Henry and Carruthers see their data as relevant to the question of the nature of the dust particles although none of the models which have been put forward so far seem to fit their observations at a first glance.

Altogether the camera took thirty photographs during a rocket flight last year, each with a seven second exposure made possible by a combined electronic and photographic system. The image is formed on a photocathode at the focus of the collecting mirror, and a solenoid surrounding the optics directs the photoelectrons onto an emulsion behind the mirror. In this system each electron has enough energy to blacken a grain in the emulsion, making possible the large return of photographs from a short flight. Nobody was surprised that one of the brightest optical stars in the sky, Betelgeuse, should be missing from the ultraviolet photographs which otherwise show the Orion constellation distinctly. Cool supergiants would not be expected to register at those wavelengths.

MYCOLOGY

Guide for Fungus Collectors



a *b* *c*

Strobilomyces floccopus (*a*), *Boletinus cavipes* (*b*) and *Suillus grevillea* (*c*) are three of the fungi described in the latest volume of the *British Fungus Flora* (R. Watling, *Boletaceae: Gomphidiaceae: Paxillaceae*, HMSO, 1970, £2 10s). Many a mycologist will doubtless be grateful to take up this book when returning from a profitable fungus foray.

VARIABLE STARS

Absorption in the Infrared

by our Cosmology Correspondent

A CORRELATION between the infrared absorption band of H_2O at 1·9 μm and the microwave emission of the same molecule in long-period Mira variables suggests details of the mass loss mechanism operating in these stars.

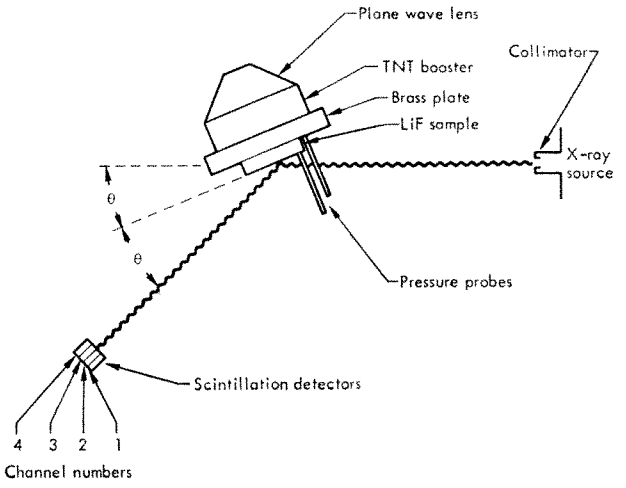
J. A. Frogel reports that absorption features characteristic of water vapour have been detected in the spectra of seventeen Mira variables, and that the variation of the intensity of the 1·9 μm band is inversely correlated with that of the radio emission of this molecule at 1·35 cm and with the optical luminosity variations (*Astrophys. J.*, **162**, L5; 1970). The infrared measurements have been carried out using a 0·5 m Ebert–Fastie spectrometer; Frogel used a comparison between the 1·9 μm band and the 2·25 μm band to determine the variation at 1·9 μm, because at 2·25 μm there is neither stellar absorption nor terrestrial atmospheric absorption, so that measurements at this wavelength provide a good indication of the variation of the total light curve of the star. Relative to the 2·25 μm feature, the 1·9 μm absorption rises to a maximum just before the minimum of the light curve, after which it decreases rapidly to a minimum value which is maintained for roughly one-fifth of the optical period before a slow rise to the next maximum. The available data at microwave frequencies suggest that the emission at 1·35 cm is positively correlated with the optical variations (compare P. Schwartz and A. Barrett, *Astrophys. J.*, **159**, L123; 1970).

The model which Frogel proposes to explain this behaviour depends in detail on the way in which mass loss occurs from these stars. He suggests that around the maximum of the optical intensity so much material is expelled above the photosphere that the water which forms (independently of pressure below 2,500 K) increases in quantity so rapidly that at first this gain outweighs the decrease in absorptivity resulting from its falling temperature. Once the star is well passed maximum intensity, the rapid cooling of the water vapour and lack of any new material soon produce a decrease in the observed absorption to a minimum. As the cycle repeats, the water vapour surrounding the star remains associated as H_2O, and just before maximum luminosity conditions are suitable for the pumping required to produce the observed microwave radiation by maser action. This model is very susceptible to observational tests, because it intimately relates the microwave and infrared behaviour of the sources. Conditions suitable for the formation of H_2O might also be expected to be close to those needed for production of OH, so that these three classes of emission or absorption objects could well be members of one basic family.

SOLIDS

X-rays and Shock Waves

ALTHOUGH X-ray diffraction has been used to study the structure of solids since near the beginning of the twentieth century, it has usually been a technique carried out over fairly long periods of time with crystals which were not subject to sudden large distortions. The desire to look at solids undergoing rapid deformations has increased with the ever growing interest in the interaction of, for example, phonons and laser radiation with matter. The problems of looking at phenomena in solids which last only a few tens of nanoseconds are, however, great and are associated chiefly with the switching of a sufficiently short and intense pulse of X-rays at the correct time.



Experimental arrangement for X-ray diffraction study of LiF under shock compression.

Johnson *et al.* report from the Lawrence Radiation Laboratory of the University of California (*Physical Review Letters*, **25**, 1099; 1970) that they have devised an experimental system which has broadly overcome these problems. They do not describe their pulsed X-ray generator in detail, but the apparatus with which they have managed to observe X-ray diffraction from a crystal of lithium fluoride under shock wave compression is illustrated here.

The transit time for the shock wave from a small TNT charge was measured with two pressure probes which defined the time of entry and exit of the shock wave and made it possible to calculate the shock pressure. Because most of the diffracted X-rays come from very close to the surface of the crystal, it is important to switch on the X-rays about 30 ns before the estimated time of breakout of the shock wave so that the compressed state can be properly observed. If X-rays were truly diffracted from the compressed state of the crystal, the signals from channel 2 would be expected to increase by a factor of about three and those from channel 4 to decrease by about the same amount. In the best experimental run, the output from channel 2 was found to be up by a factor of about 2·7 and that from channel 4 down by a factor of about 0·7. The centre of each scintillator is about 0·2 cm from its neighbour and the resolution of the apparatus is clearly not very high, but if it is assumed that a diffraction line shift from channel 4 to channel 2 has taken place, the corresponding $\Delta 2\theta$ ($\sim$2·6°) is quite close to that expected for an isotropic applied pressure of the size calculated from the shock transit time ($\sim$130 kbar). In other words, the unit cell of the lattice is being compressed from a cube into a smaller cube rather than into a rectangular box of square cross-section, as might be intuitively expected when a plane shock wave is applied to a crystal. As well as establishing a technique which will doubtless be improved in the near future, this work seems to have thrown up some interesting side issues.

Molecular Biology in the Year 2000

by
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By the year 2000 most of the problems now facing us in molecular biology will probably have been solved, but new and unexpected developments are likely to make the subject just as exciting then as it is today. This was the theme of Dr Crick's contribution to a conference held in October 1969 to help celebrate *Nature*'s centenary. This article is based on his talk.

I WANT to consider the future of molecular biology and, to a lesser extent, of cell biology*. I do not consider here applied biology nor the social implications of biological research, not because these are unimportant subjects but simply to keep the discussion within reasonable limits.

We must first consider the length of time over which it is useful to make forecasts. It is often not difficult to make short range forecasts for periods up to, say, five or ten years ahead. Molecular biologists have acquired some experience in predictions of this sort in molecular biology. During the past twenty years such forecasts have usually turned out to be correct. What has been rather variable, however, is the estimate of the precise time involved. An error in time of a factor of two is not at all uncommon. Thus at a certain stage it was possible to say, with a fair degree of confidence, that the genetic code would some day be discovered. If one made an estimate that this would happen within, say, five years, then experience has shown that the actual time might be as short as two or three years or as long as eight or ten.

In case you should think that my own judgment is peculiarly erratic, I should say that other people's judgments seem to be little better! There is an amusing example concerning the structure of proteins. After Perutz and Kendrew had discovered the structure of haemoglobin and myoglobin, Sir Lawrence Bragg forecast that it would be another ten years before a further protein was worked out to this sort of resolution. In fact, the next protein whose structure was solved was lysozyme. It took only about five years instead of the ten years expected and ironically enough it was done by David Phillips and his team working in Bragg's own laboratory. It should not be concluded from this example that things always happen more rapidly than one expects. For example, the collinearity of the gene with the protein for which it codes took a longer time to prove than we had estimated.

Naturally, as the time over which one is trying to forecast gets longer, so the problem becomes more difficult. One way of tackling this subject is to look back and try to imagine that one had made forecasts in the past about important scientific discoveries. It is easy to show that many important discoveries are of a rather unexpected nature and therefore are difficult to foresee. A good example is the work of Avery and his colleagues which showed that DNA was the chemical molecule involved in transforming *Pneumococcus*, or the discovery of Lederberg and Tatum of genetic recombination in bacteria. One would have had to be far sighted indeed to predict these discoveries more than a few years before they were made, or to realize the tremendous importance which time has shown them to hold. For this reason forecasts of fifty years or more are very difficult indeed and I feel they are hardly worth making. For a period of twenty-five years one might hope to have some success. I have therefore arbitrarily taken a period of thirty years, which brings me nicely to the year 2000.

Big Increase Likely

I shall argue that there are certain general factors which make it virtually certain that there will be a big increase in biological knowledge during this period. In the first place there is a very considerable amount of manpower available, not only at present but also on an even greater scale in the future. At the moment it is fair to say that most biological research is concentrated in the United States and parts of Western Europe, with significant contributions from the USSR and from Japan. In fact, the amount of effort seems to be strongly correlated with the standard of living. Because there are many countries in the world with a standard of living which is likely to increase, we can expect further countries to start contributing to biological research. In particular, it would not be surprising if eventually China became a major scientific power.

Until a few years ago it was possible to believe that the funds available for scientific research in any one of the advanced countries were continually increasing. A perfectly general argument shows that this rate of increase cannot be sustained over a long period and eventually saturation must set in. We are perhaps already seeing the beginning of this process in the United States, although it has been partly brought about by the war in Vietnam. From a broad point of view, however, we can be reasonably confident that, short of some nuclear catastrophe, there will be large sums of money and very large numbers of people available for biological research.

Not only are biologists themselves increasing in number, but fairly large numbers of people are moving into biology from other scientific disciplines. There is an interesting distinction that can be made here between problems and techniques. For problems, scientists seem to move upwards in the scale of complexity. That is to say, they go from physics and chemistry into molecular biology and from molecular biology into cell biology and so on. For techniques it is quite a different matter and one may find people borrowing techniques in any direction. Modern biologists are quite at home using recently developed techniques springing from the physical sciences. In spite of this it is rare for biologists to leave biology and take up problems in chemistry and physics proper.

* I do not wish to make a strong distinction between molecular and cell biology. In one or two places I have referred to other branches of biological research, on the rather doubtful grounds that molecular biology can be defined as anything that interests molecular biologists.

One result of this migration of people from the so-called more exact sciences has been the confidence which they have brought to the study of biological problems. Physics and chemistry within the past seventy years have been immensely successful and have not only revolutionized the detailed knowledge of these subjects, but have also produced theoretical foundations of great power and subtlety. Physicists in particular have been living in a revolution for so long that it has become almost second nature for them to think of their subject in this way. It is therefore not surprising that physicists who move into molecular biology have often seemed rather brash and over-confident in their approach to biological problems. In spite of the distress this may have caused to biologists proper, it has to be conceded that very great advances have been made in molecular biology from just such a point of view.

Influence of Physics and Chemistry

Another extremely important factor, which I sometimes feel is not sufficiently appreciated, has been the tremendous power of modern experimental techniques, mostly springing from physics or physical chemistry. One has only to consider such examples as chromatography, the use of radioactive tracers or the electron microscope (to mention only a few) to see how powerful and how various they are. A molecular biologist called upon to tackle almost any problem which would interest him now, using the techniques available before, say, 1935, would give up, I think, in despair. Moreover, there is little sign of the exhaustion of any one technique and there are still signs that new techniques are coming along—for example, the use of nuclear magnetic resonance on the one hand and of computers on the other. Although no one would pretend that we have techniques to solve all the problems which face us, nevertheless, there is a general feeling that either the existing techniques, or such new ones as can be generated by ingenuity and by a resolute confrontation of the problem, will be enough to see us through.

When we turn to look at the nature of biology itself we see stretching before us an almost unlimited number of important, interesting and unsolved problems. This is partly due to the inherent complexity of biology and partly due to a passionate desire to understand the world around us and our own natures in particular. There are so many things we should like to know—and like to know about in considerable detail—that we need not seriously worry at this stage that the subject will become exhausted.

For all these reasons then, that there is a large supply of both manpower and money, that very powerful techniques are available and that the problems are of absorbing interest, we can confidently predict that there will be a massive research effort in biology for a good many years to come.

Let us now consider various specific problems, first in molecular biology, and ask what state they will be in by the year 2000. The reader might find it amusing to make his own list of such problems as are closely familiar to him. One might take as examples: a detailed understanding of the replication of DNA and of the unwinding process; the structure of chromosomes; the meaning of those nucleic acid sequences which are not merely an expression of the genetic code but are used for stopping or starting or control mechanisms of one sort or another; the significance of repetitive sequences in DNA and so on. My own conclusion was that no matter what topic one considered, either in classical molecular biology or in such related subjects as oxidative phosphorylation and the structure of mitochondria or even such relatively unexplored fields as the structure of membranes, it was difficult to think of one which would not be solved by the year 2000, at least in outline. The molecular mechanism of muscular contraction; the way in which antibody variety is generated and immunological tolerance produced; the exact way in which hormones act; how synapses are modified during learning . . . all these problems, I feel, will advance very considerably towards definitive solutions by the end of the twentieth century.

Cell Biology

If we now turn to cell biology, a field which I do not know as well, one reaches broadly the same conclusion, although the period might be a little longer—perhaps up to forty or fifty years ahead in some cases. I am thinking of such subjects as: the mechanism and control of mitosis; cell movement, and axon growth in particular; cell recognition (especially as it arises in the nervous system) and also the nature of the influences which produce "gradients" in embryological development.

If the reader should think I am being over-optimistic, let him look back to 1940 and see how much more we know now than we knew then in almost all these subjects. Moreover, this period includes five years of war, during which biological research went on very slowly and for the first half of this period the manpower and money available for biology, though increasing, had still not reached the present high level.

If it be accepted that most of the problems with which we are today rather closely concerned are likely to be solved by the year 2000, it is worth while to consider what problems are likely to remain unsolved. It seems to me there are subjects of a rather general nature which are likely to fall into this class. I certainly believe that, in the intervening years, some progress will be made in them, but I rather doubt if we shall be in a position to see the answers in broad outline, let alone in great detail. Examples of such topics are: the origin of life on Earth; the existence of life on other worlds and communication with other creatures in the galaxy, assuming that they exist. Although I think a vast amount of progress will be made on the understanding of the nervous system, it would not be surprising to me if some of the more sophisticated aspects of the behaviour of the brain still remained a puzzle. I am thinking of such matters as our vivid interior three dimensional picture of the world which we build up from the light signals falling onto our eyes and other information, and also such problems as the nature of consciousness, although whether this will turn out to be a real problem or a semantic one remains to be seen.

There is also a major problem to which I believe biologists have given insufficient attention. All biologists essentially believe that evolution is driven by natural selection, but someone from the more exact sciences could well point out that it has yet to be established that the rate of evolution can be adequately explained by the processes which are familiar to us. It would not surprise me if nature has evolved rather special and ingenious mechanisms so that evolution can proceed at an extremely rapid rate—recombination is an obvious example. It may even be that if we could look back from 100 years ahead, we would realize that what we know today is not adequate to explain the rate which actually occurs. An exact estimate, if we could make it, using present known mechanisms might, for all we know, be out by a factor of 10 or even by one as large as 100. To solve this problem we may need a rather complete knowledge of many biological

systems, both at the molecular level and at the ecological level and at all levels in between. For this reason I doubt if it will become a mature subject within the period we are discussing, although it would be surprising if there were not some initial attacks on it.

In reflecting on which problems are likely to be solved by AD 2000 and which are not, it seems to me that the distinction between the two classes depends mainly on whether they can be attacked by isolating a small part of a biological system, or whether one is mainly concerned with its behaviour as a whole. Molecular biology has been successful largely because it has concentrated on the former type of problem, although certain aspects of the overall behaviour have often been used as a tool. Molecular genetics is an obvious example. But, in the long run, problems involving complex interactions can hardly be avoided, since some of the most profound aspects of biology are of this character. The development of high speed computers will help, but the difficulties are not solely computational. One needs to know so many data in order to make the calculations realistic and not merely plausible. A simple example would be the "total" behaviour of a microorganism such as *Escherichia coli*, including all its regulatory mechanisms. Such a cell can usefully be regarded as a special type of chemical factory, and it is not unreasonable to ask, for example, how efficient its construction is. All the long term problems I have mentioned involve complex interactions, except perhaps that of the origin of life, where the main difficulty is to obtain any direct experimental evidence at all of what happened so long ago.

Finally, one must consider the subjects in between; that is, problems which are not immediately in front of us on the one hand or of such a long term nature that we think they will not be solved by that time. These are by far the hardest to guess, partly because such problems depend on new and unexpected break-throughs of one sort or another. These are certain to occur. One would predict that they may, in part, depend on questions which we have not yet learned to ask. What is not clear is how much of the subject in the future will depend on such break-throughs. It is unlikely to be a negligible fraction. On the other hand, I rather doubt if research in the year 2000 will be entirely dominated by the break-throughs which have taken place in the years between now and then. Of course, it is exactly these unexpected and important developments which make a science exciting for those doing research in it, but one should not lose sight of the fact that even without them much useful science can be done following existing concepts and techniques; even in research of this later kind there will usually be a whole series of minor break-throughs to bolster up the morale and interest of the people doing the work.

New Branches of Science

However, one should consider not merely break-throughs in the existing sciences but the creation of entirely new branches of science which scarcely exist at all at the present day. In the fifties, Watson and I were rather fond of the hypothetical science of astrobotany. This, we felt, was bound to come but it seemed sufficiently far in the future that we could enjoy making jokes about it. What is remarkable is that there has already been a beginning to this study. It now seems unlikely that there will be life of any sort on the Moon and rather doubtful if there will be anything living on Mars, but experiments have already been undertaken to see what terrestrial organisms can survive in the rather bleak conditions found on these heavenly bodies.

I therefore feel an obligation to suggest a new subject in which practically no work has been done at all, and I would propose for your consideration biochemical theology*. It is not quite true that nobody has researched into such matters as the efficacy of prayer. In the last century, for example, Galton† wrote an amusing paper on the subject (Statistical Enquiries into the Efficacy of Prayer) in which he showed by a couple of ingenious statistical tests that the efficacy of prayer seemed to be rather low. This line of work does not appear to have been followed up either by the Church of England or by the Vatican. But nobody, as far as I know, has considered the problem at the biochemical level. So many people pray that one finds it difficult to believe that they do not get some satisfaction from it, and a good molecular biologist will naturally believe that this can be expressed, at least in part, in molecular terms. Part of it, of course, would involve the molecular biology of the synapse and the overall organization of the nervous system, but the principal effect is probably hormonal, and one would not be surprised to find that hormone levels were affected by prayer. No doubt before long some "with-it" church in America will take up the topic.

Today's Problems Solved

My broad conclusions, then, are that between now and the year 2000 biological research will take place on a massive scale. By then, most of the detailed problems in molecular and cell biology facing us now are likely to be solved. Certain broad but important subjects will still be in a primitive stage and there will inevitably be a proportion of novel, unexpected and significant advances the nature of which we can hardly guess. In short, the whole field is likely to be even more fascinating in the year 2000 than it is today.

Finally, I feel one should consider briefly the role of *Nature* in these developments. In the past, *Nature* has been very friendly to molecular biology, as it has to several other border-line subjects. The advantages of wide distribution and rapid publication have not gone unnoticed by people struggling to make a way for themselves in the areas between the existing scientific disciplines. Moreover, in the past few years, the standard of editorial comment on work in molecular biology has been very high and a series of correspondents have helped to keep the readers of *Nature* in touch with work published in other journals. I hope, however, that *Nature* will not become overloaded with too many detailed papers in what we now regard as the more classical parts of molecular biology. There is a need for a journal with a wide circulation containing general articles on various scientific topics which can be read with advantage and pleasure by people not working in those particular fields, in addition to papers, hot from the research front, for specialists. We are all grateful to the support that *Nature* has given to the development of our subject.

* Recently I was idly glancing at the novel *Antic Hay* by Aldous Huxley when, on the first page, I came across the following: "But if theology and theosophy, then why not theography and theometry, why not theognomy, theotrophy, theotomy, theogamy? Why not theophysics and theo-chemistry?" They form part of the musing of Theodore Gumbril Junior in the opening scene in the chapel, during which, because of the hardness of the seats, he first conceived the idea of the Patent Small-Clothes, the trousers with the inflatable seat which can be blown up or deflated at will. *Antic Hay* was first published in 1923, and I must have read Gumbril's ruminations when I was an undergraduate, or even earlier. I should point out, however, that there is a profound difference between theological biochemistry and biochemical theology. Curiously enough, I think theo-chemistry probably corresponds to the latter.

† Galton, F., *The Fortnightly Review*, August, **12**, 125 (1872).

Micrometeorite Environment at the Earth's Orbit

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Space vehicles have revealed the broad outlines of the micrometeorite mass distribution at one astronomical unit from the Sun, although some uncertainties remain. But we still know nothing about their composition and have only scant data about their origin. Such data as exist support a cometary origin for micrometeorites, in accord with most recent theoretical work.

BASICALLY micrometeorite experiments have been designed to answer one or more of the following three questions. How many are there? What are they made of? And where do they come from? We are well on the way to answering the first question and progress is being made on the third. The second, however, is a long way from solution. In this article I shall consider the experimental data on the population of sub-microgram particles in interplanetary space in the light of these questions. Theoretical questions have received adequate treatment elsewhere[1–4]. The cutoff point for this study at one microgram represents approximately the effective upper limit for satellite experiments and the lower limit for radio meteor observations[3,5,6].

Mass Distribution of Micrometeorites

Ground-based observations of the mass distribution of particles in this size range are restricted to analyses of the extent, brightness and polarization of the zodiacal light and gegenschein[7–9]. These data can be interpreted, with the aid of a suitable physical model for the nature of the scattering particles, in terms of a mass distribution[10,11]. Such a model is based on parameters such as the particle shape and albedo, which are known with less precision than is the mass distribution. Published mass distributions which have been based on zodiacal light or gegenschein observations therefore will not be reviewed here although orbital data based on these observations will be considered in answer to the third question.

A ground-based measurement which should be mentioned in this context, although it does not lead to a mass distribution, is the determination of ^{26}Al produced by cosmic radiation in deep sea sediments and Arctic ice[12–15]. From this quantity and assumptions about the mean cosmic ray flux, composition of the irradiated interplanetary dust and the orbital lifetimes of such particles, a value can be obtained for the total mass falling on the Earth per unit time in the form of dust[16]. Yokoyama has calculated an absolute upper limit to this mass of 1,000 tons per day with a probable upper limit of 100 tons per day[17], in broad agreement with the results from direct measurement of the mass distribution by means of space vehicles.

Virtually every kind of space vehicle has been used to carry experiments designed to measure the mass distribution of micrometeorites, but the yield of hard facts from the large amount of raw data has been disappointingly small. No results will be presented here from collection experiments mounted on aircraft or balloons for reasons mainly to do with contamination. Consequently the only reliable source of data from which to compute a micrometeorite mass distribution is the satellite-mounted experiment, and I have made a critical study of the results of all such experiments which could be found in the literature. I have selected from a great many experiments those which were felt to be most reliable, and to this end certain criteria were established against which each experiment and its data were measured.

First, the original data, in terms of number of micrometeorite impact events recorded, measured noise level, timing and duration of experiment, altitude and attitude of the vehicle and so on, had to be available so that independent flux calculations could be made. Second, the experimental unit should have been calibrated using a high-velocity, or preferably a hyper-velocity, facility in order to simulate micrometeorite impacts more closely. Actually in no case was the calibration procedure entirely satisfactory. The most common calibration device in recent years has been the Van de Graaff accelerator equipped with a particle injection mechanism of the kind developed at the TRW laboratory[18]. Although the Van de Graaff has enabled at least the lower end of the micrometeorite velocity spectrum to be simulated in the laboratory, many of the calibrations have suffered from two drawbacks. First, the particle filters, used to prevent more than one particle from impacting the detector at one time, have been inefficient and have allowed clusters of particles to pass into the test chamber. Second, although a small amount of work has been done using graphite particles, in order to simulate more closely the low densities thought to be characteristic of micrometeorites, by far the most common projectile material has been iron and this may be a source of error in the calibrations.

The third criterion for acceptance was freedom from known sources of noise. This was clearly a more subjective test than the others and at first it was planned to use only data from coincidence systems or experiments involving some other kind of reliable discrimination against spurious signals. Coincidence systems are those in which each micrometeorite event is recorded simultaneously by two or more transducers of different types, such as an ionization detector coupled with an acoustic sensor. Unfortunately insufficient results are available from such experiments to provide a statistically meaningful survey of the mass range of interest so that some of the more reliable single-transducer systems were also included in the results.

Fig. 1 presents the results from eight experiments which satisfied the criteria. I have followed the convention of plotting the data as a cumulative mass distribution, so that a point at mass M corresponds to the flux through unit area in unit time of micrometeorites with mass equal to or larger than M. It should be noted that, with the exception of the Gemini S-010 points, each datum point represents the number of micrometeorite impact events which exceeded a certain threshold value as most detectors used so far have had no dynamic range. The error bars correspond to one standard deviation of the recorded number of impacts and represent a measure of statistical

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strength only. It is not possible to assign error limits to mass values because of the uncertainties in calibration procedures. The line drawn through the data points is an extrapolation by eye of the radio meteor flux given by Whipple[3] which is drawn here as a definitive line though it should be noted that this, too, is the result of experimental observation and thus liable to error. For complete technical details reference should be made to the appropriate publications, but some remarks about the different systems are necessary.

The Pegasus experiment achieved an effective dynamic range corresponding to a factor of about thirty in mass by the use of three detectors of different sensitivities[19]. The large size of this experiment ensured that it recorded a great many impacts, and it is statistically very reliable. Early problems with poor calibration of the detectors have been resolved by a careful programme of hypervelocity firings so that the mass values associated with the impact data are now equivalent in reliability to those of other experiments[20].

Explorer 23 carried several micrometeorite impact experiments of which only the pressure cell system is considered here[21]. The same system was carried also on Explorer 16, but the results are essentially equivalent so that only data from the later experiment have been used. The same comments concerning calibration are valid here as in the case of Pegasus[20].

The Gemini S-010 experiment[22], in which polished stainless steel plates were exposed to space aboard Agena target vehicles, possessed the greatest dynamic range of the experiments considered here. The system was potentially capable of recording impacts over virtually the entire micrometeorite mass range, the upper limit being governed by available exposure time. But in the case of the only recovered experiment, thruster debris and handling by astronauts created a background which obliterated impact sites corresponding to masses below about 10^{-11} g. Because of the relatively small area of the experiment there were few impacts, which reduced the statistical reliability of the data.

Although acoustic sensors have received a certain amount of criticism[23], centring on their sensitivity to noise of thermal and electronic origin, very carefully controlled systems were flown on Cosmos 135 and 316 (refs. 24 and 25). An acoustically isolated sensor was used to record micrometeorite impacts while a separate sensor, attached to the spacecraft structure, monitored the noise level. As a result the count rate data are thought to be reliable although these experiments were calibrated by bead dropping and consequently their mass sensitivities must be regarded with caution. The Cosmos 163 experiment was actually a coincidence system, involving a thin film capacitor in addition to the acoustic sensor. Malfunction of the capacitor resulted in only acoustic data being obtained[25], however.

An operational coincidence system was carried on Explorer 35, consisting also of a thin film capacitor coupled with an acoustic sensor[26]. The output from this system is thought to be reliably free from noise.

The Mariner 4 micrometeorite sensor was another coincidence system in which the thin film capacitor failed to function, leaving only the acoustic data[27]. Although the noise level in this experiment was not monitored as it was in Cosmos 163, the data are felt to be reliable because of the stable thermal environment which the sensor experienced and there is no evidence that this system gave a spuriously high impact rate[28].

The detector system included in Pioneer 8 and 9 must be considered the best micrometeorite experiment yet flown[29]. In this system the micrometeorite velocity is measured directly by a time-of-flight device, its energy is measured by the output from a thin-film plasma detector, its momentum is registered by means of an acoustic transducer and finally its radiant can be determined by correlating the end points of the time-of-flight measurement. Thus the true mass of the micrometeorite can be calculated, whereas in all other experiments it was necessary to derive an effective mass based on an assumed mean micrometeorite velocity, sensor outputs being usually some function of both mass and velocity of the impacting particle. The high level of redundancy in the system also ensures that the data are virtually free of unidentified noise so that, although the count rate from these spacecraft is extremely low (nine time-of-flight measurements in more than two years), the derived flux rate is extremely reliable[30]. Finally, it should be noted that the Pioneer 8/9 experiment has an appreciable dynamic range corresponding to more than two orders of magnitude in particle kinetic energy. With the observed velocities this has resulted in a mass range of a little more than one order of magnitude. For direct comparison with the other results the Pioneer 8/9 data were degraded by reducing the real masses measured by that experiment into effective masses equivalent to those derived for the other systems.

Following the recommendation of the Cosmic Dust Panel of COSPAR, a mean value of 20 km s^{-1}, derived from radio meteor observations, has been used to compute an effective mass corresponding to each detector signal. If micrometeorite velocities are found to vary appreciably from this mean the physical significance of the computed effective masses will be clearly reduced. In fact, Pioneer 8 and 9 have measured velocities, relative to the spacecraft, of from 5·7 to 35·9 km s^{-1} (ref. 30). The significance of the effective mass is further diminished as a result of the calibration methods commonly used. Virtually all calibrations have been performed using impacting particles at normal incidence whereas in reality incident angle varies over an appreciable solid angle, frequently as much as 2π steradian. Thus, even neglecting the velocity problem al-

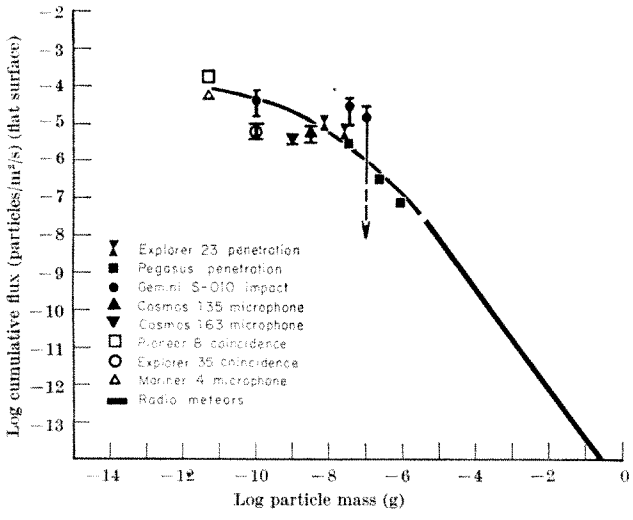


Fig. 1. The mass distribution of micrometeorites at a heliocentric distance of approximately 1 astronomical unit, expressed as a cumulative flux through a flat surface. Solid symbols represent measurements in Earth orbit, open symbols correspond to either lunar orbit (Explorer 35) or locations away from the Earth–Moon system.

ready referred to, the effective mass usually corresponds to the low end of a range of real particle masses, the extent of this range depending on the directional sensitivity of the detector concerned. It follows that, because detector response as a function of incident angle is not known usually, the flat surface flux as measured cannot be converted into a solid angle flux.

The data in Fig. 1 were all obtained at a heliocentric distance of approximately 1 astronomical unit. Solid symbols correspond to detector systems which were in Earth orbit, open symbols to those which were either in lunar orbit (Explorer 35) or away from the Earth–Moon system. Within the accuracy of the data no significant difference between different locations can be seen. An accretion rate of approximately 20 tons per day over the Earth's surface can be inferred.

Fig. 1 suggests several areas for future investigation. First, although the agreement between different experiments is now better than in the past, there are still discrepancies, some of which must be attributed to the comparison of data derived from different functions of the micrometeorite's properties, momentum and kinetic energy, for example. Second, overall determination of the shape of the mass distribution curve strictly requires the use of an experiment with sufficient dynamic range to cover the mass range of interest, in this case from some unknown lower limit up to about 10^{-6} g. Third, the data give no information about any particles which might exist smaller than about 5×10^{-11} g. Although satellite data pertaining to particles down to about 10^{-15} g do exist, none of them met the criteria given earlier so that this mass range should be regarded as unexplored. The Pioneer 8/9 data have been interpreted to show a real mass cutoff at about 5×10^{-12} g (ref. 30). Extension of this observation to the conclusion that no smaller solid particles exist in appreciable numbers in the solar system, however, involves the assumption of a monotonic distribution curve for which there seems to be no good evidence. Consequently it is worthwhile to obtain data on the population of particles in the mass range 10^{-15} g (an arbitrary lower limit) to 10^{-11} g. A fourth observation concerns the comments made earlier about the drawbacks of a distribution curve based on effective rather than real mass. It would seem that, if there are experiments which can give real mass data (plus real velocity and so on), there can be little justification for flying detectors which yield only effective mass data. With these observations in mind, three kinds of experiment are suggested as being potentially useful for increasing knowledge of the micrometeorite mass distribution.

First, the Pioneer 8/9 experiment has been proved to be a system capable of yielding noise-free data on real masses for a small but nonetheless useful mass range. Its only drawback so far seems to be the slow rate at which data are collected, a problem which could be solved by putting more units into space. In particular, a proven unit such as this would seem to be the logical choice for incorporation aboard spacecraft destined to explore new regions of the solar system, such as the asteroid belt. It would be useful to place one or more such units in Earth orbit to check more precisely the apparent absence of a dust belt around the Earth[1,31,32]. At present the experimental evidence depends on comparisons between different experiments.

Second, another kind of remote sensor which will apparently be capable of providing real mass data is the ionization detector which will be discussed in more detail in the next section. Although not a time-of-flight sensor, this detector produces one signal which seems to be a function of velocity only, plus another which is a function of both mass and velocity. Thus a real mass determination is theoretically possible. Such units are being developed in several laboratories, among them the Max-Planck-Institut für Kernphysik at Heidelberg and the University of Birmingham.

Third, the exposure to space and recovery of suitably prepared surfaces provides a method of obtaining a greater dynamic range than either of the foregoing at the expense of reduced resolution of mass and velocity. In such a study, which depends on the microscopic examination of impact sites produced in surfaces by micrometeorites, laboratory simulation experiments[33] have shown that, although the crater volume is a function of both mass and velocity, the diameter-to-depth ratio of the crater is apparently independent of mass and a function only of velocity, thus providing for a unique determination of each parameter. By a suitable combination of electron and optical microscopy, impact sites from less than 100 Å in size to an upper limit fixed only by the thickness of the sampling material or the duration of the exposure can be covered.

It can be concluded that the mass distribution of micrometeorites in our part of the solar system is reasonably well understood and that five to ten years, depending on funding levels, should see the remaining uncertainties resolved.

Composition of Micrometeorites

The determination of the elements or phases which make up micrometeorites is much less satisfactory. If criteria, similar to those of the last section, are applied to past and current experimental work, no firm conclusions can be drawn as to micrometeorite composition. These experiments have fallen into four categories: atmospheric collections (refs. 34 and 35 and paper by Brownlee and Hodge at Meteoritical Society Meeting 1969), rocket collections[36–38], recoverable spacecraft collections[22,38,39], and analysis of crater residues in recovered surfaces[40,41].

The first two categories suffer from the problems of contamination referred to previously. These are sufficiently severe to make identification of each micrometeorite impossible unless some intrinsic property is taken as proof of extraterrestrial origin. Clearly such an identification process automatically biases the results. Collection experiments mounted aboard recoverable spacecraft have been unsuccessful because the high velocity of cosmic particles has prevented them from surviving impact with the collection surface. In the case of a solid surface this impact produces a crater, and in the fourth category of experiment the electron microprobe has been used to analyse the residue left inside such a crater[40,41]. Although this technique has yielded semiquantitative compositional data on the material in the Gemini S–010 craters referred to earlier[41], some degree of fractionation probably occurred during the impact volatilization and consequently the residual composition is unlikely to be that of the original particle. The technique is of course blind to the elements making up the original surface.

So far no remote sensors have been flown capable of yielding compositional information. The next few years, however, should see the introduction of systems designed to provide *in situ* analyses of micrometeorites. The most promising of these are the ionization detectors referred to previously. In this technique a plasma pulse is produced at the site of a micrometeorite impact, the energy for

plasma formation being provided by the particle's kinetic energy. A system of electrodes close to the target surface separates ions from electrons in the plasma and the ions are then passed down a time-of-flight mass spectrometer[42].

Origin of Micrometeorites

There are three obvious sources for the production of interplanetary dust particles: comets, asteroids and interstellar dust. The objective of micrometeorite orbital determinations is to distinguish between them. Theoretical considerations have suggested that comets are the dominant source of micrometeorites in the solar system[3,4].

Zodiacal light isophots can be analysed in terms of an average value for the inclinations of the scattering particles[2]. This analysis is less dependent on assumptions as to the shape, albedo, and so on of the particles than is the mass distribution discussed earlier. A mean value of 30° has been derived for the inclination of the zodiacal particles, significantly greater than the mean for either short period comets (15°) or asteroids (9·7°). This suggests the existence of an efficient perturbing force acting on particle inclinations[2].

Although a single orbital determination on a meteorite, especially if taken in conjunction with a cosmic ray exposure age, may be of considerable genetical significance, in the case of a micrometeorite its greatly increased probability of orbital perturbation drastically reduces the significance of its orbit at any moment in time. The only meaningful way to obtain such data is to search for statistical trends among large numbers of orbital determinations and at present such determinations are few in number. The only directly measured orbital parameters have been obtained by Pioneer 8/9 (ref. 30). Although preliminary, these data have been interpreted as supporting a cometary origin. Two of the eight orbits so far coincide with actual comet orbits. Because of the low inclination of the spacecraft orbit the observations are biased towards micrometeorites in low inclination orbits. Considering the other measured parameters, however, the data are in agreement with meteor observations which have suggested positive correlations between particle mass on the one hand and semi-major axis, eccentricity and perihelion distance on the other[5,30,43,44]. It is clear, though, that more data are required before firm conclusions can be drawn regarding the original orbits of micrometeorites.

Little to Show so far

In spite of the expenditure of considerable time and money during the past fifteen years, the corpus of knowledge concerning the micrometeorite population in our part of the solar system is disappointingly small. Several factors might be responsible for this state of affairs. First, the design and construction of reliable, low-noise detection systems require the marriage of a number of sophisticated disciplines not often to be found in one laboratory.

Second, the importance of hyper-velocity calibration was late in being appreciated and it is now apparent that such calibration procedures are not only technologically difficult but also extremely expensive. A Van de Graaff accelerator equipped with adequate particle injection and filtering devices costs virtually as much as a small satellite.

Third, most attempts at direct measurement of micrometeorite flux have been influenced by the desire to provide estimates of spacecraft hazard. Consequently the emphasis has been on acquiring technical performance parameters which have subsequently been interpreted, with difficulty, to yield some scientific data concerning the micrometeorites themselves. It is obvious that, had the experiment been designed originally to provide scientific parameters such as mass and velocity, these could have readily been converted into estimates of spacecraft puncture probability.

It is to be hoped that the rather poor returns in this field so far do not inhibit further investment just at a time when adequately sophisticated experimental techniques are becoming available.

The study described here was performed while I was at the Dudley Observatory, Albany, New York. I thank the director, Dr C. L. Hemenway, for his encouragement and NASA for a research grant.

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Cooperation of Immune Lymphoid Cells with Macrophages in Tumour Immunity

by

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An immunologically specific cellular cooperation may resolve the paradox that immune spleen cells injected into normal mice prevent the growth of lymphoma cells *in vivo* but fail to do so *in vitro*.

CELLS of the L5178Y murine lymphoma carry tumour-specific transplantation-type antigens in their plasma membrane, and syngeneic DBA/2 mice become resistant to challenge by this tumour after a single immunization with L5178Y cells which have been rendered incapable of growth by exposure to X-rays[1,2]. This resistance can be transferred to untreated mice by spleen[1] and lymph node[3] cells from immune mice, but when tested *in vitro* neither the serum nor the spleen cells from such immune mice are cytotoxic or growth inhibitory to L5178Y cells. Inability to detect *in vitro* the direct cytotoxic action of syngeneic spleen cells is probably not the result of a technical failure, for the cytotoxic action of allogeneic immune spleen cells (for example, from C57/Bl or CBA mice immunized with L5178Y cells) has been readily demonstrated *in vitro*[1,4,5] and indeed the L5178Y system is one of the most convenient for studying the activity of cytotoxic lymphoid cells. The apparent paradox that immune syngeneic spleen cells can transfer activity *in vivo* but are inactive by themselves *in vitro* suggests the possibility that to destroy the L5178Y cells in the syngeneic system the immune lymphoid cells must act in cooperation with another cell. In an analogous situation in which resistance to infection by *Listeria monocytogenes* was transferred to recipient mice by spleen cells from mice immunized against the bacterium, the evidence suggested an interaction between lymphoid cells and macrophages, the latter providing the mechanism by which resistance was expressed[6,7].

We here describe experiments intended to demonstrate, first, that syngeneic peritoneal macrophages from mice immunized against L5178Y cells exert a cytotoxic effect on lymphoma cells *in vitro* and, second, that normal syngeneic macrophages may be rendered cytotoxic towards lymphoma cells following incubation with immune spleen cells.

Peritoneal exudate cells were obtained from DBA/2 mice by syringing out the cavity with Fischer's medium[1]; cultures were prepared by seeding approximately 2×10^6 macrophages into 3·5 cm Falcon plastic Petri dishes. After 15 min the medium was decanted and 2 ml. of Fischer's medium supplemented with 10 per cent heat-inactivated foetal bovine serum (FM) was added. Cultures were then incubated for 2 h, after which most of the lymphocytes present were removed by intensive washing of the monolayer with jets of tissue culture fluid. At this stage lymphocytes constituted less than 3 per cent and granulocytes—for example mast cells—less than 0·1 per cent of the culture.

Fig. 1 illustrates the growth curves of $5{\cdot}0 \times 10^4$ L5178Y cells—as determined by counting in a haemocytometer—in 2 ml. of FM when placed on such monolayers of macrophages. Although macrophages from non-immune mice were without effect on their growth, cell division was completely inhibited in the presence of immune macrophages, and the lymphoma cells began to disintegrate after 1 day. In this experiment the ratio of macrophages to L5178Y cells was 40 : 1 but effective cytotoxicity was also observed at ratios as low as 10 : 1 (see Fig. 2).

We summarize the characteristics of this reaction as follows: (1) it is immunologically specific and macrophages from DBA/2 mice immunized with BCG, with another lymphoma or after stimulation with Difco thioglycollate medium, were without effect on growth of the lymphoma. (2) The addition of complement from rabbit and rat sera was without effect. (3) The lymphoma cells were killed apparently by cell contact with the macrophages (a similar type of interaction was first described by Granger and Weiser[8] in an allogeneic system) and a confluent monolayer was essential for maximum inhibition of growth of lymphoma cells; phagocytosis only occurred after 24 h when the cells began to degenerate and in this respect this system is quite different from the intracellular killing of tumour cells by macrophages reported by Bennett, Old and Boyse[9] which required an opsonizing antibody. (4) The cells had to be in contact with the immune macrophages to be irreversibly damaged; for example, after 4 and 24 h, lymphoma cells washed off macrophage cultures grew normally when transferred into fresh dishes. (5) The medium recovered from cultures of immune macrophages previously exposed to lymphoma cells had no effect on the growth of lymphoma cells. (6) The number of contaminating lymphocytes was reduced to less than 1 per 1,000 macrophages by irradiating the monolayers of peritoneal cells with 400 r of X-rays *in vitro* before adding lymphoma cells. (The purified macrophage monolayers obtained in this way were fully cytotoxic.)

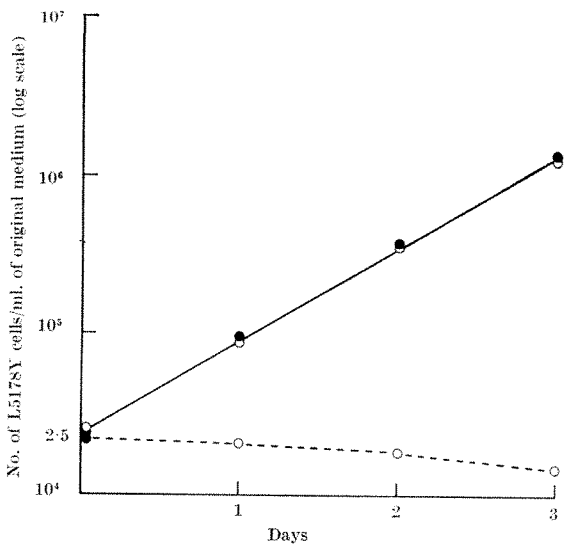


Fig. 1. Effect of immune macrophages on growth of lymphoma L5178Y cells *in vitro*. The culture medium was supplemented at daily intervals. ○—○, No macrophages; ○ - - - ○, immune macrophages; ●—●, non-immune control macrophages.

(7) Cytotoxicity was not reduced when the contaminating mast cells were removed by exposing the peritoneal exudate cells to 1·0 μg/ml. of the polyamine '48/80' (Burroughs Wellcome) which lyses mast cells[10]. (8) Monolayers of immune macrophages retained their cytotoxic action if maintained for 2 days but not if they were maintained for 7 days in culture before the lymphoma cells were added. (9) Macrophages were cytotoxic if removed from mice at any time between 3 and 21 days after two injections of irradiated cells or between 3 and 40 days after three of four injections, but after single intraperitoneal immunization with 5×10^6 irradiated lymphoma cells peritoneal macrophages were cytotoxic only if removed on day 11, and were not so if taken on days 7 or 14 after immunization. (10) By injecting thioglycollate medium 6 days before removing the peritoneal exudate cells from hyper-immunized mice, the number of macrophages in the peritoneal cavity was increased five to ten times but in spite of the greater number of macrophages which became available in this way the cells retained their full cytotoxic effect; that is, the exudate from immunized animals was not diluted and the thioglycollate medium induced the appearance of more cytotoxic macrophages. (11) Finally, monolayers of immune macrophages after they had been in contact for 1 day with lymphoma cells lost their cytotoxicity and did not retard the growth of a second inoculum of lymphoma cells.

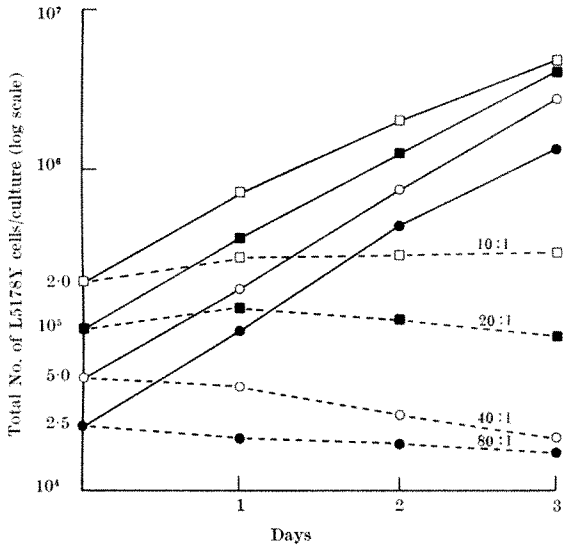


Fig. 2. The relationship between growth inhibition by immune macrophages and the inoculum of L5178Y cells. The number of macrophages remained constant: 2×10^5 per dish.

As already mentioned, spleen cells from DBA/2 mice immunized with irradiated lymphoma cells have no effect on the growth of lymphoma cells *in vitro* even when added to ratios as high as 1,000 : 1 (ref. 1 and footnote*). But the addition of such spleen cells to normal non-immune, peritoneal macrophages renders these cytotoxic. Fig. 3 illustrates an experiment in which lymphoma cells failed to grow, and subsequently degenerated, in normal macrophage cultures which had been incubated for 24 h with immune spleen cells and then thoroughly washed before the addition of lymphoma cells. Neither the non-immune macrophages nor the immune spleen cells by themselves were cytotoxic. Thus contact with immune spleen cells bestowed on peritoneal macrophages the capacity to inhibit growth of lymphoma cells, and

* Because such immune spleen cell suspensions contain as many as 5–10 per cent macrophages their failure to inhibit growth may correspond to the need of the target cells to meet a confluent monolayer or to a qualitative difference between spleen and peritoneal macrophages. In an allogeneic combination when immune spleen cells are cytotoxic *in vitro* the macrophages present do not contribute to this reaction[5].

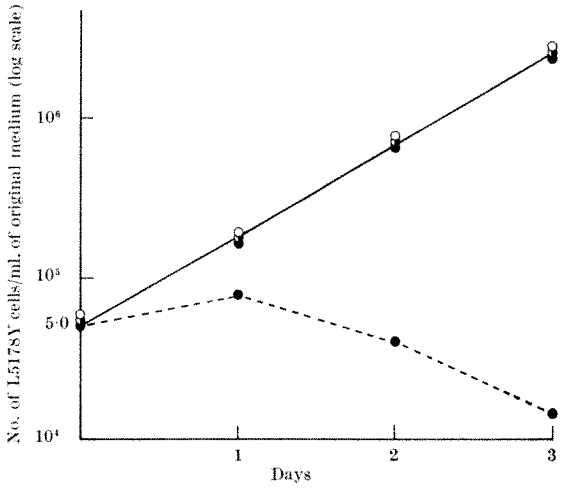


Fig. 3. The effect on growth of L5178Y cells by non-immune macrophages pre-incubated with immune spleen cells (5×10^6 pre-incubated for 24 h with non-immune macrophages and then washed off). ○—○, Culture medium only; ◑—◑, immune spleen cells only; ●—●, non-immune control macrophages; ● - - - ●, non-immune macrophages + immune spleen cells.

such "armed" macrophages could not be distinguished in this test from peritoneal macrophages obtained from immunized mice. This phenomenon is immunologically specific and has only been obtained with spleen cells from DBA/2 mice immunized with L5178Y cells. These experiments may resolve the paradox that immune spleen cells when injected into normal mice are able to prevent the growth of lymphoma cells *in vivo* but by themselves are unable to do so *in vitro*.

We are trying to determine the nature of the interaction between the spleen cells and the peritoneal macrophages and in particular to discover if transfer of an antibody-like molecule is involved. Serum from mice immunized with irradiated L5178Y cells as well as the supernatant from cultures of immune spleen cells has so far failed to render peritoneal macrophages from non-immune animals cytotoxic. The failure to find an immunologically specific humoral factor in these experiments does not exclude the possibility that cytophilic antibody produced by spleen cells is involved (see ref. 11). If such antibody has a very high affinity for peritoneal macrophages or their precursors it may be taken up so effectively *in vivo* that the amount which remains in the circulation is too low to be detected.

In view of the inability of either immune spleen cells or non-immune peritoneal macrophages to affect growth *in vitro* of lymphoma cells, the syngeneic system we have studied made it possible to demonstrate very clearly co-operation between these two components leading to a cytotoxic reaction. The same phenomenon probably also occurs in the rejection of some allografts, but because the immune lymphoid cells are toxic by themselves to allogeneic target cells an enhanced effect resulting from cooperation with macrophages will be more difficult to recognize.

These experiments may have some bearing on immunotherapy of tumours. The growth of tumours, in spite of the fact that they are immunogenic, has been attributed to a failure of the host to produce sufficient cytotoxic lymphoid cells[12,13] and attempts at immunotherapy have been designed to overcome this defect[14]. If immune lymphoid cells cooperate with macrophages, in some conditions availability of the latter may determine whether host defences fail or succeed in containing the tumour. Recent observations in man[15] and in guinea-pigs[16] that some skin tumours regress as a by-product of a non-related delayed hypersensitivity reaction could be interpreted in this way, as might the regression of dermal deposits of malignant melanoma following inoculation with vaccinia virus[17].

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Light Inactivation of Focus Formation by Chicken Embryo Fibroblasts infected with Avian Sarcoma Virus in the Presence of 5-Bromodeoxyuridine

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When RNA tumour viruses infect cells growing in the presence of 5BUdR, exposure to visible light inactivates the virus. This confirms that synthesis of DNA from viral RNA is necessary for viral transformation.

THE RNA tumour viruses (leucoviruses) seem to have a different mode of replication from other RNA-containing viruses. The replication of RNA tumour viruses is sensitive to inhibitors of DNA synthesis early in infection[1,2]; virus production by converted cells is sensitive to actinomycin D (refs. 2 and 3); and viral RNA seems to have a greater homology with DNA from converted cells than with DNA from uninfected cells[4,5]. These observations are summarized in the DNA provirus hypothesis[6]: following infection, the viral RNA is transcribed into DNA and maintained as DNA in subsequent cell generations.

Cells grown in the presence of 5-bromodeoxyuridine (5BUdR) incorporate this analogue into their DNA. This substituted DNA is more sensitive to inactivation by ultraviolet and short wavelength visible light than is unsubstituted DNA[7,8]. Kao and Puck[9] used this sensitization to select against animal cells which could undergo DNA synthesis in deficient media.

The DNA provirus hypothesis predicts that a new DNA is formed soon after infection. If this new DNA could be labelled with 5BUdR, the infection could be aborted by visible light. In addition, the 5BUdR might provide a means to separate the new DNA from cellular DNA, and thus aid in its isolation. To avoid killing cells by the exposure to light, the incorporation of 5BUdR into essential cellular DNA must be reduced. This reduction can be accomplished by maintaining the cells for several days in medium without serum, which collects the cells in the G_1-phase of the cell cycle[10–12] and reduces the number of cells entering S-phase (nuclear DNA synthesis) to 1 to 5 per cent over a 24 h period. (A similar approach is being used by Balduzzi and Morgan, personal communication.)

In preliminary experiments, stationary cultures of chicken embryo fibroblasts were infected with 100 to 1,000 focus-forming units (f.f.u.) of the avian sarcoma virus, B77, in the presence of 5BUdR (ref. 6). Focus formation was inactivated in proportion to the amount of subsequent exposure to visible light following 5BUdR treatment. (Earlier experiments suggested that treatment of stationary cultures of chicken cells with 5BUdR immediately after infection inhibited focus formation[12] but we did not observe this. The two experiments differed because here the serum-containing media were added to the cultures immediately after the removal of the 5BUdR. In the earlier experiments, there was a 24 h delay before addition of serum. It has been shown (unpublished results of D. B.) that there is an exponential loss of focus formation ($t_{\frac{1}{2}} = 9{\cdot}5$ h) during the interval between removal of 5BUdR and addition of serum.)

In these experiments, this technique was modified. Stationary cultures of chicken embryo fibroblasts were exposed to virus, treated with 5BUdR, exposed to light, and replated on a feeder layer of rat cells which are resistant to infection. This assay system gives a linear relationship between the number of foci produced and the multiplicity of infection (at multiplicities of infection of less than 0·1 f.f.u./cell (unpublished results of D. B.)[13]). For a cell to register as a focus in this assay, it must (*a*) contain non-inactivated viral genetic information, and (*b*) be capable of division and conversion. At a multiplicity of infection of 0·1 f.f.u./cell, the inactivation of focus formation by light followed single hit kinetics (Fig. 1). Essentially the same curve of inactivation by light was obtained using concentrations of 5BUdR from 2–500 μg/ml. in the post-infection incubation medium. Similar curves were obtained for cells infected with the B77 or the Schmidt–Ruppin strains of avian sarcoma virus.

In all systems where it has been examined, the effects of 5BUdR result from its incorporation into DNA in place of thymidine[8,14,15]. ^{3}H-5BUdR was used to measure the incorporation of 5BUdR into DNA. Cultures of stationary or dividing chicken cells were incubated for 20 h in medium with or without serum containing 1 μCi of ^{3}H-5BUdR. Nucleic acid was separated by a modified Schmidt–Thannhauser procedure. Less than 1 per cent of the perchloric acid precipitable counts were hydrolysed after incubation for 20 h at 37° C in 0·3 M KOH. Thus less than 1 per cent of the incorporated 5BUdR seems to be associated with RNA. Bader has reported a similar result for dividing chicken cells[16]. Also the light sensitization of focus formation induced by 5BUdR was inhibited when thymidine was added to the 5BUdR-containing medium. The 5BUdR sensitization observed in these experiments is therefore probably a direct result of incorporation into DNA.

Table 1. EFFECTS OF 5BUdR

Experiment	5BUdR (per cent)	5BUdR + 30 min exposure to light (per cent)
1. Initiation of DNA synthesis*	80·8	78·2
2. Initiation of cell division†	25	25
3. Relative plating efficiency of cells infected after 5BUdR treatment‡	100	88

* Cultures of stationary cells, infected with 3 f.f.u./cell, treated with 5 μg/ml. 5BUdR and exposed to light were incubated in ^{3}H-thymidine and serum 0–20 h following exposure to light; autoradiograms were made and labelled nuclei counted.

† Cultures of stationary cells were infected, treated with 5BUdR, and exposed to light. Serum was added. 17 h later 5×10^{-7} M colcemid was added; after an additional 8 h, cells arrested in metaphase were counted by phase-contrast microscopy.

‡ As described in the text except that the cells are infected after removal of 5BUdR and before exposure to light.

There are several explanations to account for the inactivation of focus formation by visible light after infection in the presence of 5BUdR: first, the infected cells incorporate 5BUdR into essential cellular DNA and subsequent exposure to light prevents them from dividing; second, some particular fraction of cellular DNA which is essential for cell conversion is replicated on infection, incorporates 5BUdR, and is inactivated by the exposure to light; or third, the virus genetic information is transcribed to a 5BUdR containing DNA which is inactivated on exposure to visible light. Experiments were done to test these hypotheses.

The first hypothesis was examined as shown in Table 1. The ability of cells to respond to the addition of serum by initiating DNA synthesis as examined by autoradiography or to divide as examined by counting cells arrested in metaphase by colcemid was not significantly altered by the exposure to light. When the infection is delayed until after 5BUdR treatment there is no significant change in plating efficiency after exposure to light. The lack of cell killing in these experiments (Table 1) results from the use of cultures of stationary cells in which only 1–5 per cent of the cells pass through S-phase DNA synthesis and incorporate enough 5BUdR to become sensitive to visible light. Thus cell killing and differences in plating efficiency between the cells exposed to light and those unexposed to light could account for only a small portion of the light inactivation of focus formation observed in Fig. 1.

To test the possibility that only potential focus-forming cells incorporated 5BUdR and were killed by light, the sensitivity to light of cells containing 5BUdR substituted cellular DNA was measured. Cells were exposed to 5BUdR in the presence of serum so that 5BUdR would be incorporated into cellular DNA. These cells were infected either before or after the exposure to 5BUdR. The fraction of cells able to produce foci after exposure to light was measured (Fig. 2). The inactivation followed single hit kinetics with a resistant fraction of 1–2 per cent. In another experiment cells were exposed to 5BUdR and serum for 3 days preceding infection. The resistant fraction was reduced to less than 0·1 per cent. The resistant fraction represents those cells which did not incorporate 5BUdR. The inactivation measured is the result of cell killing. The initial slope of the cell killing curve is about twenty-fold greater than that for focus formation (Fig. 1). This difference in slope demonstrates that, when stationary cultures are used, the inactivation of focus formation is not a result of cell killing. In addition the difference in slope means that there is no significant stimulation of S-phase DNA synthesis by infection with avian sarcoma viruses. The stimulation of DNA synthesis which has been reported[17] is probably an early effect of cell conversion.

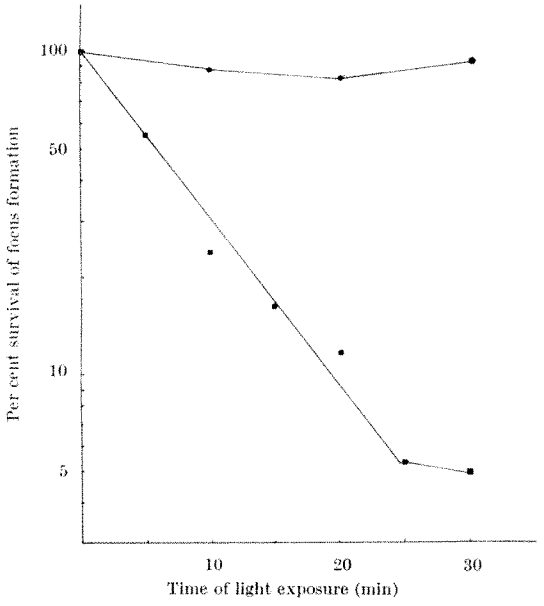


Fig. 1. Inactivation of focus formation by visible light. The protocol is explained in the text. Infection was carried out with about 8×10^4 f.f.u./culture of B77 virus and the cultures were overlaid with Eagle's medium with (■) or without (●) 5BUdR (5 μg/ml.). The cultures were exposed to light and replated on rat cells. In all cases, points represent the average of counts of duplicate cultures.

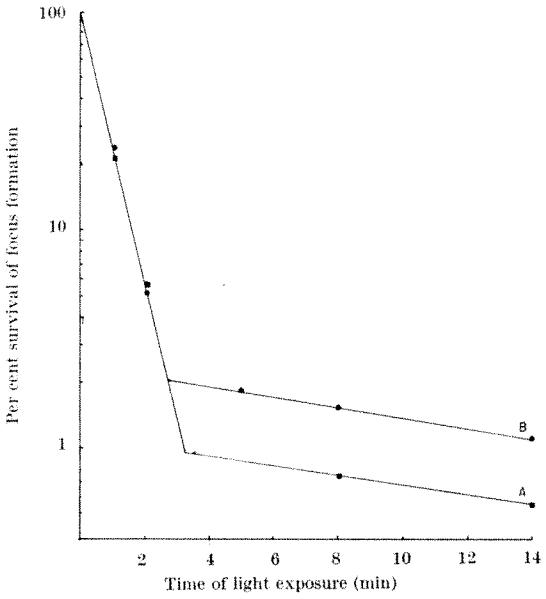


Fig. 2. The inactivation by visible light of the capacity of cells labelled with 5BUdR to produce foci. Cultures of stationary cells were either infected with 8×10^4 f.f.u. of B77 virus and incubated with 5 μg/ml. 5BUdR and 4 per cent foetal calf serum for 22 h (*A*), or incubated in the same medium and infected after incubation (*B*). The same protocol described in the methods section was followed from this point.

The difference between the second and third hypotheses is whether the new DNA is replicated from cellular DNA or whether it is transcribed from viral RNA. To distinguish between these possibilities, cultures of stationary chicken cells were infected at multiplicities of greater than 1 f.f.u./cell. If the new DNA is transcribed from viral genes, increasing the multiplicity of viral genes present in a cell might increase the number of copies of the new DNA; and the cell's focus-forming potential might be more resistant to light inactivation than that for a cell with a single copy of the new DNA. If the new DNA is cellular, the number of copies of the genes per cell would be independent of the multiplicity of infection, and the rate of focus inactivation by light would be the same at all multiplicities of infection. Parallel cultures of stationary chicken cells were infected with Schmidt–Ruppin virus at 3 f.f.u./cell or 0·15 f.f.u./cell in the presence of 5BUdR and exposed to light (Fig. 3). Focus production in cells infected at the higher multiplicity was more resistant to inactivation by light than it was for cells infected at the low multiplicity. Thus it seems that the new DNA is transcribed from viral genes.

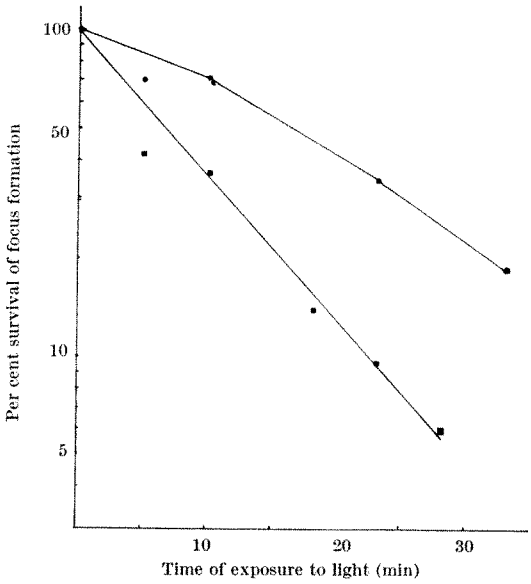


Fig. 3. The effect of multiplicity of infection on light sensitization caused by 5BUdR. Methods are described in the text. Cultures were infected with either 3 f.f.u./ml. (●) or 0·15 f.f.u./ml. (■) of the Schmidt-Ruppin virus and overlaid with medium containing 50 μg/ml. 5BUdR.

This experiment also serves as a further control for cell killing. If the light inactivation curves were the result of cell killing, increasing the multiplicity of infection should either increase the amount of cell killing or it should remain the same. But inactivation of focus formation is actually reduced by increasing the multiplicity of infection. This result is incompatible with the cell killing hypothesis.

The requirement for DNA synthesis early in infection of chicken cells by avian sarcoma viruses has been well documented[1,2,6]. There seem to be two parts to this requirement for DNA synthesis. In addition to the requirement for normal S-phase DNA synthesis and cell division[18], there is a requirement for non-S phase DNA synthesis. This non-S phase DNA synthesized following infection can be selectively labelled with 5BUdR using cultures of stationary cells. Subsequent exposure to visible light demonstrates that the non-S phase DNA required for focus formation is less sensitive to light than 5BUdR labelled S-phase DNA. The controls show that the inactivation of focus formation by light exposure is not the result of cell killing when the 5BUdR treatment is done in cultures of stationary cells.

The non-S phase DNA synthesis could involve the selective replication of cellular genes required for infection by avian sarcoma viruses, but the dependence of this non-S phase DNA synthesis on the multiplicity of infection makes this hypothesis less likely. Furthermore, because the DNA synthesized following infection by avian sarcoma viruses is specific for the viral strain used in the infection[6,19], a different set of cellular genes would be required for each strain of avian sarcoma virus.

These results are consistent with the model that the required S-phase DNA synthesized contains the genetic information for continued cell growth and division, and the required non-S phase DNA synthesized represents viral genetic information transcribed from viral RNA to a DNA intermediate.

Cultures and Irradiation

A modified Eagle's minimum essential medium (E) (obtained as a powder from Schwarz BioResearch, Orangeburg) was used. ET medium was made by adding 20 per cent tryptose phosphate broth (Difco Laboratories; Detroit, Michigan). The medium was supplemented with calf serum (Microbiological Associates) and/or foetal calf serum (Grand Island Biological Co.) as noted.

Primary cultures were made from 11–13 day old chicken embryos[20] (White Leghorn eggs were obtained from Sunnyside Hatchery, Oregon). Cultures containing $7{\cdot}5 \times 10^5$ cells were prepared in 60 mm Petri dishes (Falcon Plastics) in ET medium using cells from the first to fifth transfer generation. The medium was changed to fresh ET one day after preparation and the cultures were infected after 2 days of further incubation. Rat cell primary cultures were prepared from 12–14 day old embryos. Feeder cultures containing 5×10^5 cells were prepared in 60 mm Petri dishes in ET media supplemented with 4 per cent foetal calf serum.

B77 and Schmidt-Ruppin stocks have been described. High multiplicity of infection experiments used virus concentrated 100× by centrifugation[21]. Chicken cell cultures were exposed to 0·2 ml. virus, incubated for 40 min at 38° C, and overlaid with Eagle's medium containing 5BUdR (Calbiochem) or other analogues as indicated. After incubation for 18–22 h, the cultures were washed and overlaid with Tris buffered saline (0·14 M NaCl, 0·005 M KCl, 0·00033 M Na_2HPO_4, 0·025 M Tris, 0·0005 M $MgCl_2$, 0·001 M $CaCl_2$), exposed to the light source, resuspended with the aid of trypsin, diluted, and replated on previously prepared rat cell feeder cultures. 12–18 h later the medium was replaced with ET medium containing 0·6 per cent agar, 4 per cent calf serum and 4 per cent foetal calf serum. Foci were counted 6–8 days later. Counts on duplicate cultures differed by less than 20 per cent.

Cultures in Tris buffer were exposed from the underside at 6 cm distance to a light source consisting of two Westinghouse FS20 fluorescent sun lamps. This lamp emits a peak efficiency at 310 nm. Because irradiation from this source was found to cause considerable damage to the cells, filters consisting of 225 ml. tissue culture flasks (Falcon Plastics) filled with 0·3 mg/ml. thymidine (path length = 3 cm) were interposed. When this filter was used very little damage was observed to control cells which contained no 5BUdR.

Following incubation with ^{3}H-thymidine the cells were fixed with ethanol-acetic acid (3 : 1), washed with water, air-dried, coated with 'NTB3' nuclear track emulsion (Kodak) and exposed for 7 days at 4° C. The cultures were developed and stained with Harris's haematoxylin. For determination of 5BUdR incorporation into RNA, cells incubated in medium without serum containing 1 μCi of ^{3}H-5BUdR (Schwarz BioResearch, 10·6 Ci/mmole) were sonicated for 45 s and divided into two aliquots. Total incorporation was measured by precipitating twice with 1·5 per cent perchloric acid (PCA), resuspending in 0·3 M KOH and counting in 10 ml. of 'Scintisol' (Isolab). RNA incorporation was measured by precipitating with 1·5 per cent PCA + 100 μg bovine serum albumin, filtering on Whatman 'GF/A' filters, incubating at 37° C for 20 h in 0·3 M KOH, reprecipitating the supernatant with 1·5 per cent PCA and counting the remaining supernatant in 10 ml. of 'Scintisol'.

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Theory for the Mechanism of Chloroquine Resistance in Rodent Malaria

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The appearance of succinate dehydrogenase in the chloroquine resistant parasite suggests that resistance is acquired by a switch in the respiratory pathway to the citric acid cycle.

MITOCHONDRIA of the rodent malaria parasite *Plasmodium berghei berghei* undergo a metamorphosis in their morphology and physiology during the parasite life cycle[1]. This observation prompted us to consider whether the physiological lability of the parasite could play a part in its ability to develop resistance to chloroquine.

References to the action of chloroquine on plasmodial and other cells have been reviewed by Peters[2,3] and Aviado[4]. The literature indicates that chloroquine acts at all cell sites so far examined, by binding to RNA, DNA and cell membranes, by inhibiting hydrolytic and respiratory enzymes and by being concentrated in the lysosomes. In malarial therapy, however, it has been found that the drug is effective on the intraerythrocytic trophozoite stages but is ineffective against the exo-erythrocytic schizonts and the mosquito-borne sporogonic stages. We considered that, because chloroquine has been shown to bind to double-stranded bacterial DNA[5], to mammalian DNA and to polyadenylic and polyguanilic acids[6] and to inhibit DNA synthesis in plasmodia and bacteria[7,8], it is improbable that the DNA of the insensitive stages, or of chloroquine-resistant trophozoites of *P. berghei*, would be sufficiently different from that of the chloroquine-sensitive trophozoites to confer resistance. It has also been shown that chloroquine inhibits plasmodial hexokinase[9], but because the exo-erythrocytic schizont seems to be essentially similar to the erythrocytic trophozoite in its respiratory activity[1,10] and the former is insensitive to chloroquine we considered it unlikely that the inhibition of hexokinase is the primary action of chloroquine.

P. berghei and *P. knowlesi*[11] digest haemoglobin and several workers[12,13] have shown that the latter derives adequate supplies of most amino-acids from this source. *P. berghei*[14,15], *P. knowlesi*[16] and the avian malarias[17] ingest haemoglobin and degrade it in the phagosomes so formed. Chloroquine rapidly causes a clumping of the phagosomes (haemozoin vesicles) and later their expulsion from the parasite (reviewed by Peters[3]). Cessation of haemoglobin digestion must be related to the clumping and expulsion of the phagosomes and we therefore consider it likely that amino-acid starvation, resulting from the inhibition of haemoglobin degradation, is the primary means whereby chloroquine is effective against the trophozoite stages of the malarial parasites.

Chloroquine sensitive rodent plasmodia accumulate greater concentrations of the drug than chloroquine resistant parasites[18–20] and Macomber *et al.*[18] have suggested that chloroquine resistance may be based on an impairment of the mechanism by which the drug is accumulated. Fitch[20] has proposed that the major difference in the drug levels attained in sensitive and resistant parasites is "due to a decrease in the number, affinity or accessibility of chloroquine receptor sites" in the plasmodium. Our present observations suggest that these differences, though closely associated with the phenomenon of chloroquine resistance, are secondary to a more fundamental change in the parasite's physiology.

Because the trophozoites of chloroquine sensitive *P. berghei* lack a citric acid cycle[21,22] but the sporogonic stages may utilize this pathway[1] we postulated that chloroquine resistance in the trophozoite stages could be acquired by a simple switch to citric acid cycle activity and the synthesis of amino-acids by transamination of intermediates of this cycle.

As a preliminary step in examining this hypothesis we have investigated the occurrence of succinate dehydrogenase (SDH) in chloroquine sensitive and chloroquine resistant lines of *P. berghei*.

The lines of the parasite used in this study were the parent drug sensitive Keyberg 173 (N) strain and two others derived from it, the chloroquine resistant RC line[23] and the chloroquine resistant NS line[24]. The RC line differs from the N strain in being an obligate parasite of reticulocytes and in lacking haemozoin, whereas the NS line possesses haemozoin and, developing readily in normocytes, induces a rapidly fulminating infection similar to that of the parent N strain. The rodent hosts were *Eperythrozoon* and *Haemobartonella*-free, male TFW mice weighing about 20 g. SDH activity was located by the nitro-blue tetrazolium technique described by Pearse[25]. The following experiments were performed and repeated a minimum of ten times with each strain studied: (1) Blood-films were air-dried and unfixed; (2) blood films were quenched in acetone cooled in liquid nitrogen; (3) blood films were fixed for 5 min in 5 per cent formalin in 0·05 M phosphate buffer *p*H 7·4 at 0° C; and (4) heat denatured blood films were incubated in the following test and control media: (*a*) test medium; (*b*) medium lacking succinate; (*c*) medium containing 0·05 M sodium malonate; (*d*), (*e*) and (*f*) as (*a*), (*b*) and (*c*) respectively but containing $2{\cdot}5 \times 10^{-4}$ M phenazine methosulphate (PMS). Films were incubated for 30, 60 and 120 min at 37° C in air. In some experiments cryostat-cut sections of unfixed mouse liver were incorporated as positive controls. The ferricyanide technique of Kerpel-Fronius and Hagos[26] for the localization of SDH activity was unsuitable for the present study, for the ferricyanide was readily reduced by the mouse haemoglobin.

The results of experiments with the N strain confirmed those of Howells[1]. No demonstrable SDH activity could be observed in these parasites (Fig. 1). Following induction of reticulocytosis by administration of phenylhydrazine to mice and subsequent infection the enzyme activity in the reticulocytes was apparent as dots scattered through the cell and did not resemble the distribution observed in RC line infected reticulocytes. N strain parasites in reticulocytes were sensitive to chloroquine

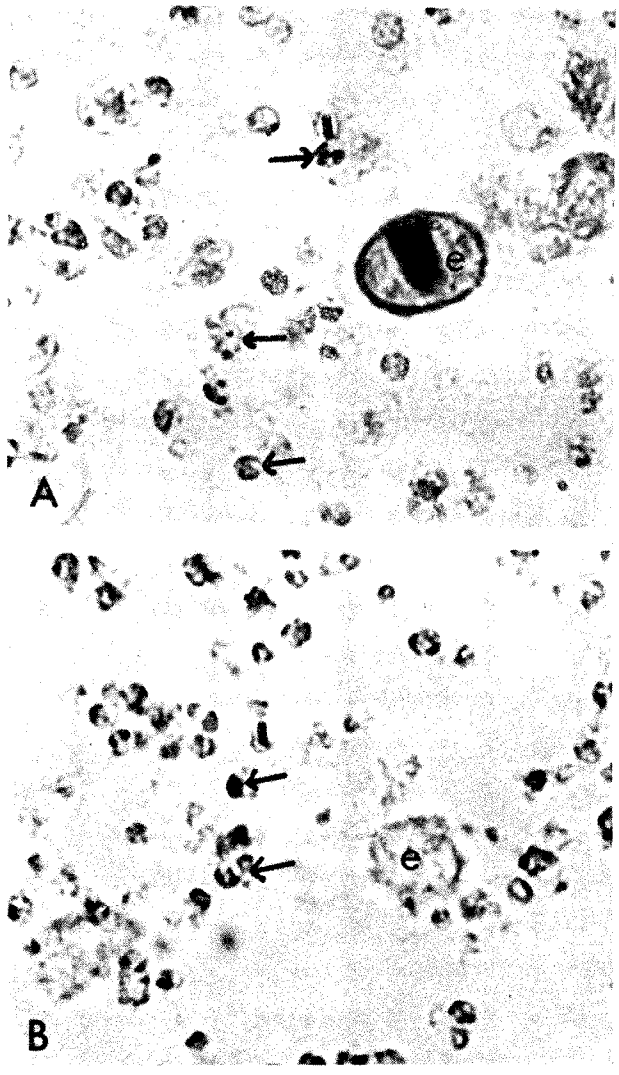


Fig. 1. Films of mouse blood infected with *P. b. berghei* N strain (×1,800). *A*, Film incubated in test medium for SDH determination. Note deposits of formazan in cytoplasm of leucocyte (e) and the dense appearance of the pigmented malarial trophozoites. In the preparation these parasites do not appear to contain a blue formazan deposit. *B*, Film incubated in test medium for SDH determination and sodium malonate. Note lack of formazan deposit in leucocyte. Trophozoites contain granules of pigment.

and did not possess SDH activity, even after chloroquine exposure.

Results with the RC line indicate that erythrocytic forms of the parasite possess a demonstrable SDH activity (Fig. 2). This activity, which was localized within the parasite, was malonate and heat sensitive and dependent on exogenous succinate. Unfixed, air-dried blood films gave a strong activity but a considerable loss of cells occurred from such slides during incubation. Fixing the films in acetone at room temperature caused a loss of enzyme activity, possibly intensified by loss of coenzyme Q from the tissues[27], but enzyme activity was preserved by quenching in cold acetone. This technique also prevented cell loss. SDH activity was located most accurately by the formalin fixed slides because little or no non-specific absorption of formazan to cell membranes occurred in these preparations.

Initial experiments on 5 day old infections of the NS line in undrugged mice indicated that the pigmented, normocyte dwelling parasites lacked SDH activity. Further tests on 18 to 21 day old infections in undrugged mice gave positive results. At this stage of the infection the parasites were apigmented and occurred in reticulocytes. The effect of chloroquine on the development of the parasitaemia in the NS line was investigated by Peters *et al.*[24]. These authors demonstrated that though the parasites invade normocytes during the early stages of



Fig. 2. Films of mouse blood infected with *P. b. berghei* RC line (×1,800). *A* and *B*, Film incubated in test medium for SDH determination. Note deposits of formazan in leucocyte (e) and in the malarial trophozoites (arrows). *C*, Film incubated in test medium for SDH determination and sodium malonate. Note absence of pigment and formazan from these para sites (arrows).

infection in undrugged mice, if chloroquine is administered to mice at the time of infection the parasites invade immature red cells for the first few days, but later invade normocytes. The SDH activity of parasites in drugged mice was therefore investigated. Parasites maintained under drug pressure from day zero possess SDH activity but are restricted to reticulocytes and lack pigment. If the parasitaemia is allowed to develop without chloroquine and the drug administered on day 4 (Fig. 3), within 24 h a 60 per cent mortality occurs and pigmented, normocyte dwelling parasites are largely lost from the circulation. The surviving parasites possess SDH activity. Within 3–4 days after the chloroquine dose some parasites again do not possess SDH activity. Further drugging induces a further switch to SDH activity. It is significant that administration of chloroquine on day 4 does not reduce the number of parasites in reticulocytes. The habitation of reticulocytes, however, does not itself confer resistance to the parasites, for drugging of the parasites on day 9 causes a significant (approximately 30 per cent) mortality of reticulocyte parasites. Exposure of a population of N strain parasites within reticulocytes to chloroquine for 24 h does not induce SDH activity nor are such parasites resistant to the drug.

These simple experiments demonstrate a significant difference between the chloroquine sensitive and chloroquine resistant strains of *P. b. berghei*. The key role of SDH in the citric acid cycle, and the improbability that this enzyme represents the only difference between the chloroquine-sensitive and resistant strains, indicates that a considerable difference occurs in their respiratory pathways. These results also indicate that, although the RC and NS lines appear to be morphologically distinct variants in undrugged mice, under drug pressure both have a similar appearance and, in possessing SDH activity, presumably possess a common mechanism of chloroquine resistance. We therefore consider that the physiologically labile NS line represents an intermediate stage between the parent N strain in which the trophozoites catabolize glucose by glycolysis[28] and the RC line in which all trophozoites possess SDH activity and presumably catabolize glucose aerobically.

In older infections of the NS line the trophozoites develop SDH activity even in the absence of the drug. Such a change might be induced by the immune response of the mouse. The occurrence of the enzyme in the sporogonic stages of drug sensitive *P. berghei*[1] further indicates that the enzyme does not appear *de novo* in the chloroquine resistant organisms but that its appearance may be related to the respiratory switch which, in sensitive parasites, occurs only in association with gametocytogenesis. This may explain why the N strain, which had lost the ability to produce gametocytes during twenty years of syringe passage in the laboratory, on repeated treatment with chloroquine not only developed resistance but also began to produce gametocytes[24]. It may also relate to the enigmatic observation of Ramkaran and Peters[29] that the infectivity of a chloroquine resistant line of *P. berghei* to *Anopheles stephensi* was enhanced by chloroquine.

We consider that the presence of SDH in the chloroquine resistant parasites indicates a respiratory difference between the sensitive and resistant strains of *P. berghei*. The lability of the NS line and induction of SDH activity by chloroquine further suggests that the utilization by the parasites of the respiratory pathway involving SDH is not simply an incidental factor but is in some manner directly associated with chloroquine resistance.

The avian malarias present an apparent obstacle to this hypothesis in that they utilize the citric acid cycle, synthesize amino-acids by transamination[28], but still produce pigment and are sensitive to chloroquine[2,30]. There is a widely held belief that chloroquine resistance cannot develop in avian malarias. This, however, has been disproved by the study of chloroquine resistance in

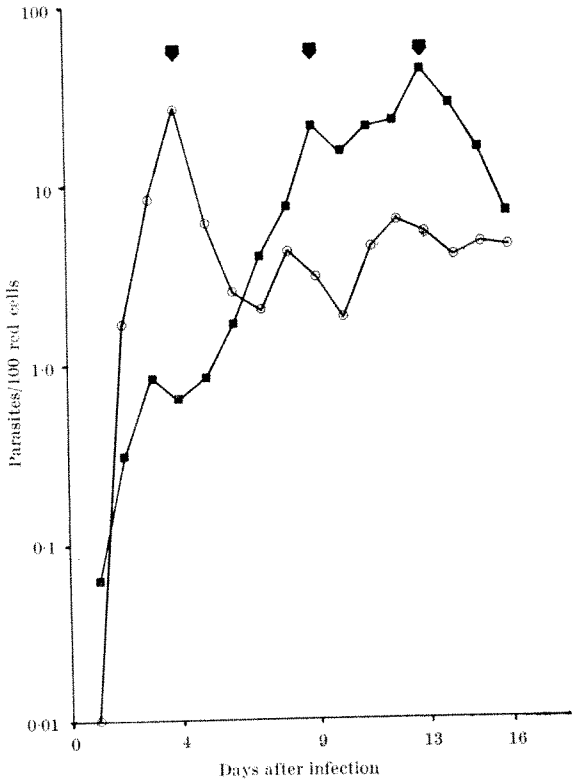


Fig. 3. The course of parasitaemia in mature and immature erythrocytes in mice infected with the NS line of *P. b. berghei* treated with chloroquine. Arrows indicate days on which chloroquine was administered (100 mg/kg subcutaneously). Note rapid decline in the number of normocyte parasites after dosing with chloroquine on day 4. ○—○, Parasites in normocytes; ■—■, parasites in reticulocytes.

P. gallinaceum[31]. Pyridoxine, the precursor of pyridoxal phosphate, an essential cofactor in many transamination reactions, does not inhibit the effect of chloroquine and mepacrine on *P. berghei* N strain (R. E. H. and D. C. W., in preparation). But pyridoxine does inhibit the effect of mepacrine (and quinine) on the avian parasite *P. lophurae*[32]. (Mepacrine and quinine resistant strains of *P. berghei* show cross resistance to chloroquine[33,34]. Observations in this laboratory indicate that there is a common mechanism of resistance to mepacrine and chloroquine.) *P. lophurae* has been shown to be dependent on its host cell for coenzyme A (refs. 35, 36). Similarly it may be that the amount of host cell pyridoxine limits the amount of transamination which avian parasites may perform and is a controlling factor in the response of the parasites to chloroquine.

The results presented indicate that the chloroquine resistant line of *P. berghei* differs from the sensitive strain in utilizing the citric acid cycle. Further work is in progress on the respiratory processes of these strains. An immediate objective is the fine structural location of the SDH activity. The transamination of Krebs cycle intermediates by the parasites is being investigated.

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Amnesic Syndrome: Consolidation or Retrieval?

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Amnesic patients can remember verbal material if they are tested with a method of partial information. The present experiments show that such retention depends more on the method of retrieval than on the method of acquisition. It is suggested here that long-term memory can be demonstrated in the patients if methods are used that eliminate incorrect and interfering responses.

Amnesic subjects are not so forgetful as was once thought. Learning and retention of motor skills such as rotary-pursuit tasks and tracking tasks were possible if not altogether normal in one severely amnesic subject[1]. Perhaps more strikingly, amnesics can learn to recognize a series of fragmented pictures and words and show evidence of retention over days and even weeks[2,3]. Moreover, the principle of providing partial information as a stimulus for retrieval has been shown to operate for verbal as well as perceptual stimulus material[4]. Why, then, do amnesic subjects learn and retain verbal material using this method when the same items are relatively unavailable using conventional methods? Is such retention by amnesics made possible by their having learned the material in a particular form, or by being presented with material in that form at the retention stage? Moreover, is it merely a favourable retrieval technique for all subjects, amnesics and controls alike, or is there a special factor which disproportionately affects the amnesics' performance?

The aim of this experiment was to manipulate independently both method of learning and method of retrieval. The results suggest that in amnesic subjects the retrieval procedure is more relevant than the method of learning and that the method of retrieval itself is critical in determining their performance on tasks of retention. The effectiveness of partial information in eliciting stored items of information raises various theoretical questions and allows one to relate the human amnesic syndrome to the relevant animal experiments.

Four patients with severe amnesic states, one following a temporal lobectomy and three with alcoholic Korsakoff psychosis (Nos. 1, 2, 5 and 6 reported in a previous study[3]), were available for these experiments. They had normal intellectual capacities other than memory defect. There were fourteen control patients with extra-cerebral neurological disease who took part in these experiments, six in experiment 1 and eight in experiment 2. No control subject did both experiments. These control subjects were tested in two subtests of the Wechsler Adult Intelligence Scale (WAIS) (Vocabulary and Block Design) and were matched as closely as possible for age and intellectual level with the amnesic group.

In the first experiment the learning conditions (using the fragmented word technique, see Fig. 1) were kept constant, and subsequent retention was measured using three different retrieval methods: recall, recognition and one partial information technique (fragmented words). Because amnesic subjects can learn by this method, the three retrieval methods may be directly compared.

Three lists of nine words, all of high frequency, were drawn from the Thorndike–Lorge word count. Two



Fig. 1. Example of fragmented word.

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fragmented versions of each word were prepared as described previously[3]. On each learning trial the most incomplete version of the word was presented first, then the less incomplete version followed by the complete word, and so on through the list of nine words. Subjects identified each word as soon as possible. The error score was defined as the number of additional versions (other than the most fragmented version) required for identification. Five learning trials plus a retention test were given on each of the three lists.

There were three retention conditions, each being attempted by all subjects in a counterbalanced order. Retention was assessed 1 min after each learning task was completed; the interval was filled by counting backwards from 100. There was an unfilled interval of 5 min between each condition. (1) Recall: the subject gave a spoken report of words from the test list. (2) Recognition: the subject was required to respond "Yes" or "No" sequentially at self-paced rate to each of eighteen words (printed in the same size and type of lettering as the stimulus list), nine new words and the nine old arranged in a random order. (3) Partial information: a further sixth trial using the method of fragmented words was given. The mean error score for each group in experiment I on the five learning trials for all lists combined is shown in Table 1. The score on trial 1 is an indication of the perceptual difficulty of the task before any further memory component has been introduced. The word lists for each condition were of approximately equal difficulty and it can be seen that the controls were reasonably well matched with the amnesics on the perceptual measure.

The retention scores (expressed as percentage correct) for each method of retrieval are given in Table 2. The recognition score was calculated by subtracting the number of false-positive errors from the number of items from the stimulus list correctly identified. The fragmented word retention score is expressed in terms of a savings measure between the fifth and sixth trials.

Though the amnesic group learned nearly as efficiently as the control group (Table 1), there was a clear-cut deficit on retention, both by recall and by recognition, in the amnesic group ($P < 0.002$ and 0·01 respectively). Neither group showed any significant degree of decrement on retention by partial information. As there was a ceiling effect in the control group with the recognition procedure, it is not clear from these findings whether or not retention elicited by partial information is differentially affected in amnesic subjects. It is clear, nevertheless, that learning the material with the partial information technique does not make the information normally available to recall and recognition by amnesics.

In the second experiment different methods of retrieval were again used to assess retention after a constant learning condition, but in this case a conventional word learning task (reading a word list three times) was followed by one of four retention conditions. It is known that retention of such material measured by recall and recognition in amnesics is very poor[5]. The question is whether material originally presented in this conventional manner can be shown to be retained using methods of partial information.

Lists of eight five-letter words, all of high frequency in the Thorndike–Lorge count, were used as test stimuli. Subjects were given three learning trials followed by a retention test. There were four retention conditions and each subject learned three lists for each retention condition. A learning trial consisted of reading aloud a list of eight whole words (printed in large black capital letters on white cards) presented at a two-second rate. Retention was assessed in all subjects (after a 1 min delay filled with counting backwards) by each of the following methods in a counterbalanced order: (1) recall; (2) recognition; (3) partial information—fragmented words; (4) partial information—initial letters of words. The procedure for the first three retrieval conditions was the same as described for experiment 1; in condition (4) the first three letters of each word were presented and the subject required to identify the stimulus word. For each retention condition there were no inter-list intervals, though between each retention condition there was a 5 min unfilled interval.

A preliminary task preceded the preparation of the word lists for the two retrieval methods using partial information. First, subjects were shown fragmented words (20 per cent patterned) used in method 3, and any word which could be identified in this incomplete version was rejected from the test list. Each test list for each individual subject was made up of eight words which thus could not be read in the fragmented form. Second, the subjects were shown the first three letters used in method 4 and asked to think of a five-letter word. The final lists were constructed individually for each subject using only words which were not responses in the guessing task.

Mean retention scores for each of the four methods of retrieval for each subject group are given in Table 3. An analysis of variance was computed using arcsine transforms of the mean retention scores for subject groups and retention conditions. With conventional methods of assessing retention (see Table 3), that is, by recall and by recognition, the amnesics were significantly worse than the controls ($P < 0.02$ and < 0.001 respectively by the one-tailed t-test; the differences were also significant by the Mann–Whitney U test, $P < 0.02$ in both cases). No significant difference between subject groups was found for either of the methods of partial information (see Table 3) and in neither case could a ceiling effect account for the similar level of performance in the two groups. Although the overall difference between the amnesic and control group was not significant, the difference between retention conditions was significant ($P < 0.001$). Furthermore, the group x retention conditions interaction term was significant ($P < 0.05$) indicating that the retention conditions had a differential effect on the amnesics and the control groups. Retrieval by recognition was superior to retrieval by recall in the control group ($P < 0.0001$) but not in the amnesic group, while retrieval by partial information was superior to retrieval by recall in the amnesic group ($P < 0.05$) but not in the control group.

Table 1. MEAN ERROR SCORE ON LEARNING AND RETENTION TRIALS

	Trial No. 1	2	3	4	5	6
Amnesic group N = 4	6·0	2·9	1·8	2·1	1·4	1·5
Control group N = 6	7·9	3·0	1·7	1·1	0·7	0·8

Table 2. RETENTION SCORES—EXPERIMENT I

	Controls	Amnesics
Recall	48 per cent	14 per cent
Recognition	94 per cent	59 per cent
Fragmented words	96 per cent	94 per cent

Table 3. RETENTION SCORES—EXPERIMENT II

	Controls N = 8	Amnesics N = 4
Recall	13·0	8·0
Recognition	18·7	10·5
Fragmented words	11·1	11·5
Initial letters	16·0	14·5

It can be seen that the method of learning did not differentially affect retention in amnesic subjects, in contrast to the method of retrieval, which did. The fact that information in long-term memory is available even after learning by conventional methods, if a particular retrieval method is used, is further evidence that it is inappropriate to characterize the amnesic syndrome as being a failure of registration or consolidation. We have shown previously that the normal relationship between recall and recognition may not be valid for amnesics; there was a relatively greater deficit on the recognition task than the recall task in the amnesic group[6]. The present experiment is consistent with this finding and furthermore has shown that retention by partial informa-

tion is a particularly favourable retrieval method for the amnesics but not the controls.

It is paradoxical that partial information in the form of fragmented letters or the initial letters of the word should be a more effective retrieval method than a conventional "yes–no" recognition task where the whole word is presented. The paradox can be resolved if one considers the proposition that to store or retrieve too much is as disastrous as to store or retrieve too little for efficient and effective memory. One special property of retrieval by partial information is that the subject can eliminate incorrect false-positive responses. The number of alternatives which would match the fragmented words must be very limited if not unique and in the case of the parts of words the number of alternatives is finite and can be counted. It has already been shown that the number of alternatives that could be formed from the same first three letters is a significant variable for both amnesic and control subjects[4]. This possibility of rejecting incorrect responses would be evident if either long-term memory information were not appropriately categorized or if there were too many items of stored information. It has been shown that organizational processes are important in normal memory[7,8] and it can be shown that at least some classification processes are possible for amnesics. It is therefore suggested that in the amnesic syndrome information in storage is either not inhibited or not dissipated in a normal fashion. This would account for the observations of a high incidence of prior list intrusions in amnesic subjects in tests of recall[6,10,11]. Performance on recognition tasks is subject to proactive interference effects, and it has been shown that there is a very rapid build-up of proactive interference in a continuous recognition memory task[12], which might account for the fact that recognition is no more favourable a measure of retention than recall in the amnesic group. It may not be too far-fetched to suggest that effective normal day-to-day memory demands that previous events be forgotten or suppressed and the inability to do so in the amnesic subject produces responses analogous to prior-list intrusions recorded in formal verbal learning experiments. From this point of view normal forgetting may be a positive benefit for efficient memory.

We now have two types of task, motor learning and retention by partial information, which are relatively well preserved in amnesic subjects. Is there a common factor linking performance on these apparently dissimilar tasks? Learning of new motor skills in normal subjects is not necessarily rapid, but retention over long periods of time is good. One property of motor skills is that retention is remarkably unaffected by interference effects in general[13] and by proactive interference in particular[14]. It is therefore suggested that both types of task, not necessarily for the same reason, are resistant to proactive interference.

Hitherto there has been an apparent discrepancy between amnesic deficits in man, as judged clinically, and the results of animal experiments that have attempted to generate amnesia in the laboratory. The present interpretation, based on disinhibition and interference phenomena rather than on faulty consolidation, allows one to bring the human amnesic syndrome, in which the hippocampus has been implicated[5], directly into line with the results of experimental medial–temporal and hippocampal damage in animals. For example, hippocampectomized rats and monkeys are slower to extinguish, slower in discrimination reversal tasks and under-react to novel stimuli when performing strongly established responses. Kimble[15] suggests that a failure of internal inhibition, Douglas and Pribram[16] that a failure of dissipation of awareness of unrewarded events would account for these findings. No doubt the cognitive capacities of man are different, and the defect would therefore have a different manifestation, but it is possible that the underlying functional disorder is the same.

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Experimental Production of Transients in Human Interaction

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A review of some methods for measuring aspects of human interaction, based on the rhythmic patterns of activity and inactivity which two people display when they meet.

In the measurement of interaction, the beginnings and endings of actions are operationally defined by observing the associated contractions and relaxations of muscle systems (in the human, chiefly those of the face and head, often associated with sound and usually coincident with movements of the mouth). The actions are, in effect, made up of the fixed action patterns of the animal[1]. With the criteria of contraction and relaxation, the flow of fixed action patterns can be broken into separable "action" units; this procedure contrasts with much ethological description. Durations, as well as frequencies, thus become available for quantitative analysis.

The sequence of measurements demonstrates that their regularities are rhythmic in character. Though these rhythms are the fundamental property of the actions (and inactions) of humans and other animals, uniformities are obscured by environmental factors, particularly by the interference of the rhythms of other individuals. If individual rhythms are not completely synchronized, transients are set in motion. One can, however, define the experimental conditions through which a perfectly synchronized or "free-running" state can be achieved, in a first approximation at least, as being parallel to such states in the circadian rhythms[2,3].

Components (Variables) of the Interaction Sequences

Only two-person interaction will be considered here. Given two persons, A and B, only four states are possible: (1) A acting, B inactive; (2) A inactive, B acting; (3) A and B both acting; and (4) A and B both inactive. Because these states occur in sequences, one can then define and differentiate sequences by their components; in effect, one determines the order of the transition states and whether A or B is responsible for their appearance. By definition, any given sequence is begun and ended when the two individuals assume opposite states.

The free-running state may be a natural consequence of the complementarity of the two individuals' basic rhythms, or deliberately contrived by programming one individual's pattern to be the converse of the other's. In free-running, the rhythms show minimal variance. If one assumes that the pulses (the beats or tempos) are approximately equal, and the durations of the component actions and inactions are the quantitative alternates, then A and B will never interrupt each other or fail to respond. Runs of such synchronized interaction occur with partners of long standing—old friends or spouses[4]; they are extremely uncommon (and very brief) in the world of those with emotional disorders[5].

Interactional Asynchrony as Stress

In everyday human interaction, asynchrony is predominant, varying widely in its quantitative values. By hypothesis, each instance of asynchrony is regarded as an interactional stress, the stress being defined and differentiated by the components of the pattern (sequence) involved. One might argue that a brief non-response or interruption (or other sequential pattern) hardly has the dramatic implications which are ordinarily applied to the term "stress"; yet observation demonstrates that very small amounts of asynchrony can be sufficient in some people to trigger off a violent and sustained pattern of "acting-out". For many others, thresholds for very brief and irregular patterns of asynchrony are high enough for no appreciable disturbance of the rhythms to be demonstrable. Moreover, it should be emphasized that the threshold for each pattern of stress is usually different. Although one stress pattern leaves the subject "unruffled", another can quickly bring about substantial changes in his basal rhythms.

Thus stress, as here used, has no connotations of magnitude or intensity. It simply means that a particular type of asynchrony has occurred with specific quantitative properties in an interactional sequence; the stress may be repeated with the same or different durations, interspersed with other types of stress and with intervals of synchronization. When such a single stress is imposed on a basal rhythm, there may or may not be evidence of distortions of the rhythm; once ended, shifts in the interaction rate may or may not be evident. Obviously the constant repetition of such stresses—the mother not responding to the child, the wife constantly dominating the husband—brings about severe reactions, outbursts of temper, violence or withdrawal.

The quantitative properties of the various patterns of asynchronous interaction can be investigated by programming the interaction of the experimenter (the interviewer, B) in the durations of each component and the frequency of their repetition (and sequential position). This includes intervals of synchrony and the asynchronous sequences of stress experimentally introduced. Further, content is rigorously non-directive; intensity, pitch, timbre and articulation of the voice are held within narrow limits, as are their expressive movement cognates. The synchronous state is the first stage of the interview, and establishes the basal rhythm. A period of synchrony, following the period of stress, provides a "recovery" period. This then becomes the means through which the type, severity and extent of the stress and its transients are determined.

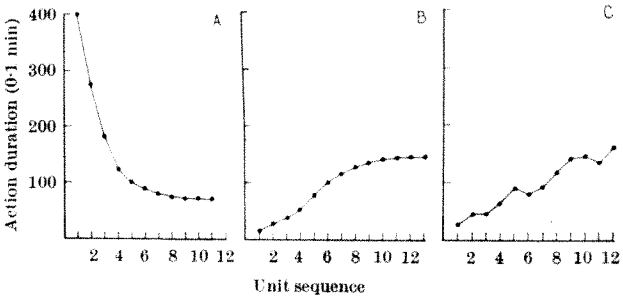


Fig. 1. Non-linear patterns of transients.

Initial State Transients

Interaction measurements seem best described as coupled relaxation oscillators[6]. Even though one expects the starting up of an oscillator to involve a transient state, this is very definitely the case when two oscillators (the two people) are coupled in the interaction sequence. These initial state transients are themselves worthy of comment, because they are so fraught with the emotional concomitants of entering a new situation, meeting a new person, or even shifting gears from one interpersonal relationship to another.

Thus, either person, or both, may be shy, bashful, pompous or pretentious. To "translate" these references to subjective (emotional) states and the behaviour which validates them, one has to trace the sequence of interaction patterns shown when interaction begins. One then observes a remarkable regularity in the quantitative values of the interaction measurements; a sequential shift in values (idiosyncratic but replicable) which underlines the variegated elements such as shyness or pretentiousness.

To a large degree, these can be defined as functions of the action and inaction variables. Thus, for example, if one plots the durations against their sequential order, it is common to find the values of the transients arrayed in a positive or negative non-linear function, as Fig. 1 indicates (for action durations).

Not all individuals, of course, show transients with any sequential frequency. They attain their basal level at the first or second action duration and remain there, though they usually exhibit a regular variation in durations with a rhythmic form, to be commented on shortly. These rhythmic variations may also modify the exponential form of the transients illustrated in Figs. 1*A* and *B*, as *C* demonstrates. But, further, in severely hostile individuals or those who are depressed the action durations may be extremely short, their variation being only a function of the low numbers themselves (a deviation from 3 to 5 s, for example).

The inactions (silences, if the term can include the absence of non-verbal responses) show similar transient properties. These are complicated by significant deviations in the initiative and dominance variables. Asynchrony may occur either by latency of response or by interruptive actions. It is ended when one or the other re-establishes synchrony, either by ending the mutual silence and initiating an action, or by persisting against the opposing action until the other falls silent.

Because human subjects can react with intense emotional severity to the introduction of the forcing oscillation (*Zeitgeber*), only threshold amounts (in durations and frequencies) are used. It is hypothesized that true entrainment and the appearance of a new synchronized endogenous oscillation in the individual are a function of a sustained and continuously repeated imposition of the forcing oscillation over long periods of time[1]. In re-establishing what seem to be basal levels, several months of exposure to forcing oscillations are usually required[7].

The criteria for determining the manifestations of the basal rhythm (the free-running period) of the individual

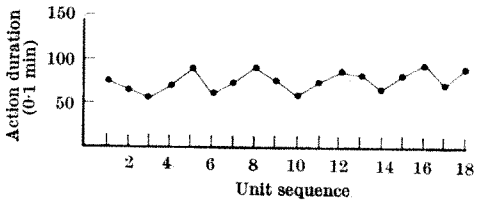


Fig. 2. Periodicity in sequential values of action duration.

are more complicated than those in circadian studies of activity, because the number of interaction variables is larger. Frequency, ratio of action duration to inaction duration and total amount of activity are present, but the endogenous impact of the initiative and dominance variables (analogous to what Wever calls the "special behaviour rhythms", as with an animal deciding to go on to a running wheel) also have to be taken into account[1]. They have their own periodicities: for example, the occurrence of asynchrony when B, the experimenter, does not respond builds up internal "pressures" to initiate and bring the silence to an end, which can bias the values of the basic variables.

As a consequence, a necessary further condition for evidence of the free-running period is the absence of asynchronous patterns of interaction on the part of A, the subject. Such patterns do not merely indicate the presence of biasing variables with their own periodic properties; they are also evidence of fixed emotional states which, by assumption and empirical evidence, seem to be the resultant of prior entrainment to a pathological oscillatory rhythm.

When they have achieved their base, most people show, as the figures indicate, a minor but regular variation; so much so that, after plotting the durations as in Fig. 1, the sequential values follow an apparent periodic curve (Fig. 2). For reasons described elsewhere, this internal rhythm within the duration sequence seems to be a function of variations in the intensity (amplitude) of the sound or expressive movements of the non-verbal response[1,6].

Experimental Stress

With the exception of the stress provided by the coming together of two people and the starting up and coupling of their oscillators, stresses are experimentally introduced only after the free-running oscillation is established (or its pathological form in the psychiatric states). The quantitative properties of the *Zeitgebers* used can be varied almost infinitely. Essentially, however, the sequential patterns involve either the initiative (latency) or dominance (interruption) variables.

In either category, one can vary the number of sequential trials, the duration of action or silence of A before B acts (initiates or interrupts), the length of B's action when initiating or persisting against A's opposition and, in the case of dominance, how long he continues after winning the competition for control. Further, in the event that a single programme sequence has been completed but A remains in his prior state, either being mute or continuing to talk, one can vary the interval before the sequence is repeated. Permutations within each of these categories of variables are manifold; further complications can be introduced if switching from one category to the other is programmed (as in ordinary free interaction give-and-take).

Figs. 3 and 4 illustrate two such experiments, for initiative and dominance respectively. Only enough of the basal rhythm is given to indicate its values and, in both, the same number of asynchronous patterns is used to make up the sequence. These experiments were conducted on the same day with the same individual, but not consecutively, to avoid carry-over of the transient reaction. Clearly the transients shown here have a very different quantitative form. It is important to emphasize that extreme reactivity to one type of stress does not mean that it will occur after a different stress pattern; neither does it follow that a positive slope for one will mean a positive slope in all transients.

Fig. 5 illustrates what happens to three persons with approximately equivalent basal rhythms when the same stress sequence given to the individual in Fig. 4 is administered. The subject of Fig. 5*A* is clearly "unflappable", at least to this amount of asynchrony. Though his values shift during entrainment, afterwards he returns immediately to his base. The subject of Fig. 5*B* is disturbed chiefly in his basal rhythms, with relatively small distortions of his other variables; that of Fig. 5*C* reacts so severely that the interview had to be ended before any significant shift took place.

Autonomic–Somatic Interdependence

In considering the numerical values it should be remembered that these interactional changes are not some kind of abstract aberration of the skeletal muscles—they are the behavioural manifestations of the autonomic–somatic system[1]. The nature of the forced oscillation (the stress) imposed on the system sets in motion, by hypothesis, specific (and idiosyncratic) autonomic changes. These provide the parametric values by which the severity and length of the reaction are determined. In relatively primitive investigations, pronounced changes in interactional rhythms have been reported after injections of adrenaline or acetylcholine (Mecholyl)[8]. Modern studies of emotion in physiology and biochemistry have made clear the fine tuning of the reaction patterns even if, surprisingly, little sophisticated measurement has been used[9].

As a consequence, in fear or anger or love, and all their variants (semantically and operationally), the measured interaction patterns change and can be identified together with concomitant physiological changes. These physiological changes involve both neurological and biochemical activators and combinations thereof, and have different temporal properties.

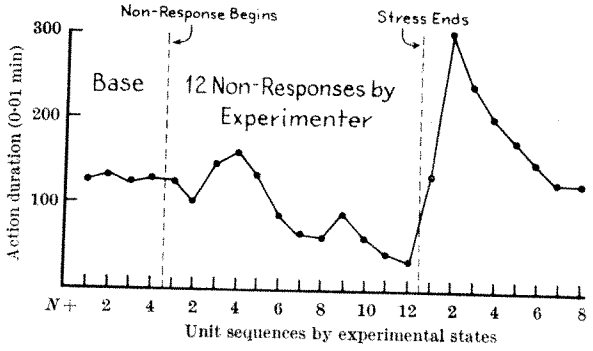


Fig. 3. Results of interaction experiment for initiative.

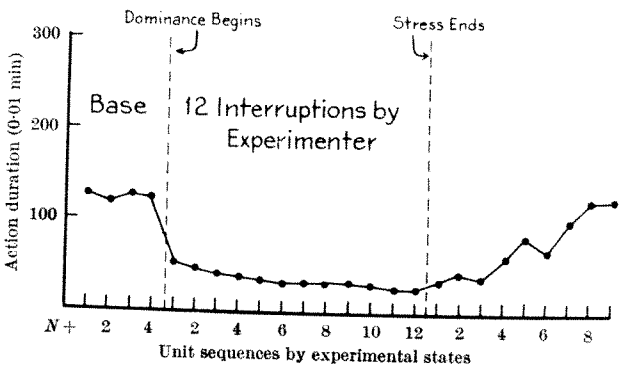


Fig. 4. Results of interaction experiment for dominance.

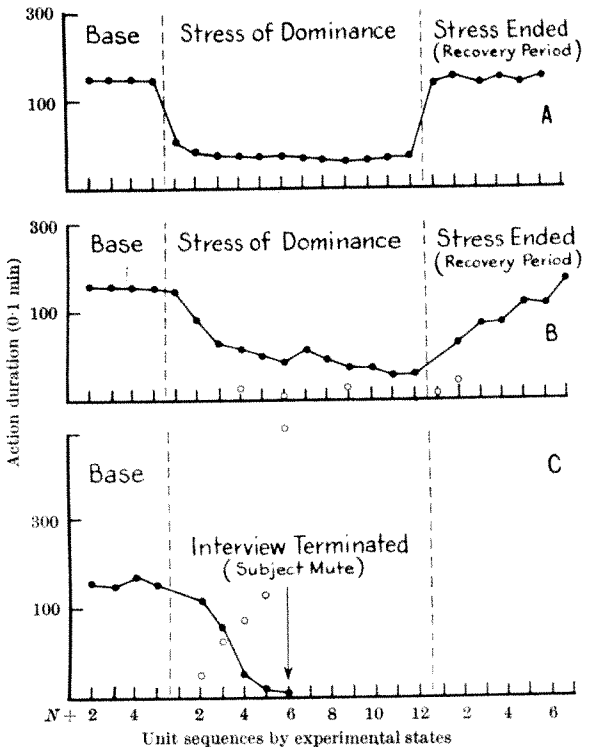


Fig. 5. Three different reactions to the stress sequence applied to the subject of Fig. 4. ●, Action duration; ○, latency.

As Fig. 5 illustrates, the transients are made up not merely of the action–inaction durations themselves but of the asynchronous initiative and dominance variables, those characteristic patternings of latencies of response and of the interruptions when one person breaks in on the other's action interval and begins to act himself. These variables are not merely elements or artefacts of oscillatory shifts; they are the stresses (for the other person) and the additional evidence of disfunctioning of the autonomic nervous system.

Thus basic shifts in the action-silence rhythms occur when a *Zeitgeber* is set in motion. They continue even after the entrainment sequence ends, but they are transformed in their emotional impact by the inability of the individual to manage to adjust, to synchronize, to avoid being disturbed and to be disturbing to those others with whom he comes in contact. Hostility, aggression, depression, in their interactional patterns, are thus compounded of the disturbances to the basal rhythms and the latencies or competitive actions of the asynchronous states. These variables are then variations of the primary oscillatory sequence; they serve to differentiate the transient reactions even further.

If one bears in mind the significance of transient multiplication when entrainment begins and the carry-over which may continue after the *Zeitgeber* is removed, then the emotional–interactional combinations which make up the many transients so far identified can be understood. If a transient appears after a stress situation is ended, its properties are varied emotionally (internally) by the prior transient manifested when the forced oscillation was imposed on the individual's basal rhythm. The mathematical formulation of two transients may thus be equivalent, but there is a carry-over from one transient which sets off the next, a kind of hysteresis which alters the nature and properties of the subsequent reaction.

Thus the magnitude of each transient, and the length of time it lasts, is a function of the magnitude of the forced oscillation and the duration of the entrainment period. In addition, the idiosyncratic differences in threshold and reactivity have to be borne in mind; these are the boundary conditions within which transients have their effects.

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Increased Frequency of Deletions in DNA Polymerase Mutants of *Escherichia coli*

by

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Mutants of *E. coli*, deficient in DNA polymerase activity *in vitro*, spontaneously generate chromosomal deletions at a high frequency. Such deletions may arise from the inability of these strains to repair single-strand breaks in DNA efficiently.

SPONTANEOUS deletion of genetic material could involve one or more of the enzymatic processes in DNA repair, replication or recombination. We have determined the frequency of appearance of deletions in different types of *E. coli* mutants which are defective in the repair of damage to DNA resulting from ultraviolet irradiation. Mutations in a DNA polymerase structural gene(s) markedly increased the frequency of occurrence of deletions. The average frequency of spontaneously occurring *tonB* and *tonB-trp* deletion mutants in stationary phase cultures was determined as described in Table 1. The ultraviolet-sensitive strains examined and the frequency of *tonB-trp* deletions in each are listed in Table 1, experiment (*a*). Our previous studies[1,2] have indicated that even in closely related strains, differences in the structure of the chromosome in the vicinity of the *trp* operon can influence the frequency of *tonB-trp* deletions. The deletion frequency of each ultraviolet-sensitive strain was

therefore compared directly with its ultraviolet-resistant parent. Of the ultraviolet-sensitive strains examined, only strain P3478(W3110), which carries the *polA1* mutation[3,4], exhibited a *tonB-trp* deletion frequency significantly different from its parent. To determine whether the apparent increase in deletion frequency in strain P3478 was related to the presence of the *polA1* mutation, Pol$^+$ revertants of P3478 were isolated by selecting for resistance to methylmethanesulphonate (MMS)[3]. When the *tonB-trp* deletion frequency of several of these revertants was determined, we found that deletion frequencies were now similar to the low frequency characteristic of the W3110 parent. Values for one Pol$^+$ revertant (PR3478) are shown in Table 1, experiment (*a*). This revertant was used as a Pol$^+$ control in subsequent experiments. The magnitude of the increase in frequency of *tonB-trp* deletions in the *polA1* strain, relative to the Pol$^+$ revertant, varied between fifteen and forty-fold in separate experiments. The factors responsible for this variation are unknown. Thy$^+$ transductants of P3478 produced deletions at the increased frequency.

produced *tonB-trp* deletions at a frequency at least twenty-fold higher than the average frequency of the Pol$^+$ strains.

The *polA1* mutation causes no gross alteration in the position of the endpoints of *tonB-trp* deletions in the *trp* operon. The percentages of deletions terminating in genes *trpA*, *trpB*, *trpC*, *trpD* and *trpE* (or beyond the *trp* operon) of the parental strain (W3110 Thy$^-$) were 11, 23, 8, 8 and 50 per cent, respectively (35 deletions examined). For strain P3478 the percentages were 9, 22, 22, 7 and 40 per cent, respectively (45 deletions examined), and for strain PR3478 they were 7, 24, 20, 25 and 24 per cent, respectively (51 deletions examined).

PolA1 is an amber mutant which responds to the suppressors *suI*$^+$, *suII*$^+$ and *suIII*$^+$ (ref. 4), that is, sensitivity to ultraviolet light and MMS is suppressed and polymerase levels are increased. If the *polA1* mutation is responsible for the increased frequency of deletions in strain P3478, this high deletion frequency might be suppressible by various amber suppressors. To test this expectation, we introduced a *trpA* amber mutation (A9761) into strains

Table 1. FREQUENCY OF *tonB-trp* DELETIONS IN VARIOUS ULTRAVIOLET SENSITIVE STRAINS

Expt.	Strain	Relevant allele	Other markers	Source	*tonB*/viable cell ($\times 10^7$)	Percentage Trp$^-$	*tonB-trp* deletions/viable cell ($\times 10^7$)*
(*a*)	AB2497	*uvr*$^+$	F$^-$*thi-leu-arg-pro-his-thy-lac-ara-gal-mtl-xyl-str^r T6^{r}thr$^-$*	K. Smith	2·0	19	0·38
	AB2500	*uvrA6*			1·3	18	0·24
	AB2499	*uvrB5*			2·5	13	0·33
	AB2498	*uvrC34*			2·1	15	0·32
	AB2480	*uvrA6recA13*	F$^-$*pro-thi-lac-gal-str^{r}T6^r*		2·1	14	0·29
	W3623	*uvr*$^+$	F-*thy-trp*$^-$	H. Shizuya	6·2	25	1·55
	N14-4	*uvrD3*	F-*trp*$^-$		6·3	21	1·32
	AX100	*ras*$^+$	F-*lac-thi-leu-str^{r}suII*$^+$	J. R. Walker	6·3	28	1·76
	AX83	*ras*$^-$	F-*lac-thi*$^-$		7·7	24	1·85
	W3110	*pol*$^+$	F-*thy*$^-$	J. Cairns via P. Hanawalt	2·2	18	0·39
	P3478 (W3110)	*polA1*	F-*thy*$^-$		29·5	36	10·6
	PR3478	*pol*$^+$	F-*thy*$^-$	Reversion from P3478 on MMS†	2·2	23	0·51
(*b*)	P62	*pol-4*	F-*thy-met*$^-$	J. Cairns via P. Hanawalt	12·6	39	4·9
	P94	*pol-5*	F-*thy-met*$^-$		14·5	34	5·0
	P108	*pol-6*	F-*thy-met*$^-$		13·3	25	3·4
	P112	*pol-3*	F-*thy-met*		23·3	42	9·8
	P250	*pol-2*	F-*thy-met*$^-$		3·6	30	1·1
	PR112	*pol*$^+$	F-*thy-met*$^-$	Reversion from P112 on MMS†	4·7	26	1·2
(*c*)	MEP8	*pol*$^+$	F$^-$*cys*$^-$	Constructed as described in text	3·2	11	0·35‡
	MEP3	*polA1*	F$^-$*cys*$^-$		26·8	27	7·3‡

* Each *tonB-trp* deletion frequency is an average of four or more independent determinations.

† Approximately 5×10^8 cells from a fresh L-broth culture were spread on nutrient agar–cysteine plates supplemented with either 0·04 or 0·06 per cent MMS. After 48 h at 37° C, MMS-resistant colonies were picked and checked for resistance to ultraviolet irradiation (540 ergs/mm^2).

‡ Two additional Pol$^+$ and Pol$^-$ isolates were examined and they exhibited similar *tonB-trp* deletion frequencies.

To measure deletion frequencies, four or more cultures (5·0 ml.) of the strain to be examined were grown from a small inoculum (< 100 cells/ml.) to stationary phase (1×10^9–3×10^9 cells/ml.) in L-broth (or L-broth + thymidine for Thy$^-$ strains) at 37° C. An aliquot of each culture (usually 0·1 ml.) was spread on nutrient agar + cysteine (30 μg/ml.) plates with approximately 10^9 particles of φ80vir and 10^9–10^{10} particles of a colicin V, B preparation. The plates were incubated at 37° C for 24–48 h. The resulting *tonB* mutants were then replicated on a medium lacking tryptophan (minimal medium supplemented with 0·1 per cent acid-hydrolysed casein). Trp$^-$ colonies were scored as *tonB-trp* deletions. For strains carrying a *trpA* mutation, the *tonB* colonies were replicated on minimal + casein + indole (10 μg/ml.) plates. In this case, only deletions extending through the *trpA* gene can be detected. (The order of genes in the vicinity of the *trp* operon is *trpEDCBA–tonB–*.) The *tonB–trp* deletion frequency for a particular strain is calculated from an average of all cultures examined and is expressed as the number of *tonB–trp* deletion cells/10^7 viable cells in the stationary phase cultures.

Deletion frequencies were also determined for five other strains carrying independent *pol*$^-$ mutations (Table 1, experiment (*b*)). (These strains are MMS^s, UV^s and exhibit low DNA polymerase activity *in vitro*: unpublished observations by J. Gross and J. Cairns.) All five Pol$^-$ mutants showed a deletion frequency greater than the parental W3110 strain (Table 1, experiment (*a*)). A MMS^r, UV^r revertant of strain P112 (PR112) showed a diminished deletion frequency.

To prove that the increased frequency of *tonB-trp* deletions in P3478 is the result of a genetic alteration in the *polA* region, *polA1* was introduced into a *polA*$^+$ *cysB*$^-$ *metE*$^-$ stock by cotransduction with *metE*$^+$ (ref. 4). (Repeated attempts to grow phage Plkc on strain P3478 were unsuccessful. Lysates with titres of about 10^8/ml. were, however, obtained with Plc*lr*.) Met$^+$ transductants of each phenotype (Pol$^+$ or Pol$^-$) were examined for *tonB-trp* deletion frequencies. Values for a Pol$^+$ derivative (MEP 8) and a Pol$^-$ derivative (MEP-3) are listed in Table 1, experiment (*c*). All the Pol$^-$ strains examined P3478 and PR3478 by transduction. It was then possible, in subsequent experiments, to construct Pol$^-$ and Pol$^+$ strains carrying *suI*$^+$, *suII*$^+$ or *suIII*$^+$ by transducing in the appropriate suppressor and selecting for suppression of the *trpA* amber mutation. The average *tonB-trp* deletion frequency determined for each *polA1* Su$^+$ strain was compared with a Pol$^+$ strain (revertant) carrying the same suppressor. The data of Table 2 show that all three amber suppressors reduced the relative increase of *tonB-trp* deletions (*polA1*/*polA*$^+$) in the *polA1* strains. Only *suIII*$^+$, however, completely suppressed the *polA1* mutation. Furthermore, whereas all the suppressors reduced the frequency of deletions in the *polA1* strains relative to the appropriate Pol$^+$ Su$^+$ control, only *suIII*$^+$ significantly reduced (85 per cent) the absolute deletion frequency of the *polA1* strain.

The response of the *polA1* strains to the different amber suppressors was variable, and therefore the same suppressor strains were examined for their sensitivity to ultraviolet irradiation. Ultraviolet survival curves are shown

in Fig. 1. *SuIII*$^+$, which efficiently eliminated the increased frequency of *tonB-trp* deletions in the *polA1* strain (Table 2), also totally suppressed the ultraviolet sensitivity of that strain while *suI*$^+$ and *suII*$^+$, which only partially suppressed the increased frequency of deletions, incompletely relieved ultraviolet sensitivity. Thus the ability of the different suppressors to reduce the frequency of deletions in the *polA1* strains correlates somewhat with their ability to suppress ultraviolet sensitivity. Strain PR3478, although exhibiting a low deletion frequency (Table 1), is still slightly ultraviolet sensitive (Fig. 1); this suggests that this strain has not reverted to the wild-type configuration. The *polA1* mutant was examined for a mutator effect by determining the reversion frequency of *trpA46* in Pol$^+$ and Pol$^-$ strains. (*TrpA46* is reverted by many base pair changes[7].) The results obtained indicate that *polA1* has little or no mutation activity.

Table 2. EFFECT OF AMBER SUPPRESSORS ON THE DELETION FREQUENCY OF *polA1* STRAINS

Suppressor*	*tonB-trp* deletions/viable cell ($\times 10^7$)† *polA*$^+$	*polA1*	*polA1*/*polA*$^+$	Percentage of control
Control (Su$^-$)	0·24	7·3	30·0	—
suI$^+$	0·51	5·3	10·0	33
suII$^+$	0·98	15·2	15·5	52
suIII$^+$	0·82	1·1	1·3	4

* *polA*$^+$ and *polA1* strains carrying each suppressor type were constructed as described in the text.

† Each *tonB-trp* deletion frequency is an average of twelve or more independent determinations.

How can the effect of the *polA1* mutation on the frequency of *tonB-trp* deletions be interpreted in terms of what is known about the processes involved in the repair of ultraviolet damage? Dark repair probably involves three processes: an endonuclease makes a single strand nick[8,9]; the damaged region is excised and repaired[6,10]; the repaired segment is rejoined to the parental DNA strand by a polynucleotide ligase[11–13]. Table 1 shows that impairment of any of these processes does not necessarily result in an abnormal frequency of deletions. Strains with mutations in genes *uvrA*, *B* or *C* and which therefore presumably lack the nicking function[14] have deletion frequencies comparable with their Uvr$^+$ parent. The frequency of deletions is also unchanged in ultraviolet-sensitive strains defective in genes *uvrD* or *ras*. These strains are capable of excising pyrimidine dimers[15,16] and therefore presumably are deficient in some process subsequent to nicking. In addition, ultraviolet-sensitive strains exhibiting a Rec$^-$ phenotype produce deletions at a frequency indistinguishable from that of Rec$^+$ strains[1,17–19]. Of the ultraviolet-sensitive strains examined therefore only strains defective in DNA polymerase activity *in vitro* exhibit an abnormal deletion frequency.

The properties *in vitro* of the DNA polymerase of *E. coli* suggest that this enzyme could participate in the excision-repair function *in vivo*[6]. In this case, a mutant deficient in DNA polymerase activity should be extremely sensitive to ultraviolet light and should be unable to perform normal repair replication. The results of a study of ultraviolet repair in a *PolA1* mutant strain[20] indicated that, following ultraviolet irradiation, the *polA1* mutant could still excise pyrimidine dimers and was as capable as its parent of performing repair replication (Cooper and P. Hanawalt, unpublished observation). The average molecular weight of the single strands of the DNA from the mutant (in an alkaline sucrose gradient), however, remained lower than that of the parent for at least 90 min after irradiation. Thus the *polA1* mutant was unable to rejoin efficiently the repaired DNA fragments. Assuming that DNA polymerase does catalyse excision-repair, these workers suggest that the defective polymerase of the *polA1* mutant may be capable of carrying out repair replication, *in vivo*, but may impair normal ligase function.

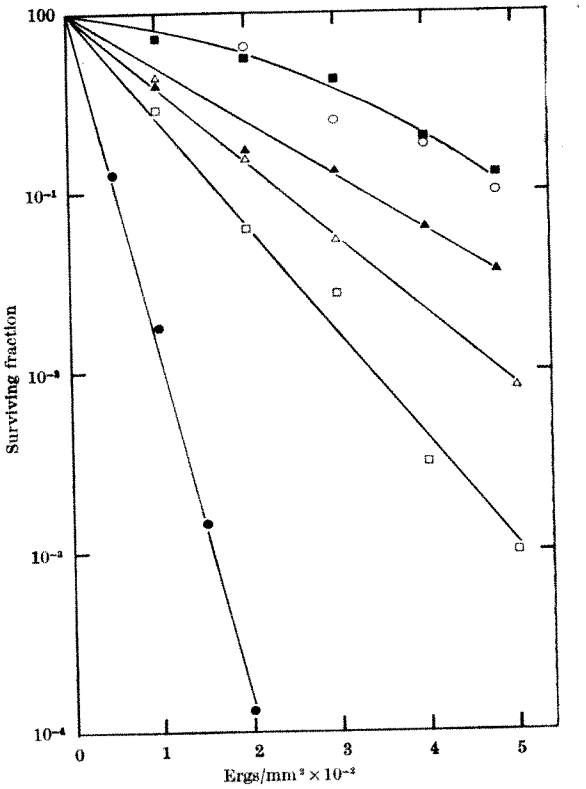


Fig. 1. Ultraviolet survival curves of various suppressed *polA1* strains. Cultures were grown at 37° C in L-broth to approximately 10^8 cells/ml., centrifuged and resuspended in 10 volumes of saline. 10 ml. samples were placed in glass Petri plates and irradiated with a dose of 10 ergs/mm^2/s. At appropriate intervals, aliquots were removed, diluted in saline and spread on nutrient agar–cysteine. The plates were counted after 20 h at 37° C. ○, W3110 (Pol$^+$); ●, Su$^-$*polA1*; △, Su$^-$Pol$^+$ (revertant); ▲, *suI*$^+$*polA1*; □, *suII*$^+$*polA1*; ■, *suIII*$^+$*polA1*.

On the basis of this interpretation we tentatively conclude that the high frequency of *tonB-trp* deletions observed in Pol$^-$ mutants results from an increased number of unrepaired single-strand breaks in DNA.

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Prebiotic Synthesis of Polypeptides by Heterogeneous Polycondensation of Amino-acid Adenylates

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Certain types of clays may have played a catalytic part in the evolution of polypeptides. The active forms of amino-acids, concentrated by adsorption on the clay particle surface, are shown to condense to form polypeptides of different but discrete sizes.

THE prebiotic synthesis of biopolymers probably involved the condensation and dehydration of active monomers under the conditions prevailing in the primaeval ocean[1]. Various models have been proposed to explain the polycondensation process and several experiments have been carried out on peptide formation in aqueous media. We have studied the polymerization of free and substituted amino-acid phospho-anhydrides[2-5] which have an energy content high enough to permit the formation of peptide bonds even at high dilution and physiological *p*H, and at room temperature. Steinman *et al.*[6] obtained diglycine from the condensation of dicyandiamide at acid *p*H values. Because dicyandiamide might be produced from the primitive atmosphere, and because its reactivity is similar to the cyclodiimide of Khorana[7], it may well have been the starting material for primaeval biosynthesis. In our experiments, however, extensive thermal polymerization of phosphates of amino-acids and of dicyandiamide only occurred in anhydrous conditions[8,9]; it is unlikely that such conditions existed.

The primary difficulty in imagining the polymerization process going so far in the free ocean is, as J. D. Bernal suggested, the extreme dilutions of the system. A large scale concentration of the products would be an absolute necessity for evolution, but it occurred to him that adsorption on very fine clay deposits of marine or fresh water could provide the favourable conditions for concentration. Movement of the clay might then have blocked further transformations of the primary photochemical products[10].

On the basis of this idea, some workers have used clays as a support for amino-acid synthesis[11]. Here, we show that certain clays, in particular the montmorillonites, can act as heterogeneous catalysts of the aqueous polycondensation of active forms of amino-acids. As predicted by Bernal, the presence of clay particles in the polymerization medium strongly inhibits competitive hydrolysis of the active groups and enables rapid formation of peptide bonds in physiological conditions.

Because the first step in the cellular synthesis of proteins is the formation of amino-acid adenylates, we chose the adenylates as model substances for active forms of amino-acids. It was assumed that amino-acid adenylates were synthesized spontaneously in the primaeval ocean from amino-acids formed in prebiotic conditions[12-14] and from ATP which can be synthesized spontaneously in an aqueous medium equilibrated with the primaeval atmosphere[15].

A series of polycondensation experiments was done in the presence of materials such as ion exchangers (cellites, amberlites and so on), silica gel, colloidal metal suspensions (copper, cobalt, iron and ultrasonicated mercury), inorganic polyphosphates and phosphate esters having long aliphatic chains. In all these trials only the cobalt and copper colloids and the aliphatic phosphate esters enabled the formation of higher peptides, and they were formed in rather low yields.

Among the clays, appatites and kaolinates were found not to affect polymerization, and only the highly swelling clays, such as the illites and montmorillonites, exhibited a marked catalytic activity. The permutites, which are regarded as one of the geological precursors of the montmorillonites but which do not swell, displayed no catalytic activity.

Free adenylic acid does not adsorb to the surface of montmorillonite particles; amino-acids adsorb at acid *p*H values but not at *p*H values higher than seven[16]. Amino-acid adenylates, however, adsorb up to an adsorption value of 10^{-3} moles/g montmorillonite. Assuming that montmorillonite disperses completely to single platelets, the area per g would be 7×10^6 cm^2. If the area per adsorbed alanine-adenylate is 50 Å^2, the maximum adsorption at full coverage would be 14×10^{20} molecules/g or $2{\cdot}3 \times 10^{-3}$ moles adenylate/g. The maximal surface coverage, therefore, corresponds to approximately 30 per cent of the total montmorillonite area.

Montmorillonite samples (Montmorillonite No. 25, John C. Lane Tract, Upton, Wyoming), prepared according to Posner[17], were fractionated centrifugally into platelets of diameters ranging from 550 Å to 2000 Å. The thickness of the platelets is about 10 Å. Although both the extent of adsorption and the polymerization rate depend on the particle size, the variation is not very great, so that a particle size of 700 Å was chosen for the experiments reported below.

Some polycondensation experiments were carried out with prolyl adenylate and diprolyl adenylate, but most of the data were obtained from studies with alanyl adenylate. It seems that copolymerization between alanyl adenylate and prolyl adenylate proceeds very easily in the presence of montmorillonite. It was found that the mechanism of polycondensation in homogeneous solution is different from that with heterogeneous catalysis. Homogeneous polymerization is always preceded by a hydrolytic liberation of the free amino-acid. This is represented by equation (1) in which A represents amino-acid, P represents adenylate and A∼P represents active amino-acid adenylate

$$\mathrm{A{\sim}P} \xrightarrow[k_h]{\mathrm{H_2O}} \mathrm{A+P} \qquad (1)$$

The free amino-acid subsequently interacts with the adenylate to give, in consecutive steps, short peptides according to the scheme

$$\begin{aligned} &\mathrm{A+A{\sim}P \rightarrow A_2+P} \\ &\mathrm{A_2+A{\sim}P \rightarrow A_3+P} \quad \text{and so on} \\ &\mathrm{A_i+A{\sim}P \rightarrow A_{i+1}+P} \end{aligned} \qquad (2)$$

where A_i denotes an i-meric peptide.

Because hydrolysis is considerable, the product is largely free monomeric amino-acid and, according to equation (2), all the peptides formed are free of adenylic

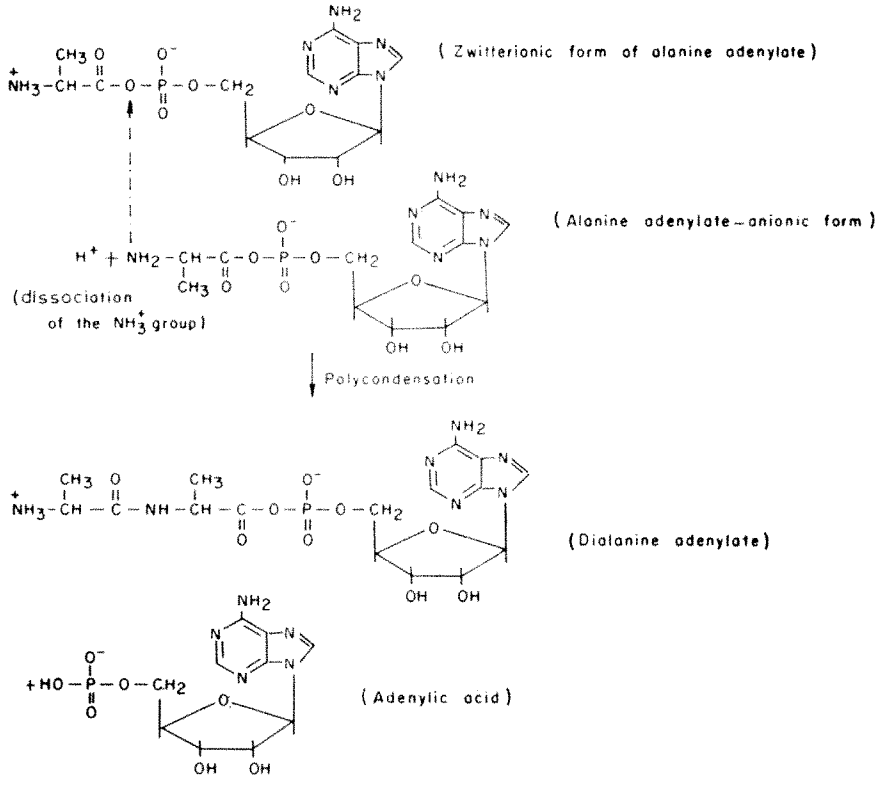


Fig. 1. The first propagation step in the heterogeneous polycondensation of alanyl-adenylate.

acid residues. Heterogeneous polycondensation proceeds in a different manner: generally, no free amino-acid is obtained, so that hydrolysis is largely inhibited by the surface adsorption. Moreover, a high percentage of the peptides obtained carries terminal adenylic acid. Unlike the anhydrides, these terminal groups do not interact with hydroxamic acid at neutral *p*H but do react at very high *p*H and seem to consist of an ester of an amino-acid with the adenylic OH groups of the ribose residue. A plausible reaction scheme for the catalysed polycondensation is therefore

$$\begin{aligned} &A\sim P + A\sim P \rightarrow A_2\sim P + P \\ &A_2\sim P + A\sim P \rightarrow A_3\sim P + P \\ &A_2\sim P + A_3\sim P \rightarrow A_5\sim P + P \quad \text{and so on} \end{aligned} \tag{3}$$

or the general propagation process:

$$A_i\sim P + A_j\sim P \rightarrow A_{i+j}\sim P + P \tag{4}$$

The termination step can be written as

$$A_i\sim P \rightarrow A_i - P \tag{5}$$

where the active phosphoanhydride bond $\sim P$ is converted to the inactive ester bond $-P$.

A closer inspection of the process reveals a few other important points. The organic formulae for the process of equation (3) are depicted in Fig. 1. It will be observed that an essential condition is the dissociation of the ammonium groups, which can take place only in an alkaline medium; polymerization would be inhibited in an acid medium. Fig. 1 demonstrates that each polymerization step liberates the strong adenylic acid which acts as a negative feed-back control on the polycondensation process. To maintain a constant rate of polymerization, therefore, alkali has to be added. In a similar manner, the termination reaction (equation (5) and Fig. 2) is an acidifying process which has to be controlled.

On the basis of these observations, the polycondensation was carried out in the following way: 25 mg montmorillonite was suspended in 50 ml. of water brought to *p*H 7·8–8·5. To the suspension was added 1 mM alanyl adenylate (418 mg), in portions of 10 mg dry powder at time intervals of 10–12 min. The amount of catalyst is sufficient to absorb all of the monomer; within 10 min turnover is complete and a new batch of material can be added. Throughout the experiment a constant *p*H value was maintained in the reaction mixture by means of a *p*H-stat which controlled the addition of 1 M NaOH or Na_2CO_3 to the reaction mixture. Because the alkali additions are a measure of the reaction rate, the time course of the reaction could be followed. It was typical of a heterogeneous polymerization: the rate developed during a few hours from a low to a constant stationary value. When no further alkali addition was required a stationary concentration of active $\sim P$ groups had been reached.

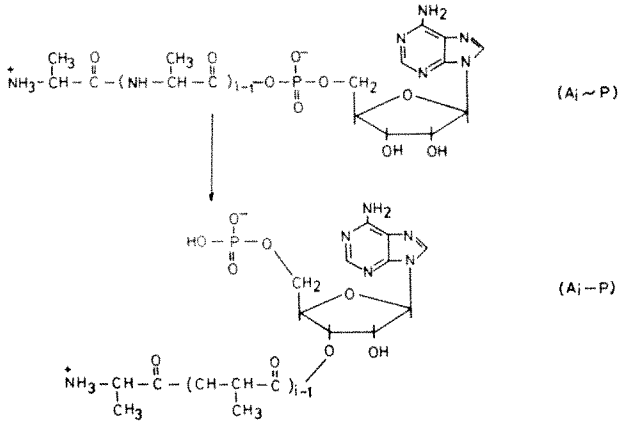


Fig. 2. The termination step in the heterogeneous polycondensation of alanyl-adenylate.

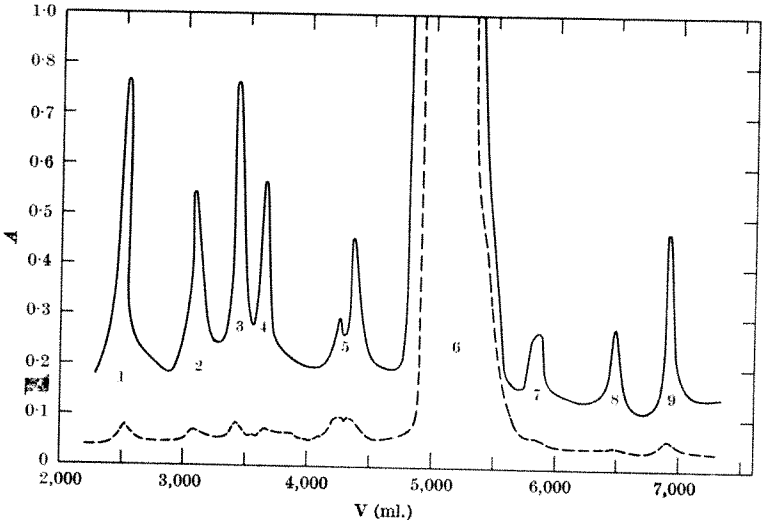


Fig. 3. Chromatography of fraction II on 'Sephadex G-25', with gel bed 3×4×150 cm. The peptides of each peak are described in Table 2. In addition, peaks 5 and 8 represent chiefly adenylic acid; the materials of peaks 7 and 9 are inorganic salt and residual alanyl-adenylate respectively. Elution solvent, 0·05 M HCl; - - - -, absorbance at 216 nm; ———, absorbance at 258 nm.

At the end of the reaction the montmorillonite was separated by centrifugation, and washed a few times with distilled water. The total aqueous reaction medium was ultrafiltered through membranes of various pore sizes and then subjected to chromatographic separation on columns of 'Sephadex', with water and dilute HCl as solvents.

To follow the efflux out of the column, a double beam spectrophotometric recorder was set up at the entrance to the fraction collector. One beam, of wavelength 258 nm, recorded the exit of only the free or bound adenylic acid; the other beam (216 nm) recorded the exit of both peptides and adenylic acid. Thus it was possible to determine simultaneously bound adenylic acid groups and total peptide bonds.

In a typical experiment, 1 g alanyl adenylate containing 172 mg alanine was added in 10 mg portions to 25 mg montmorillonite in 50 ml. water at *p*H 8·5. After 24 h the montmorillonite was removed by centrifugation and the reaction mixture was passed through a 'Sephadex G-25' column and eluted with water to give two fractions, I and II.

Table 1. DATA ON PEPTIDES

Peptide molecular weight	Degree of polymerization	Weight (mg)
640	9	10·3
1,120	16	35·2
1,900	27	20·0
Adenylic acid	1	470·5

The fractions were passed through a second similar 'Sephadex' column and eluted with 0·05 M HCl. A major part of fraction I was adenylic acid and the rest was free alanyl peptides. Data are presented in Table 1. The molecular weights of the peptides were determined in an analytical Spinco ultracentrifuge and the values obtained were corroborated by end group determination. High voltage paper electrophoresis showed that each of the peptide fractions was practically monodisperse.

The elution profiles of both fractions were unusual, and suggested an irregular but discrete distribution of molecular weights. A typical elution profile is shown in Fig. 3. Here, high polypeptides of molecular weight up to 4,000 and a degree of polymerization greater than 50 are found. In this molecular weight range all the peptides carry terminal adenylic acid residues in accord with the mechanism discussed above. It is also remarkable that practically no hydrolysed monomer was found in the reaction mixture. This indicates that montmorillonite protected the adenylates efficiently and allowed their full polymerization.

Similar behaviour was observed with a more primitive form of clay called illite, which is regarded as one of the geological precursors of montmorillonite, and which is able to swell to some extent. It was found that in the presence of illite, all the monomers polymerized, although the degrees of polymerization were lower than in the present experiments, ranging between 10–16 alanine residues.

As pointed out by Calvin[18], the selection of chains of certain molecular weights from a large number of possibilities is typical of living processes. The finding that heterogeneous clay polycondensation is selective prompts further consideration. In an attempt to explain the grouping of molecular weights around 9, 16 (18), 27, 32 (36), 42 (45) and 54 (56), which seem to be multiples of a basic nonamer, we assumed some periodic nine membered structure in the montmorillonite surface or the particle edges. We failed to discover such a repeating unit and, moreover, the repeating number was different at other concentrations or with other particle sizes. Another possibility is that the discontinuous distribution is a case of a dissipative structure based on the coupling of diffusional and chemical flows. It will be recalled that during the polymerization process free adenylic acid is

Table 2. PEPTIDES OBTAINED ON POLYCONDENSATION OF ALANYL ADENYLATE

Peak No. from Fig. 3	Molecular weight of peptide adenylate	Degree of polymerization	Weight (mg)
4	2,130	30	8
2	2,310	32	5·2
1	3,020	42	10·8
3	4,000	56	17·1
6	Adenylic acid	1	260·1

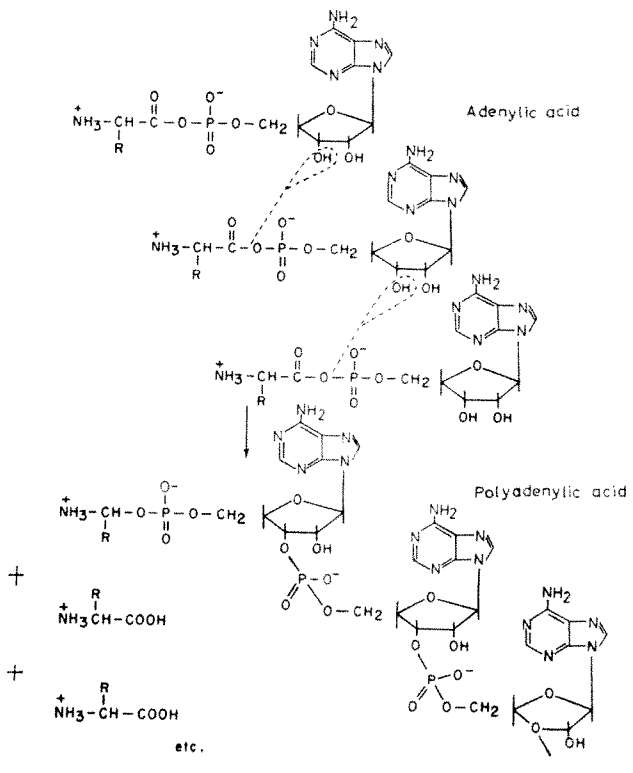


Fig. 4. Hypothetical formation of polyadenylic acid from amino-acid adenylates.

liberated which inhibits polycondensation by preventing the non-reactive ammonium group from dissociating with release of a proton and thereby producing the highly reactive terminal $-NH_2$. Thus we can readily envisage that a pulse of rapid polycondensation will produce a high local concentration of acid molecules which will stop the process until the adenylic acid diffuses out. After this diffusion, a second pulse will occur which will again be stopped after, say, nine steps by the accumulated acidity. For the joint operations of reaction and diffusion to give a periodic phenomenon therefore a correct matching of the processes and a suitable time lag are required.

Although many features have still to be studied, for example the dependence of the rate of polycondensation on the nature of the amino-acid and the nucleotide, the production of copolymers and their selective evolution from random monomeric mixtures, and, indeed, the influence of various physical factors on the process as a whole, it seems that the dissipative structures may play a part in all these reactions. The variable *p*H on the surface of the montmorillonite suggests other possibilities: it will be noted that the polycondensation and termination reactions described by equations (4) and (5) do not exhaust the combination possibilities of amino-acid nucleotide anhydrides and a possibility is the formation of polynucleotides with the liberation of free amino-acid.

The conditions for this process are unknown. It is, however, conceivable that in the periodic variation of *p*H the scheme according to Fig. 4 will predominate in one phase while the scheme according to Fig. 1 will predominate in another. Thus we could envisage the simultaneous formation of peptides and polynucleotides during an oscillatory process of clay catalysis.

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Mapping of the Combining Sites of Antibodies specific for Poly-L-alanine Determinants

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By modulating the structural features of a series of haptens and observing their strengths of interaction with antibody, conclusions are drawn which cast doubt on previous interpretations of hapten–subsite fitting.

THE hapten inhibition method is commonly used to study the size and nature of the combining sites in antibodies[1,2]. Because an antibody combining site is rather large (it can accommodate, for example, up to six glucose units[2], or three to seven amino-acid residues[3–9]), it is suggested that, for the purpose of mapping, it be divided into several subsites[10], defined as regions on the antibody molecule which interact with a particular portion of the antigenic determinant (or hapten). Knowing the size of the binding site (or the number of subsites), it is possible to map it in terms of the particular interactions which the various subsites can exhibit. This approach was used with advantage in exploring binding properties of chain-splitting enzymes such as lysozyme[11,12], papain[13–16], carboxypeptidase A[17], and others[18–20]. For example, it was found that the active site of papain can interact simultaneously with a stretch of seven amino-acid residues, and the binding energies of different amino-acid side chains in two subsites (out of a total of seven) were evaluated.

I have studied the properties of antibodies specific to poly-L-alanine chains (carrying free α-amino groups). In this case the combining site is visualized as being composed of several subsites, each accommodating one amino-acid residue of the antigenic determinant (Fig. 1). The extent and properties of the binding site are deduced from the correlation between the structural features of series of haptens and their strength of interaction with the antibody (as manifested by their capacity to inhibit the precipitin reaction). The types of structural changes applied here were: (1) elongation of the peptide chain of the hapten; (2) blocking of the amino function or the

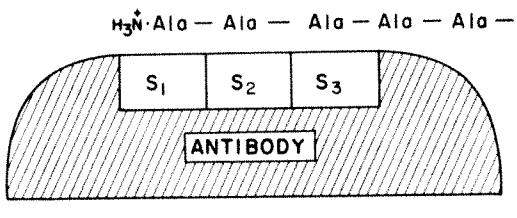


Fig. 1. Schematic representation of the complex formed between a poly-L-alanine chain and its specific antibody. The combining site of the antibody is composed of three subsites (S_1 to S_3) each accommodating one amino-acid residue of the antigenic determinant. The N-terminal L-alanine residue occupies S_1, the penultimate residue S_2, the third residue S_3, and the other alanine residues are outside the region of the combining site.

carboxyl function of the peptides; (3) replacement of an L-alanine residue in the peptide by its D antipode; (4) replacement of one L-alanine residue by a glycine, L-phenylalanine or L-valine residue. The influence of these changes on inhibitory power is taken as evidence of interaction of the modified portion of the peptide with the combining site.

It is well known that antibodies even to a unique determinant are heterogeneous, but this work demonstrates that it is possible, nevertheless, to attribute specific properties to the various subsites of the combining site. The results therefore represent average properties of subsites in different antibody molecules. The detailed structure of the subsites in different molecules should vary and work is in progress to evaluate the degree of variation of these properties by studying the combining sites of subfractions of this antibody population.

Antibodies to poly-L-alanine chains were obtained from rabbits immunized with poly-L-alanyl–human serum albumin[5]. A molecule of antigen contained on average 25 chains of polyalanine, each composed of 4·8 L-alanine residues. Pooled antisera were absorbed on poly-L-alanyl–rabbit serum albumin–cellulose conjugate according to Robbins *et al.*[21]. The purified antibodies were eluted from the immunoadsorbant with 0·010 M tetra-L-alanine in phosphate-buffered saline (*p*H 7·3) and then dialysed against the solvent to remove the peptide. The amount of purified antibody was 36 per cent of the total present in the original serum, and 72 per cent of it was precipitated by poly-L-alanyl–rabbit serum albumin. Quantitative inhibition studies of the precipitin reaction were performed as described[5]. The synthesis of alanine peptide diastereoisomers used as inhibitors has been reported[22]. Other peptides were prepared by the hydroxysuccinimide method[23]. The purity of these peptides was higher than 99 per cent as checked by high voltage electrophoresis at *p*H 1·4 and by chromatography in butanol–acetic acid–water.

Table 1. INHIBITION OF THE PRECIPITIN REACTION OF ANTIBODIES TO POLY-L-ALANINE BY ALANINE PEPTIDES

	Peptide concentration (mM) at 50 per cent inhibition				
Ala_n	H-L$_n$-OH	H-L$_{n-1}$D-OH	H-DL$_{n-1}$-OH	H-L$_n$-NH$_2$	Ac-L$_n$-OH
Ala_1	> 8·5 (6%)			> 8·5 (42%)	
Ala_2	8·5	> 8·5 (25%)		0·32	> 3 (0%)
Ala_3	0·32	2·4	> 3 (13%)	0·08	> 3 (10%)
Ala_4	0·09	0·11	> 3 (31%)	0·07	> 3 (22%)
Ala_5	0·08	0·07	> 3 (30%)	0·07	

Reaction mixtures contained purified antibodies, poly-L-alanine–rabbit serum albumin (serving as precipitating antigen) and peptide. A number of concentrations of peptides were used to determine the point of 50 per cent inhibition. When 50 per cent inhibition was not achieved, the percentage inhibition obtained at the maximal peptide concentration used is given in parentheses. H-L$_n$-OH, free L-alanine peptide; H-L$_{n-1}$D-OH, free alanine peptide with a D residue at the C terminus; HDL$_{n-1}$-OH, free alanine peptide with a D residue at the N terminus; H-L$_n$-NH$_2$ amide of alanine peptide; Ac-L$_n$-OH, N-acetyl alanyl peptide.

The degree of inhibition of anti-poly-L-alanyl antibodies by various alanine peptides is given in Table 1. For the purpose of analysis of the data we define subsite S_1 as the one which interacts with the N-terminal L-alanine residue (which is the most exposed in the antigenic determinant), whereas the adjacent residues in the antigenic determinant (or hapten) occupy subsites S_2, and so on (Fig. 1). The inhibitory capacity of free L-alanine peptides increases with their size up to the tetrapeptide, Ala_5 being as good an inhibitor as Ala_4. The common interpretation of these results would be that the combining site can accommodate a tetrapeptide[2,5], and in terms of the subsite concept the conclusion would be drawn that it is composed of four subsites. As I shall show, however, a more rigorous procedure shows the presence of only three subsites.

Precise information on the properties of the combining site can be derived from comparing haptens of equal size which differ from one another in one particular feature, rather than from studying the effect of elongation in a homologous series of peptides. This is because the stronger binding caused by elongation does not necessarily arise from additional interaction by the extra piece, but can also be attributed to the abolition of unfavourable interactions in the smaller hapten. Thus the finding that Ala_4 is a better inhibitor than Ala_3 by a factor of 3 does not prove the existence of subsite S_4, for it may reflect a lack of interaction between the carboxylate of Ala_3 and subsite S_3. Indeed, the inhibitory capacity of the amide of Ala_3, which is equal in size to Ala_3 but without the carboxylate ion, is much better than that of Ala_3 and similar to that of Ala_4 and Ala_5 (see Table 1). Other erroneous conclusions could be drawn from the use of the free peptide series alone. For example, the contribution of the third residue is over-estimated by using data obtained with free peptides, for elongation from di to tripeptide increases inhibitory efficacy by a factor of 26. The data obtained with the corresponding amides give a factor of 4 (Table 1), which seems to be a better estimate for this elongation.

The effects of systematic variations in the structure of tetra-L-alanine are shown in Tables 2 and 3. Replacement of an L-alanine residue by D-alanine reduces binding when it occurs in the first three positions, but it is not effective in the fourth position (from the N-terminal end). The effect of diastereoisomeric replacement is stronger the closer it is to the N-terminal end (Table 2). Substitution of L-alanine by L-phenylalanine (or L-valine) in the N-terminal position reduces binding markedly. This effect diminishes gradually with distance from the N-terminal end, and disappears in the fourth position (the C-terminal end). A similar pattern is observed by substituting glycine for L-alanine (Table 3).

Table 2. THE EFFECT OF DIASTEREOISOMERIC REPLACEMENTS ON THE INHIBITORY CAPACITY OF TETRA-ALANINE PEPTIDES

H-Ala_4-OH configuration	Concentration (mM) at 50 per cent inhibition
LLLL	0·09
LLLD	0·11
LLDL	1·8
LDLL	> 3 (28%)
DLLL	> 3 (31%)

Reaction mixtures as in Table 1. When 50 per cent inhibition was not achieved, the percentage obtained at the maximal peptide concentration used is given in parentheses.

Because the peptide determinants in the antigen have no free carboxyl groups, more precise information on the combining site will be obtained by means of peptide amides than by the use of free peptides. A comparison between the amides and the free peptides shows that the binding of H-Ala_n-NH_2 is almost equal to that of H-Ala_{n+1}-OH (Table 1). This means that in free peptides either the carbon portion of the C-terminal residue does not interact with the combining site, or that interference caused by the carboxylate of H-Ala_n-OH is counterbalanced by the interaction of the carbon portion of the C-terminal alanine in H-Ala_{n+1}-OH. The latter conclusion is correct, for Ala_3(L$_3$) is a better inhibitor than Ala_3(L$_2$D). The finding that amidation improved the inhibitory capacity of alanine, Ala_2 and Ala_3 (but not of Ala_4 and Ala_5) shows that the carboxylate ion interferes with binding up to the third residue.

Thus, diastereoisomeric replacements, the presence of carboxylate ion and changes in bulkiness of the side chain (an increase Ala→Phe, or decrease Ala→Gly) are felt over three residues from the N-terminal end of an L-alanine peptide. The size of the combining site is therefore such as to accommodate three L-alanine residues; in other words, it is composed of three subsites (S_1, S_2 and S_3, Fig. 1). Stereospecificity is very strong in S_1 and S_2, and somewhat less in S_3 (Table 2). The structural requirements for the interaction of a ligand with S_1 are very stringent, less so in S_2 and even less in S_3. The strict requirements of S_1 are deduced from findings that N-acetyl-Ala_4(L$_4$) and Ala_5(DL$_4$) (which contain uninter-

Table 3. THE EFFECT OF SIDE CHAIN SUBSTITUTION ON THE INHIBITORY CAPACITY OF TETRAPEPTIDES

Peptide	C (mM)	Peptide	C (mM)	Peptide	C (mM)
Ala Ala Ala Ala	0·09				
Ala Ala Ala *Phe*	0·10			Ala Ala Ala *Gly*	0·09
Ala Ala *Phe* Ala	0·18			Ala Ala *Gly* Ala	0·18
Ala *Phe* Ala Ala	2·1	Ala *Val* Ala Ala	> 3 (32%)	Ala *Gly* Ala Ala	0·70
Phe Ala Ala Ala	> 3 (24%)	*Val* Ala Ala Ala	> 3 (24%)	*Gly* Ala Ala Ala	2·9

Reaction mixtures as in Table 1. C represents the concentration at 50 per cent inhibition. When 50 per cent inhibition was not achieved, the percentage inhibition obtained at the maximal peptide concentration used is given in parentheses. All amino-acids are L.

rupted sequences of four L-alanine residues) are very poor inhibitors compared with Ala_4 (Table 1): a non-N-terminal L-alanine residue does not bind efficiently in S_1. Also, the highest degree of space limitation towards L-phenylalanine and L-valine residues is exhibited by subsite S_1 (Table 3).

Because a glycine residue occupies less space than an alanine residue the inhibition data can be used, by a first approximation, to calculate the binding energy of the methyl side chain of L-alanine ($-\Delta^2F$) in the various subsites. For this purpose the inhibitory capacity of pairs of peptides of equal size is compared, using the relation $-\Delta F = RT \ln(C_{Pep}/C_{Ala})$[24]. For example, the free energy of binding of the β methyl in S_2 is calculated by using the concentrations, at 50 per cent inhibition, of $AlaGlyAla_2(C_{Pep})$ and $Ala_4(C_{Ala})$. The $-\Delta^2F$ values so obtained are summarized in Table 4 and also show that in spite of the different inhibitory potencies of Ala_3 and Ala_4, the same $-\Delta^2F$ values for the binding of a methyl group in S_1 are obtained from the pairs $GlyAla_2$ and Ala_3 as well as $GlyAla_3$ and Ala_4. These findings strongly support our assumption that the peptides are lined up on the combining site in such a way that the free N-terminal residue always occupies the same subsite, that is, S_1. In all cases examined, the binding energies of the methyl groups in the various subsites were found to be independent and thus additive. The example given shows that the interaction of the methyl group in S_1 is independent of the presence of a charged carboxylate in S_3. In this connexion it can be mentioned that binding of a methyl in S_3 is independent of the presence of β-methyl in the fourth position (compare $Ala_2GlyAla$ and Ala_2Gly_2 with Ala_4). The additivity is demonstrated by the fact that in peptides in which two alanine residues were replaced by glycines the $-\Delta^2F$ values calculated were equal to the sum of the $-\Delta^2F$ values of the appropriate single replacements. For example, the change in binding energy between Gly_2Ala_2 and Ala_4 was found to be 3·38 kcalories mole^{-1}, which is the same as the sum of the $-\Delta^2F$ values determined separately for S_1 and S_2 (2·1 + 1·25 = 3·35 kcalories mole^{-1}; see Table 4 for other examples).

Table 4. BINDING ENERGY OF THE METHYL SIDE CHAIN OF L-ALANINE IN THE VARIOUS SUBSITES OF ANTIBODIES TO POLY-L-ALANINE CHAINS

Peptide	C (mM)	C_{rep}/C_{Ala}	$-\Delta^2F$ (kcalories mole^{-1})	Subsite explored
Ala Ala Ala Ala	0·09			
Gly Ala Ala Ala	2·9	32	2·1	S_1
Ala *Gly* Ala Ala	0·70	7·8	1·25	S_2
Ala Ala *Gly* Ala	0·18	2·0	0·42	S_3
Ala Ala Ala *Gly*	0·09	1·0	0	
Gly Gly Ala Ala	24	266	3·38	S_1+S_2
Ala Ala *Gly Gly*	0·17	1·9	0·39	S_3
Ala Ala Ala	0·32			
Gly Ala Ala	10	31	2·09	S_1
Ala *Gly Gly*	5·0	16	1·67	S_2+S_3

Reaction mixtures as in Table 1. C represents the concentration at 50 per cent inhibition. The free energy of binding of the methyl side chain (at 36° C) was calculated from the relation $-\Delta^2F = RT \ln(C_{rep}/C_{Ala})$, using peptides of equal size. All alanine residues are L.

The finding that the methyl groups of an alanine peptide are bound in the three subsites with different energies (2·1 kcalories mole^{-1} in S_1, 1·2 kcalories mole^{-1} in S_2 and 0·4 kcalories mole^{-1} in S_3) may give an important clue to the architecture of the binding site. Because it is reasonable to assume that hydrophobic interaction is the main source of binding energy, the differences observed seem to reflect differences in the degree of contact. In other words, one might say that in S_1 a methyl group of the hapten fits into a depression which completely surrounds it, in S_2 the depression is either shallower or wider, producing less contact, whereas in S_3 contact is still weaker. This would also account for the fact that S_1 cannot contain a phenyl group, S_2 accommodates it with difficulty, and in S_3 the interference is rather slight. The varying degrees of stereospecificity of the three subsites also fall into the same pattern.

A similar situation was found to exist in the binding of peptide inhibitors at the active site of papain which specifically binds aromatic residues in S_2 (refs. 14, 15). Binding energies of various amino-acid side chains in two subsites (S_1 and S_2) were determined and correlated with preliminary X-ray diffraction data of an enzyme-inhibitor complex[16]. The methyl side chain of alanine was found to be bound in S_1 with 0·85 kcalories mole^{-1}. The model showed that only about half of it is in hydrophobic contact with the enzyme surface. The phenyl group of phenylalanine was bound with 0·85 kcalories mole^{-1} in S_1 but with 3 kcalories mole^{-1} in S_1. Moreover, in S_1 contact is rather slight whereas in S_2 hydrophobic contact is extensive. It is also of interest that a binding energy of 4 kcalories mole^{-1} for a phenyl group was determined by Karush in the anti-L (or D)-phenyl-(*p*-azobenzoyl-amino) acetate system[25]. In terms of the lock and key theory one may say that a given key (methyl or phenyl group) may fit one lock better than another.

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Proton Magnetic Resonance Studies at 220 MHz of the Histidine Residues of Staphylococcal Nuclease

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The imidazole C2-H resonances in the 220 MHz proton magnetic resonance spectra of staphylococcal nuclease have been identified with the aid of computer curve-fitting. Spectra were fitted to Lorentzian curves for the measurement of peak area, and chemical shifts were fitted to theoretical *p*H titration curves for the precise determination of *pK* values. Such a high resolution analysis makes a conformational equilibrium hypothesis involving histidine unlikely in the case of nuclease.

HIGH resolution proton magnetic resonance (PMR) spectroscopy can potentially resolve signals from one kind of amino-acid located in different chemical environments within the three dimensional structure of a protein. At a resonance frequency of 100 MHz, however, only the C2 protons of the imidazole groups of histidine residues have been fully resolved in unmodified proteins[1,2]. Imidazole protons are known to absorb in the low field portion of the spectrum and to have a large *p*H dependent change in chemical shift[3]. It is for these reasons that PMR has been useful in obtaining the microscopic *pK* values of individual histidine residues in proteins[4,5] and in studying their interactions with substrate analogues[6] Because of the low signal strength, however, the crossing of peaks as the *p*H is changed and the presence of other resonances in this region of the spectrum, ambiguities occur which may make identification of resonances equivocal. We present here a computer-assisted study of the low field region of the PMR spectrum of nuclease, using the greater sensitivity and resolution obtainable at 220 MHz[7].

Computer curve fitting[8] was carried out with a least squares program (MODELAIDE)[9], using an IBM 360/50 computer with the IBM 2250 display. The computations were done in two stages. First, the PMR data were fitted to a sum of Lorentzian curves[10], in order to determine peak area (a), chemical shift (δ) and width (σ), even where resonances overlap. Then, the midpoints δ were fitted to theoretical titration curves derived from the Henderson–Hasselbach equation[11]

$$pH = pK + \log [B]/[HB] = pK + \log (\delta_{max} - \delta)/(\delta - \delta_{min}) \quad (1)$$

so that

$$\delta = (\delta_{max} + 10^{(pH - pk)}\, \delta_{min})/1 + 10^{(pH - pK)} \quad (2)$$

where chemical shift is considered a linear function of concentration. This computer analysis facilitates the construction of complete histidine titration curves and the determination of precise *pK* values. To be identified with imidazole C2 protons, resonances must satisfy two criteria: they should have equal area (because each arises from a single proton) and they should titrate between *p*H 5 and *p*H 8 (ref. 3).

The low field portions of 220 MHz PMR spectra of nuclease at various *p*H values are shown in Fig. 1. Although nuclease has only four histidine residues[12] five peaks are clearly visible in many spectra (see, for example, Fig. 1*b*). An example of computer curve fitting of the spectrum in Fig. 1*b* is shown in Fig. 2. This spectrum was fitted with six Lorentzian curves, the baseline being approximated by a single large upfield Lorentzian peak. Initial values of a, δ and σ were estimated; the program then altered these parameters until convergence was achieved. The relative areas of the peaks in this spectrum are indicated in Fig. 2. (No experimental accuracy for area measurements can be quoted in view of different signal/noise ratios in individual spectra and the potential degeneracy of solutions where two or more Lorentzian peaks overlap.)

With increase in *p*H, peaks H-1 to H-4 are observed to shift upfield while the peak labelled X-1 undergoes little change in chemical shift. (X-1 broadens and is difficult to resolve above *p*H 6.) The chemical shifts of these resonances as a function of *p*H are shown in Fig. 3, at two

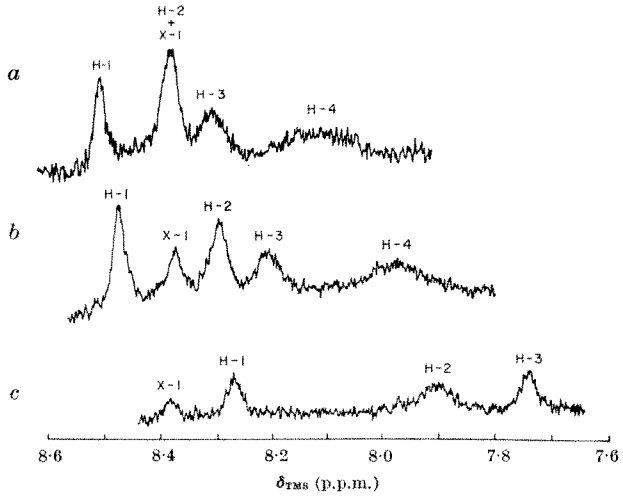


Fig. 1. Spectra were recorded on a Varian Associates 'HR-220' spectrometer at the ambient probe temperature of 22 ± 1° C. Each spectrum was calibrated with the same 6 per cent TMS in CCl_4 sample using the sideband technique, and chemical shift values are in p.p.m. downfield from this standard. Values of *p*H are uncorrected meter readings. All other experimental details are as reported previously[4]. 220 MHz time-averaged PMR spectra of staphylococcal nuclease at 100 mg/ml. in 0·1 M NaCl-^2H_2O (100 per cent). *a*, *p*H 4·56; *b*, *p*H 4·97; *c*, *p*H 6·00. The four peaks H-1 to H-4 are identified as the four C2 imidazole resonances; the peak X-1 does not shift with *p*H. H-3 and H-4 are broader than usual in this sample, but sharpen with increase in *p*H, while H-2 broadens as normal.

concentrations of NaCl. The computer-drawn plots of the Henderson–Hasselbach relationship which gives the best fit in each case and the computed apparent pK values are also shown.

Fig. 3 demonstrates that the titration curves of H-1 and H-2 cross each other. Such chiasmata are fairly common where there are multiple histidine resonances. We have examined both possible continuities after the point of superposition, and find much poorer fits to the theoretical Henderson–Hasselbach relationship in the case where no crossover is assumed. This is indicated by the computer fit in which the relative value of the residual sum of squares without crossover is thirty times greater than with crossover. Thus the computer assisted analysis aids determination of the true continuity.

The different histidine pK values indicate differences in the microscopic environment of each of the imidazole side chains. Ease of protonation is determined by the local dielectric constant and the proximity of positive and negative charges[13]. Attempts to quantitate these effects, as in the Linderstrøm–Lang model of spherically symmetrical charges[14], are inadequate at the molecular level. We have therefore left the results as individual apparent pK values. The effect of increasing salt concentration on these microscopic pK values is difficult to predict because it depends on the sign of charges near the imidazole ring, binding of the cations and anions and possible changes in protein conformation. The major effect of high salt concentration, however, would probably be to shield the imidazole from the increasing number of positive charges on the molecule as pH is lowered. This would explain the rise in the apparent pK.

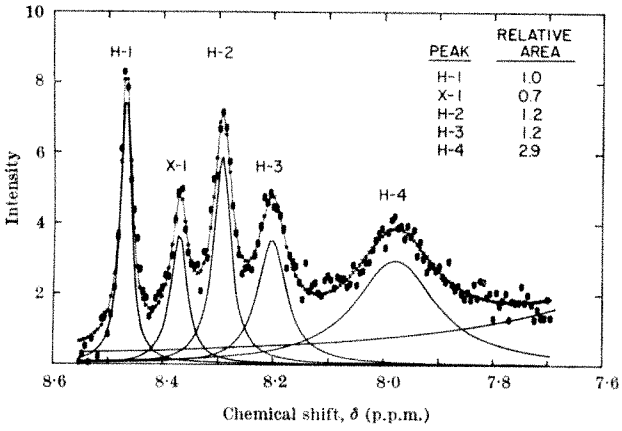


Fig. 2. CALCOMP plot of 220 MHz nuclease spectrum 1*b* fitted with Lorentzian curves. ●, Observed; ★, calculated; ———, components. The computer calculated the areas of the component peaks which gave the best fit to the data; these areas are indicated by their ratio to the area of H-1 (taken as 1·0). The area of peak H-4 is increased because of the presence of several smaller broad resonances absorbing in the same region.

The net result of the curve fitting process applied to many spectra shows that the four peaks H-1 to H-4 are all of essentially equal area (see also ref. 8). When a histidine peak is in the region of the spectrum from 7·8 to 8·1 p.p.m., however, its area is increased (as for H-4 in Fig. 2) by superposition on several smaller resonances in this region. The X-1 resonance characteristically has an area about half that of peaks H-1 to H-4. Further, the peaks H-1 to H-4 titrate as expected, and are therefore identified as the four imidazole C2-H resonances. The peak X-1 and other smaller resonances seem to be of non-histidine origin.

In previous PMR studies at 100 MHz of the same low field region of the nuclease spectrum, Jardetzky and his co-workers[15–18] observed the resonances described here. But they interpreted[17] their data as indicating that one

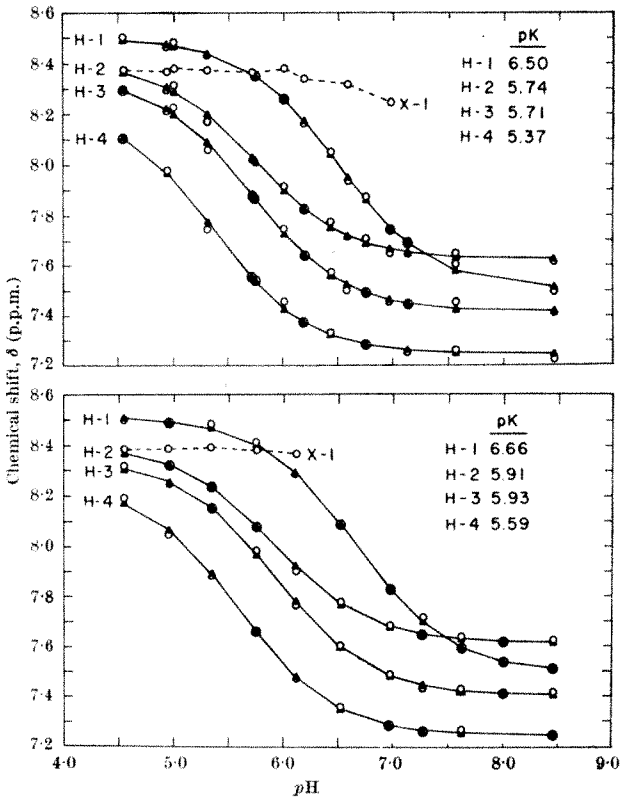


Fig. 3. Titration curves of the imidazole C2-H resonances of nuclease in 0·1 M NaCl (upper) and 0·3 M NaCl (lower). ○, Observed points; ▲, computer calculated points for the best fit to equation 2. The lines connect the calculated points. The separate curves have been traced from CALCOMP plots on the composite figure.

histidine residue is located in two different, slowly exchanging conformational environments. Their interpretation asserts (*a*) that two peaks (which they call H2*a* and H2*b*) have partial proton areas, and sum to unity relative to the other discrete C2-H resonances; (*b*) that the two titration curves* of H2a and H2b are related by the exchange phenomenon, and that neither curve can be described by a single Henderson–Hasselbach equation[8,19]. We correlate their H2a resonance with X-1 and their H2b resonance with H-2. (A number of spectra were also run at 32° C, the temperature used in the 100 MHz study[17], with no alteration in the findings.) We have found that the sum of the areas of these resonances is significantly greater than unity (Fig. 2). This can also be seen in Fig. 1*a*, where the superposition of peaks H-2 and X-1 results in a relative area (2·2) significantly larger than those of H-1 and H-3 (both 1·0). The computer fitted results indicate no significant deviation from the theoretical titration relationship for H-2 (Fig. 3).

A conformational equilibrium in ribonuclease involving a histidine residue has been postulated[8]. The demonstration of such equilibria in several proteins would be important in the interpretation of protein structure in solution. We believe, however, that the higher resolution at 220 MHz, together with the computer assisted analysis of the data, has yielded a more complete understanding of this region of the PMR spectrum which makes this conformational equilibrium hypothesis involving histidine unlikely in the case of nuclease. Other spectroscopic studies show evidence of structural transitions only below pH 5 and above pH 10 for this protein[20,21].

The ability to measure areas of peaks has enabled us to follow the process of denaturation in some detail. The

* In addition to X-1, another resonance of partial proton area was seen with some samples. This corresponds in chemical shift to the chemically equivalent C2 protons of denatured nuclease, and titrates with pH. This peak can be a source of ambiguity in the identification of resonances.

capability of fitting titration data, ascertaining continuity, and determining apparent pK values with precision[8] should extend the usefulness of PMR in studying histidine residues of small proteins and their interactions with substrate analogues.

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Stability of Binuclear Complexes

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Empirical arguments lead to a relationship between the stability constant for the formation of a binuclear complex $MXM^{(2m-x)+}$, and that for the corresponding mononuclear complex $MX^{(m-x)+}$. As K_{MX} increases along a series of metal ions M^{m+}, with a given ligand X^{x-}, it is predicted that K_{MXM} will pass through a maximum value, in agreement with available data.

BINUCLEAR complexes are frequently postulated as intermediates in reactions between metal ions in aqueous solution, especially redox reactions. Stabilities of such complexes are clearly important in the discussion of reaction rates[1] and they can also be relevant to the assignment of mechanisms. In reaction 1, for example, a binuclear intermediate is assumed but its lifetime is not known; only the second-order rate constant, K_1k_1, can be measured. By analogy with complexes of other metals, however, K_1 has been estimated[29] to be of the order of $10^{0\pm1}$ M^{-1} (at 25° C), whence $k_1 = 10^{6\pm1}$ s^{-1}. This shows that the redox process, although rapid, is not as fast as the rate of replacement

$$Co^{III}[NH_2(CH_2)_2NH_2](H_2O)OH^{2+} + Cr^{2+} \overset{K_1}{\rightleftharpoons} \{Co^{III}[NH_2(CH_2)_2NH_2](H_2O)OHCr^{II}\}^{4+} \xrightarrow{k_1} Co^{II} + Cr^{III} \quad (1)$$

of water molecules in the coordination sphere of the Cr^{2+} ion. If it had been otherwise, the suggested mechanism would have been untenable. In reaction 2 the binuclear intermediate was observed directly[2] but the valency states had still to be determined

$$cis\text{-}Ru^{III}(NH_3)_4Cl_2^+ + Cr^{2+} \overset{K_2}{\rightleftharpoons} [(NH_3)_4RuCl_2Cr]^{3+} \rightarrow Ru^{II} + Cr^{III} \quad (2)$$

It was argued that the observed value of K_2 (460 M^{-1} at 25° C) was too large for an Ru^{III}–Cr^{II} binuclear species and the assignment Ru^{II}–Cr^{III} was preferred.

Many other cases could be cited where a knowledge (or even a rough estimate) of the tendency to form binuclear species would be of value, but the data are still comparatively sparse. The aim of this article is to propose a general relationship by which stabilities of certain types of binuclear complex may be estimated from those of the corresponding mononuclear species.

Order of Stabilities

Consider two metal ions M^{m+} joined by a ligand X^{x-}; suppose that X^{x-} is a sufficiently strong base to give two conjugate acids $HX^{(x-1)-}$ and $H_2X^{(x-2)-}$, and further

Table 1. COMPARISON OF PROTON AFFINITIES OF METAL COMPLEXES $MX^{(m-x)+}$ WITH THOSE OF THE PROTONATED LIGANDS $HX^{(x-1)-}$

	Ligand X^{x-}	Central ion* M^{m+}	log K_{HX}	log $K_{H,XM}$	log K_{HXH}
(*a*)	CH_3O^-	$Co(NH_3)_5^{3+}$	> 15·7	5·6	−2·5
				(ref. 34)	(ref. 28)
	HO^-	Cr^{3+}	15·7	3·8	−1·7
	HOO^-	Fe^{3+}	12	2·96	−3
	PO_4^{3-}	$Co(NH_3)_5^{3+}$	11·8	8·5	6·3
		Sr^{2+}	11·7†	8·7	6·8†
	CO_3^{2-}	$Co(NH_3)_5^{3+}$	10·4	6·4	3·8
	$HN(PO_3)_2^{4-}$	Ca^{2+}	10·4	8·9	4·6
	HPO_4^{2-}	$Co(NH_3)_5^{3+}$	6·3	3·6	1·7
	CH_3COO^-	Cr^{3+}	4·5	0·7	−6·2
				(ref. 35)	(ref. 36)
	$H_2PO_4^{2-}$	$Co(NH_3)_5^{3+}$	1·7	−0·67	−3
		UO_2^{2+}	1·7	0·0	−3
	SO_4^{2-}	Fe^{3+}	1·0	−0·4	−3
					(ref. 37)
		Fe^{2+}	1·1†	0·6	−3
(*b*)	HPO_3^{2-}	$Cr^{III}(H_2PO_3)_2^+$	6·6	2·7	1·3
	N_3^-	$Co^{III}(CN)_5^{2-}$	4·5	0·7	−6·2
	$H_2PO_4^-$	$UO_2.PO_4H_2^+$	2·1	1·1	−3
		$UO_2.PO_4H_2^{2+}$	2·1	1·5	−3
		$UO_2(PO_4)_2H$	2·1	1·4	−3
		$UO_2(PO_4)_2H^{4+}$	2·1	2·3	−3
		$UO_2(PO_4)_2H_2^{2+}$	2·1	0·5	−3
	SO_4^{2-}	$ThSO^{2+}$	1·1	0·45	−3
					(ref. 37)
		$FeSO_4^+$	1·0†	−0·6	−3
		$PbSO_4$	2·0†	1·3	−3
(*c*)	$TeO_6H_3^{3-}$	$Mn^{IV}(TeO_6H_3)_2$	10·4	2·2	8·8
	$TeO_6H_3^-$	$Mn^{IV}(TeO_6H_3)_2^{2-}$	14·5	7·5	10·4
	$TeO_6H_2^{4-}$	$Mn^{IV}(TeO_6H_3)_2^{2-}$	?	11·5	14·5
	OH^-	CH_3^+	15·7	−2·5	−1·7
				(ref. 28)	

Data from reference 3 except where otherwise shown.
* Coordinated water molecules not shown.
† Where different values are quoted for the same constant, they refer to different ionic media.

that the complex $MX^{(m-x)+}$ can be protonated to yield $MXH^{(m+1-x)+}$. Species of this kind are occasionally found where X^{x-} is a multidentate or highly charged anion[3]; they have also been recognized as intermediates in acid catalysed aquation reactions[4], and a fair number of pK_a values are available. Taking the proton as central ion there is a series of three complexes, $H^+ X^{x-}$, $H^+XM^{(x-m)-}$, $H^+XH^{(x-1)-}$, with stability constants defined by

$$K_{HX} = [HX^{(x-1)-}]/[H^+][X^{x-}] \quad (3a)$$

$$K_{H,XM} = [HXM^{(x-m-1)-}]/[H^+][XM^{(x-m)-}] \quad (3b)$$

$$K_{HXM} = [HXH^{(x-2)-}]/[H^+][XH^{(x-1)-}] \quad (3c)$$

It turns out that in most cases stabilities decrease in the order $K_{HX} > K_{H,XM} > K_{HXH}$ (that is, the hydrogen ion which is to be attached to X is repelled either by an existing metal ion, or by a hydrogen ion, but more so by the latter). Table 1, section *a*, gives some idea of the variety of metals and ligands which obey this rule, and section *b* of the Table shows that the same holds good (with one exception) in complexes with negative ligands already attached to the metal.

It might be expected that a small and highly charged metal ion would have a greater repulsive effect than the proton, but although this can happen it is quite rare. Examples (Table 1, section *c*) include manganese(IV) in certain complexes with tellurate ion, and the alkyl carbon atom when considered as a solvated "metal" in ions of the type $CH_3OH_2^+$ (the latter expressing the familiar fact that alcohols are less basic than water).

Similar comparisons can be made taking M^{m+} itself as central ion. The complexes are $M^{m+}{\cdot}X^{x-}$, $M^{m+}{\cdot}XM^{(x-m)-}$, $M^{m+}{\cdot}XH^{(x-1)-}$, with stability constants defined as

$$K_{MX} = [MX^{(m-x)+}]/[M^{m+}][X^{x-}] \quad (4a)$$

$$K_{MXM} = [MXM^{(2m-x)+}]/[M^{m+}][XM^{(x-m)-}] \quad (4b)$$

$$K_{M,XH} = [MXH^{(m+1-x)+}]/[M^{m+}][XH^{(m-1)-}] \quad (4c)$$

and by analogy with the previous results, the stability sequence is expected to be $K_{MX} > K_{MXM} > K_{M,XH}$, though again, with small and highly charged metal ions, the order might change to $K_{MX} > K_{M,XH} > K_{MXM}$.

Free Energies

The assumption now proposed is that in both series the repulsive effects of M^{m+} and H^+, expressed as free energies, are in the same ratio. In other words, the value of log K_{MXM} divides the interval (log K_{MX}–log $K_{M,XH}$) in the same ratio that log $K_{H,XM}$ divides the interval (log K_{HX} − log K_{HXH}).

$$\frac{\log K_{HX} - \log K_{H,XM}}{\log K_{HX} - \log K_{HXH}} = \frac{\log K_{MX} - \log K_{MXM}}{\log K_{MX} - \log K_{M,XH}} \quad (5)$$

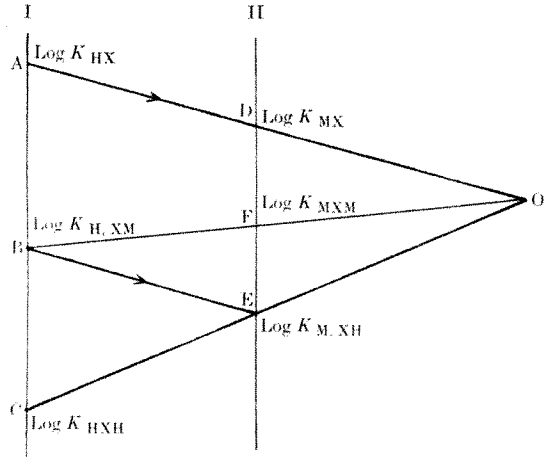


Fig. 1

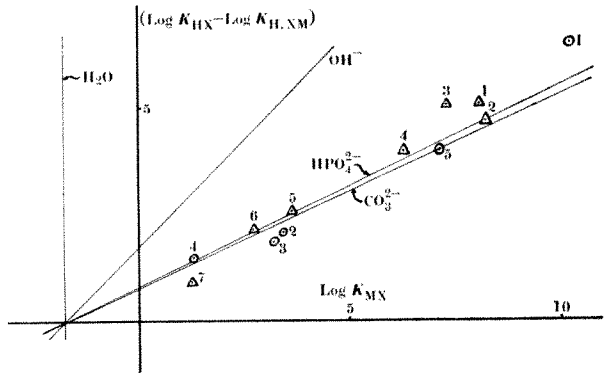


Fig. 2. Effect of coordinated metal ions M on the proton affinity of ligands X. △, Complexes of HPO_4^{2-}: 1, Al^{3+}; 2, Fe^{3+}; 3, $Al(H_2PO_4)_2^{2+}$; 4, $Al(H_2PO_4)^{2+}$; 5, $Co(NH_3)_5^{3+}$; 6, Ca^{2+}; 7, Sr^{2+}. ⊙, Complexes of CO_3^{2-}: 1, H^+; 2, Mg^{2+}; 3, Ca^{2+}; 4, Na^+; 5, $Co(NH_3)_5^{3+}$. Data from ref. 3.

and because, by definition,

$$K_{HX} \cdot K_{M,XH} = K_{MX} \cdot K_{H,XM} \quad (6)$$

this gives

$$\log K_{MXM} = \log K_{MX} - \frac{(\log K_{HX} - \log K_{H,XM})^2}{(\log K_{HX} - \log K_{HXH})} \quad (7)$$

Fig. 1 shows that the basic assumption is reasonable. On a vertical axis I, values of log K_{HX}, log $K_{H,XM}$ and log K_{HXH} are plotted as points A, B, C and on an adjacent parallel axis II, log K_{MX} is plotted as point D. From equation 6, a line through B parallel to AD gives E, corresponding to log $K_{M,XH}$. Let AD and CE meet at O. Then, if equation 5 is valid, joining OB gives F on line II corresponding to log K_{MXM}. The assumption is thus equivalent to saying that $MX^{(m-x)+}$ can be treated as a ligand and that there are linear free energy relationships between the stabilities of its complexes and those of the other ligands X^{x-} and $XH^{(x-1)-}$.

Equation 7 contains two independent variables, K_{MX} and $K_{H,XM}$, but in certain cases one of them can be eliminated. For a given ligand X^{x-} with a series of metals M^{m+}, it is suggested that the acidity of the H in $MXH^{(m+1-x)+}$ will be related to the stability of $MX^{(m-x)+}$. This is clearly to be expected if the bonding is predominantly electrostatic; but, if not, it can still be argued that a highly polarizing metal M^{m+} will take a disproportionate share of electrons and so weaken the X-H bond. Fig. 2 appears to support this assumption, and in the special case $X = OH^-$ we obtain a straight line relationship by definition. Taking

$$K_{MX} = [MOH^{(m-1)+}]/[M^{m+}][OH^-] \quad (8a)$$

and

$$K_{MXM} = [MOHM^{(2m-1)+}]/[MOH^{(m-1)+}][M^{m+}] \quad (8b)$$

the other equilibrium constants become

$$K_{M,XH} = a^{-1} \quad (9a)$$

$$K_{HX} = a/K_w \quad (9b)$$

$$K_{H,XM} = (K_w K_{MX})^{-1} \quad (9c)$$

$$K_{HXH} = a^{-1} \quad (9d)$$

where $K_w = [H^+][OH^-]$ and $a = [H_2O]$; and therefore

$$\log K_{HX} - \log K_{H,XM} = \log K_{MX} + \log a \quad (10)$$

which corresponds in Fig. 2 to a line of unit slope cutting the horizontal axis at log $K_{MX} = -\log a$. The further special case $X = H_2O$ gives a vertical line cutting the horizontal axis at the same point (that is, species $MOH_3^{(m+1)+}$ are expected to have varying acidities depending on the nature of M, but K_{MX} is always equal to a^{-1}). For complexes with monohydrogen phosphate ion the data are sufficient to suggest a linear relationship; for carbonate ion there are fewer points, but it is notice-

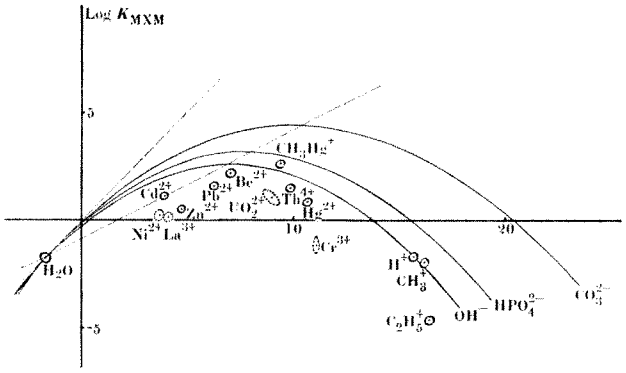


Fig. 3. Stabilities of complexes $M_2X^{(2m-x)+}$. The points are data for $X^{x-}\equiv OH^-$. A dotted line surrounding a point indicates approximate limits of error; a full line indicates that the limits of error are too small to be shown clearly. The curves are calculated from equation 13 and the straight lines have slopes 1 and ½.

able that in both cases the best straight line through the available points gives a negative intercept on the log K_{MX} axis. This suggests the general relationship,

$$\log K_{HX} - \log K_{H,XM} = \alpha(\log K_{MX} + \log a) \qquad (11)$$

where α is a constant depending only on the nature of X. In particular

$$\log K_{HX} - \log K_{HXH} = \alpha(\log K_{HX} + \log a) \qquad (12)$$

so that equation 7 becomes

$$\log K_{MXM} = \log K_{MX} - \alpha \frac{(\log K_{MX} + \log a)^2}{(\log K_{HX} + \log a)} \qquad (13)$$

Thus, as K_{MX} varies over a series of metals, K_{MXM} will pass through a maximum value

$$\log K_{MXM} = \left(\frac{1}{4\alpha}\right)(\log K_{HX} + \log a) - \log a = \log K_{MXM}^{max} \qquad (14a)$$

say at

$$\log K_{MX} = \left(\frac{1}{2\alpha}\right)(\log K_{HX} + \log a) - \log a = \log K_{MX}^{max} \qquad (14b)$$

then

$$(\log K_{MXM}^{max} + \log a) = \tfrac{1}{2}(\log K_{MX}^{max} + \log a) \qquad (15)$$

independently of the nature of the ligand.

Using these equations, curves predicting log K_{MXM} for different metals and ligands are shown in Fig. 3. Geometrical features of the diagram are that all maxima fall on the line defined by equation 15, and all curves pass through a common point with unit slope. The common point can be assigned to the "metal" H_2O, with log K_{MX} = log K_{MXM} = − log a. With carbonate ion as ligand, maximum stability of the bridged binuclear complex is expected to occur when K_{MX} is of the order of 10^{11} M^{-1}, but unfortunately most of the stability constants so far reported[3] are considerably less than this and it seems doubtful whether the predicted turnover in log K_{MXM} will be verified experimentally. A similar difficulty arises with the monohydrogen phosphate ion, though log K_{MX}^{max} is appreciably lower and given a sufficient number of experimental results some curvature might become apparent. For hydroxide ion, however, there is a fairly extensive series of data, and with the special values (equations 9) of the equilibrium constants, equation 7 becomes

$$\log K_{MXM} = \log K_{MX} - (\log K_{MX} + \log a)^2 / (2 \log a - \log K_w) \qquad (16)$$

predicting a maximum log k_{MXM} at log $K_{MX} = \frac{1}{2}\, pK_w \cong 7{\cdot}0$, well within the range of reported values.

The hydroxide data are entered in Fig. 3. Wherever possible, values of K_{MX} and K_{MXM} are taken from studies[3,5] in which both MOH and M_2OH were detected in the same medium, and limits of error are those suggested by the authors. The data for Be^{2+} are probably the most reliable of any. At least seven independent studies[6–12] confirm the existence of Be_2OH^{3+} and all but one[11] are agreed on the absence of $Be_2(OH)_2^{2+}$ under the conditions of the experiments. The difficulty at first was to establish the stability of the mononuclear species. Mattock[6] postulated $BeOH^+$ with log $K_{MX} = 7{\cdot}3$ in 1 M $NaClO_4$, and Kakihana and Sillén[7] invoked $Be(OH)_2$ with log $\beta_2 = 17{\cdot}5$ in 3 M $NaClO_4$, but Mesmer and Baes[12] have shown that species of this basicity are more probably polymeric. Schwarzenbach, however, had obtained log $K_{MX} = 8{\cdot}2$ in 0·1 M $NaClO_4$, in rapid reaction conditions and free from polymerization[13], and recalculation of the original data of Kakihana and Sillén has also given[14] the upper limit log $K_{MX} \leqslant 8{\cdot}1$ which (allowing for the different ionic media) is consistent with Schwarzenbach's value. The converse situation arose with chromium(III). The monomer $CrOH^{2+}$ has been studied many times[3] by conventional methods, but the singly bridged dimer is always metastable towards other species. It has now been prepared separately, however, and its stability obtained from kinetic studies[15]. Data[16,17] for $PbOH^+$ and Pb_2OH^{3+}, and[18,19] for $ThOH^{3+}$ and Th_2OH^{7+}, also came from different sets of experiments, but only because stabilities of monomer and dimer necessitated different concentration ranges of total metal ion. With UO_2^{2+}, polynuclear complexes, and $(UO_2)_2OH^{3+}$ in particular, are well established, but mononuclear $(UO_2)OH^+$ is a very minor constituent and its stability has only been roughly estimated[20]. Because the authors report an overall formation constant for the binuclear ion, log K_{MXM} depends partly on log K_{MX} and the "point" for UO_2^{2+} in Fig. 3 is a straight line of slope minus one.

The point for $C_2H_5^+$ refers to equation 17*c*, calculated as follows:

$$2C_2H_5OH(g) \rightleftharpoons (C_2H_5)_2O(g) + H_2O(g) \qquad (17a)$$

$$2C_2H_5OH(aq) \rightleftharpoons (C_2H_5)_2O(aq) + H_2O(aq) \qquad (17b)$$

$$C_2H_5OH_2^+(aq) + C_2H_5OH(aq) \rightleftharpoons (C_2H_5)_2OH^+(aq) + H_2O(aq) \qquad (17c)$$

Kabel and Johanson[21] determined the thermodynamic equilibrium constant of reaction 17*a* in the gas phase at 120·8° C (compare with ref. 22). Heats of combustion of ethanol[23] and diethyl ether[24] give $\Delta H^\circ = -5{\cdot}55$ kcalorie mole^{-1} at 25° C, and from the variation of C_p with temperature for gaseous ethanol[25], ether[26] and water[25], $\Delta G^\circ = -2{\cdot}68$ kcalorie mole^{-1} in the gaseous phase at 25° C. Equilibria of ethanol and ether between the gaseous and dilute aqueous phases were measured by Butler[27] and together with the free energy of evaporation of liquid water[25], these data yield $\Delta G^\circ = +2{\cdot}84$ kcalorie mole^{-1}, or log $K = -2{\cdot}08$ for equation 17*b*. The thermodynamic pK_a of protonated diethyl ether is reported as $-3{\cdot}59$[28]; for protonated ethanol no value is available, but for protonated methanol and *n*-butanol $pK_a = -2{\cdot}5$ and $-2{\cdot}3$ respectively[28]. These values seem typical of the respective functional groups, and there is little doubt that ethers are less basic than the corresponding alcohols by about one pK unit. Log $K \cong -3$ for equation 17*c*, and log $K_{MXM} \cong -4{\cdot}7$.

Diethyl ether is apparently the only ether for which this calculation can be carried out in full, but a fairly confident estimate can be made for dimethyl ether. Published free energy data[25] yield $\Delta G^\circ = -4{\cdot}21$ kcalorie mole^{-1} for the reaction corresponding to equation 17*a*. The free energy of hydration is not known, but for diethyl, di-*n*-propyl, and ethyl-*n*-propyl ethers, Butler[27] found it to be almost the sum of those for the two corresponding alcohols (actually less, by $0{\cdot}17 \pm 0{\cdot}04$ kcalorie mole^{-1}).

Assuming that the same is true for dimethyl ether, we obtain log $K = +1{\cdot}0$ for the reaction corresponding to equation 17*b*, so that[28] log $K_{MXM} \simeq -2{\cdot}0$.

All the data (except for $C_2H_5^+$, CH_3^+ and H^+) relate to high ionic strengths, and the appropriate values of log K_w vary from $-13{\cdot}7$ to $-14{\cdot}2$; the concentration of water also varies, but for convenience the curve in Fig. 3 is calculated from equation 16, using log $K_w = -14{\cdot}0$, and $a = 55{\cdot}5$ M.

It was stated previously[29] that, with the exception of Be^{2+}, values of K_{MXM} do not vary greatly from one metal to another even though K_{MX} varies over eight orders of magnitude, and that a value of log $K_{MXM} = 0{\cdot}0 \pm 1$ would cover most M_2OH complexes. This is still true, but it also seems that there is a relationship between K_{MXM} and K_{MX}, and that a maximum in log K_{MXM} occurs somewhere near the middle of the series. (Although the scatter is large, it can be pointed out that the points which for various reasons can be considered most reliable are by themselves sufficient to indicate a strongly curved dependence of log K_{MXM} on log K_{MX}. These are the reference point (H_2O) and the data for Cd^{2+}, Be^{2+}, CH_3Hg^+, Hg^{2+}, Cr^{3+}, H^+ and $C_2H_5^+$.)

Although the points follow the shape of the calculated curve fairly well, most of them lie significantly below it. Neglect of ionic strength effects may be one reason for this, but other correlations can also be pointed out. For example, the ions closest to the curve include those with the smallest radii (or alternatively those with lowest coordination number with respect to the solvent). Schwarzenbach[5] has particularly emphasized the analogy between CH^3Hg^+ and H^+, both having a coordination number of 1.

A non-linear relationship between the stability of mono and binuclear complexes was predicted by Martell[30] for dihydroxodimers of the form $LM(OH)_2ML$, where L is a non-bridging polydentate ligand. Martell pointed out that up to a certain point, increasing the nuclear charge of M would increase the stability of the dimer, but beyond that point repulsion between the two metal ions would become more important.

Simple Ionic Model

It is interesting to note that a simple ionic model leads to a relationship of the same form as equation 13. Assigning radii r_m and r_x, and effective charges $+z_m e$ and $-z_x e$ to metal and ligand respectively, the coulombic interaction energy in the mononuclear complex MX is given by

$$U_{MX} = -z_m z_x e^2/(r_m + r_x) \qquad (18)$$

Introducing a second metal ion M, with bond distances unchanged, and M-X-M bond angle 2θ, the additional coulombic terms are given by

$$U_{MXM} = z_m^2 e^2/2(r_m + r_x) \sin\theta - z_m z_x e^2/(r_m + r_x) \qquad (19)$$

$$= U_{MX} + (r_m + r_x) U_{MX}^2/2 z_x^2 e^2 \sin\theta \qquad (20)$$

For a series of complexes with constant r_m, variation in stability can be attributed to variation of z_m, and there is a maximum U_{MXM} when

$$z_m/z_x = \sin\theta \qquad (21)$$

$$U_{MXM} = -z_x^2 e^2 \sin\theta/2(r_m + r_x) \qquad (22)$$

$$= \tfrac{1}{2} U_{MX} \text{ (compare equation 15)} \qquad (23)$$

On the other hand, for a series of complexes in which r_m and z_m both vary, but U_{MX} remains constant, U_{MXM} is expected to become less negative with increasing r_m:

$$U_{MXM} = U_{MXM}^{\circ} + (U_{MXM}^{\circ} - U_{MX}) \frac{r_m}{r_x} \qquad (24)$$

where U_{MXM}° is the value of U_{MXM} corresponding to $r_m = 0$. Similar considerations apply when the ligand X is polyatomic, and the two metal ions are attached at different points. Thus the gaseous ionic model correctly predicts both the occurrence of the maximum stability, and the scatter of points according to ionic radii. On the other hand, it is certainly not realistic, for it predicts $z_m/z_x = 0{\cdot}87$ to $1{\cdot}0$ (taking θ from 60° to 90°), and total energies in the gas phase bear little relation to free energies in solution; a more detailed treatment would involve consideration of ion-solvent interactions.

Another factor is repulsion between non-bridging ligands[32,33]. This may be important with ions such as La^{3+}, if the positive charge is effectively delocalized on to the coordinated water molecules, or with Cr^{3+} in view of the well known rigidity of the octahedral structure. The difference in stability between methyl and ethyl ethers can also be attributed to steric effects, because it is equally pronounced in both gaseous and aqueous phases.

It must be emphasized that these arguments apply only when all four species MX, MXM, MXH, HXH have analogous structures. For instance, if X is a multidentate ligand giving a chelate MX, a relationship of the type of Fig. 3 may exist, but it can be shown that K_{MXM} will always be lower than the prediction of equation 13 by a factor depending on the magnitude of the chelate effect for the ligand in question. In a number of cases the acid H_2X is known or believed to have both protons attached to the same atom of X, whereas in M_2X this is precluded by steric factors. In such cases, only an upper limit can be obtained for the value of log K_{HXH}, and equation 5 can no longer be used.

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Crystal Structures of $[(CO)_3OsO]_4$ and $[(C_2H_5)_3PtCl]_4$ and a Discussion of Known and Possible Related Tetrameric Compounds

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Compounds with a cubane skeleton are found in nearly every group of the periodic table. The structure of two of these compounds has been determined.

RECENT X-ray diffraction studies in these laboratories have enabled us to determine the structure of the two compounds $[(CO)_3OsO]_4$ (ref. 1), Fig. 1, and $[(C_2H_5)_3PtCl]_4$ (ref. 2), Fig. 2; both of these are similar to the tetrameric "cubane—Type I" structure of Fig. 3 with an octahedrally coordinated metal atom and a trigonal ligand. ("Cubane", C_8H_8, has a structure in which there are eight C–H groups at the corners of a cube, Fig. 4 (ref. 3).)

The osmium compound is obtained in small yield in the preparation of $Os_3(CO)_{12}$ from OsO_4 and carbon monoxide in xylene[4,5]. The platinum compound was synthesized by Kettle[6]. Both structures are similar to that of $[(CH_3)_3PtCl]_4$ (Fig. 3, Type I, M=Pt, X=Cl, L=Me) reported some years ago[7]. This similarity and the fact that Os(II) and Pt(IV) are isoelectronic caused us to consider whether this structure is widespread for the other isoelectronic metals (W(0), Re(I) and Ir(III)) in the sequence. In fact, the compound $[(CO)_2(NO)Mo(OH)]_4$ (ref. 8) is formally of this type if one assumes that the NO group acts as a uni-positive radical with OH being the negative radical; this is of Type I with one NO and two CO groups on each metal atom.

Re(I) and Mn(I) of group VII form compounds $[(CO)_3M(I)SR]_4$ which were shown[9] by infrared spectroscopy to have structures with cubic symmetry. So far, no compounds of this type have been reported for the triad Co^{III}, Rh^{III} and Ir^{III}; in Table 1 we summarize examples of known compounds with the skeleton depicted schematically in Fig. 3. The metal may be 3, 4 or 6-coordinated.

In the transition metal series considered so far the metal has a d^6 configuration with low spin. Some of the compounds crystallize with $\bar{4}3m$ symmetry for the molecule while in others it approximates to this symmetry, although this is not a crystallographic requirement. For these compounds the general formula may be written $[L_3MX]_4$. For non-transition elements, however, the same MX skeleton with the same symmetry is found, although the general formulae correspond to $[MX]_4$ or $[LMX]_4$. A particularly interesting example is the non-metal compound "cubane" itself. The geometrical similarity between "cubane" and alkoxides and alkyl-amides such as MeZnOMe[10] (Fig. 5), TlOMe[11] (Fig. 6) and PhAlNPh[12] was pointed out by Tzschach and Balszuweit[13].

In Table 1, X covers a wide range of entities. It may be uni-negative Cl^-, I^-, OH^-, OR^-, SR^- or bi-negative O^{2-}, S^{2-}, NR^{2-}; it is even possible to describe $[PhSnP]_4$ formally in terms of a tri-negative P^{3-} and tri-positive $PhSn^{3+}$ but the ionic picture is no longer applicable to cubane itself (Fig. 4) in which each CH group forms three single bonds to other groups. In his review of polymers formed by alkoxo and alkyl-amido bridges, Bradley[14] considered that the number of bonds possible

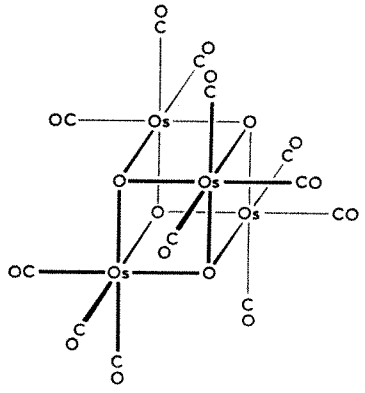


Fig. 1. The structure of $[(CO)_3OsO]_4$, tetra-μ_3-oxotetrakis[tricarbonylosmium (II)]. In this, as in most of the diagrams, the structure shown is idealized. As discussed in the text, the angles at the corners of the "cube" are not necessarily 90°.

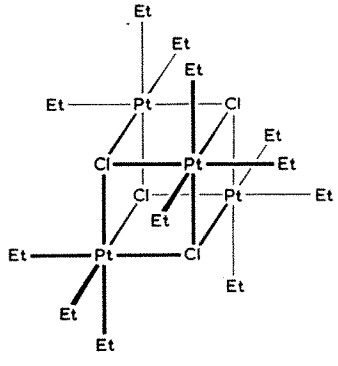


Fig. 2. The structure of $[Et_3PtCl]_4$, tetra-μ_3-chlorotetrakis[triethylplatinum (IV)].

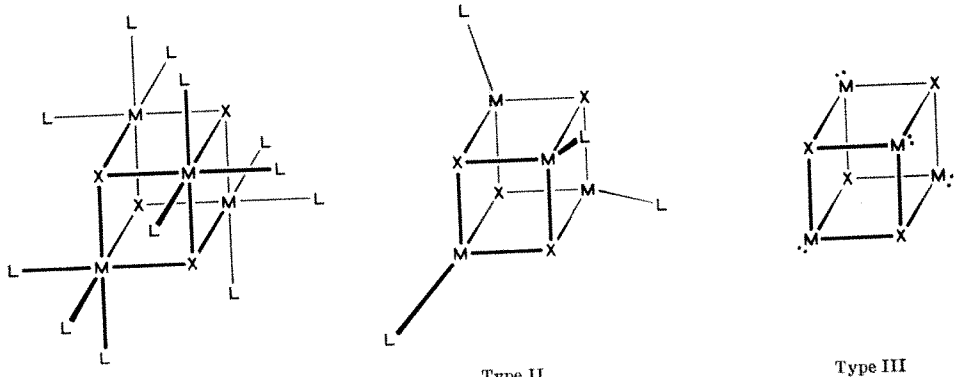


Fig. 3. Cubane-like structures. Type I, L_3MX, metal atom M, octahedral. Type II, LMX, metal atom M, tetrahedral. Type III, MX, metal atom M, trigonal. (In all cases X may be tetrahedral, for example, CH_3O^-; or pyramidal, for example, I^-.)

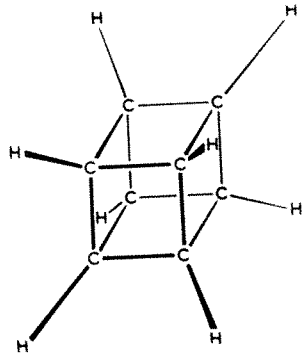


Fig. 4. The molecule of "cubane", C_8H_8.

was equal to the number of lone pairs available, that is, three in the uni-negative ions. Our description of L_3M, LM or M in Table 1 in terms of the number of electrons is based implicitly on an ionic model for alkali metals, for aluminium, and for the transition metals where the anionic entity is uni- or bi-negative. For the elements with d^{10} electron shells there are $[Me_3AsCu^+I^-]_4$ (ref. 15) (Fig. 7) in group IB and many examples of $[LMX]_4$ for group IIB where M is Zn, Cd or Hg (ref. 14), all of which may be reasonably written in the ionic form. For the compounds of Tl(I) and Pb(II), the neutral thallium alkoxides and the charged $[PbOH]_4^{4+}$, one may postulate a cation with a lone pair of electrons occupying the corner, as shown in Fig. 6. Recent calculations[16] indicate that less than 0·1 of an electron is involved in the metal–metal bonding postulated to explain the high intensity of the low-frequency Raman bands.

There is a lack of X-ray diffraction measurements on these interesting heavy metal compounds. In the only one studied in the crystalline form with the empirical formula TlOMe, a tetrahedron of thallium atoms was found[11] although no atoms of the methoxyl groups were located. Similar tetrahedra of metal atoms have been found in solutions of other thallium alkoxides[17] and of $[PbOH]_4^{4+}$ (ref. 18). For PhSnP the bonding is presumably covalent with sp^3 hybridized tin being bound to one phenyl group and three trivalent phosphorus atoms. The dimensions of this molecule are not known, the evidence[19] in support of the proposed tetrameric structure being the cryoscopic molecular weight and the infrared spectrum which is consistent with high symmetry. In addition to the possible electrostatic, covalent and metal–metal bonding mentioned, the "cubane" structure is also found with electron deficient bonding in lithium methyl[20]. Here X ($=CH_3^-$) has only one lone pair of electrons so it must form three electron-deficient bonds. Ironically the first reported compound with a methyl group acting in this way, $[(CH_3)_3PtCH_3]_4$, which led to the late R. E. Rundle's excellent work on electron-deficient compounds, has since been shown to be $[(CH_3)_3PtOH]_4$ (ref. 21).

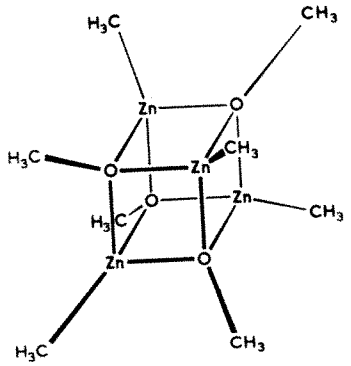


Fig. 5. Methylzinc methoxide tetramer.

Table 1. COMPOUNDS WITH THE "CUBANE" SKELETON

Group	I	II	III	IV	V	VI	VII	VIII	
d^n on M	d^0	d^0	d^0			d^6	d^6	d^6 d^8	d^8
Type	MX		L M X	LMX (X=CH)		L_3 M X	L_3 M X	L_3 M X	L_3 M X
Formula	LiMe (ref. 20)		PhAlNPh (ref. 12)	HCCH (ref. 3)		$(CO)_3(NO)MoOH$ (ref. 8)	$(CO)_3MnSR$ (ref. 9)	$(CO)_3OsO$	Me_3PtCl (ref. 7)
	$KOBu^t$ (refs. 22, 23)						$(CO)_3ReSR$ (ref. 9)		Et_3PtCl (refs. 1, 2)
	$RbOBu^t$								Me_3PtOH (refs. 29, 30)
	$CsOBu^t$								Me_3PtI (ref. 31)
	Ib	IIb	IIIb	IVb	IVb				
	d^{10}	d^{10}	d^{10} s^2	d^{10}	d^{10} s^2				
	L M X	L M X	M X	L M X	M X				
	Me_3AsCuI (ref. 16)	MeZnOMe (ref. 10)	T10Me (ref. 11)	PhSnP (ref. 19)	$PbOH^+$ (ref. 18)				

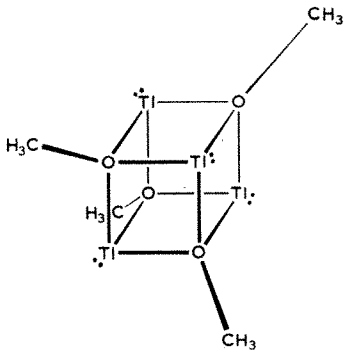


Fig. 6. Thallousmethoxide tetramer.

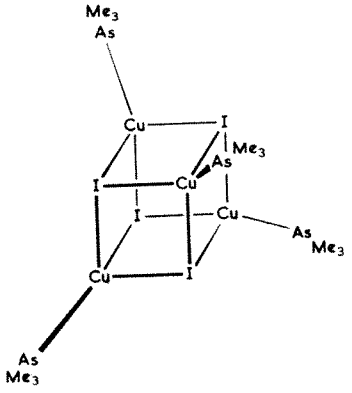


Fig. 7. Iodotrimethylarsine copper (I).

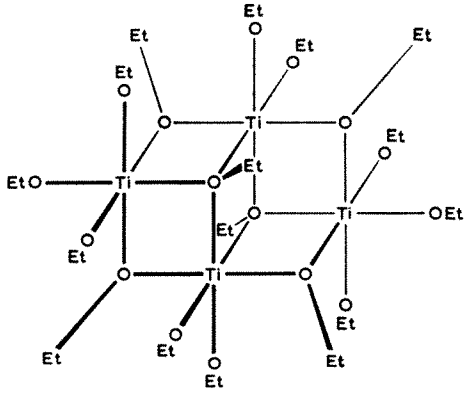


Fig. 8. Titanium ethoxide tetramer.

The compounds can be considered as tetrahedra of four M atoms with X in a position along the normal at the centre of a face of the tetrahedron. If X were at zero distance along the normal it would lie in the face and M–X–M would be 120°. If the distance along the normal is 0·409 times the metal–metal distance the angles M–X–M and X–M–X would both be 90°, that is, the ideal "cube". Greater distances can lead to a description in terms of X being "above the faces of the tetrahedron". If the distance along the normal is very long there is presumptive evidence of metal–metal bonding. Comparison of M–X with M–M gives the simplest criterion, M–X = 0·433 × M–M for X in the face of the tetrahedron; MX = 0·707 M–M for the ideal cube, and M–X > 0·707 M–M for a tetrahedron with X above the faces.

For the d^6 metals M–X ≈ 0·65 M–M in trialkyl platinum compounds[2,7] this corresponds to angles of 80° at Pt and 100° at X, in both cases 10° smaller than the ideal octahedral and tetrahedral angles respectively. Potassium tertiary butoxide (K–O = 0·707 × K–K) is described as having an "only slightly distorted cubic" structure[22,23]; PhAlNPh is similar[12] but the Li–C distance[20] is 0·89 × Li–Li in the electron-deficient $LiCH_3$ and this may indicate some Li–Li bonding. Similar evidence, that is, Cu-I = 1·02 × Cu-Cu, giving an acute angle at iodide[15], suggests metal–metal bonding in the d^{10} compound. In the Zn(II) compound[10], MeZnOme, however, Zn-O = 0·66 × Zn-Zn so that the acute angle is at the metal. This is distorted more from a tetrahedron than is the oxygen.

We should point out that there are compounds which might be expected to have this structure but do not; for example, titanium ethoxide could be written $[(EtO)_3TiOEt]_4$ but actually has the arrangement[24] shown in Fig. 8. There are also compounds which have the MX skeleton without forming part of our series, for example, [salicylaldehydato(ethanol)nickel(II)OMe]$_4$ (ref. 25) (Fig. 9).

Another series of compounds containing one set of atoms, M, at the corners of a tetrahedron have six, not four, groups joining them so that the bridging groups occupy the edges of the tetrahedron. For group IV these include the covalent compound adamantane $(CH)_4(CH_2)_6$ and compounds which may have some ionic character for d^0, $(MeSi)_4S_6$ (Fig. 10) and for d^{10}, $(MeSn)_4S_6$ (ref. 26). In group V compounds of this shape correspond to M(III) and may have a lone pair of electrons in the fourth position, for example, hexamethylenetetramine and $As_4(NMe)_6$ (Fig. 11)[27]. In the transition metal series a compound $(R_3PNi)_4(CO)_6$, where $R = C_2H_4CN$, has been found[28] with this geometry; the >C=O groups are bridging and Ni–Ni bonding is also postulated to complete a close-shell configuration.

Finally, we consider extending the series of compounds for transition metals. The simplest method is a small change from a known one, for example, $[(CO)_3OsNR]_4$ prepared with NR^{2-} instead of O^{2-}. Next we try to find isoelectronic metals; for Co, Rh, and Ir the d^6 configura-

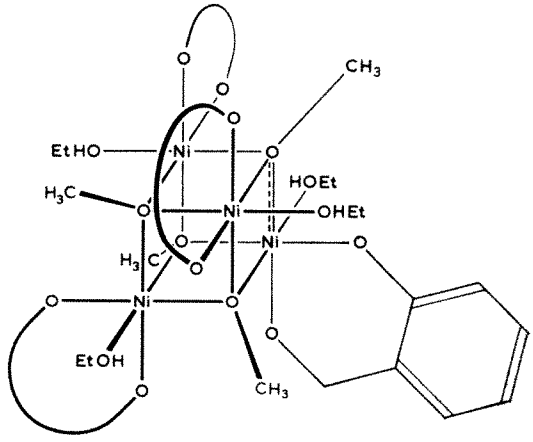


Fig. 9. Tetra-μ_3-methoxytetrakis[salicylaldehydato(ethanol)nickel (II)]. Only one salicylaldehydato ligand is shown in full, the other three are indicated schematically.

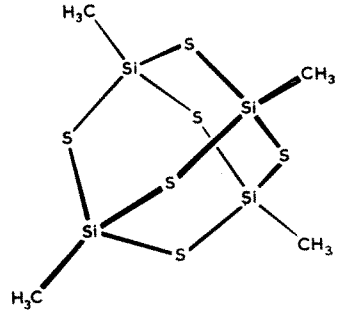


Fig. 10. Methylsilicon sulphide.

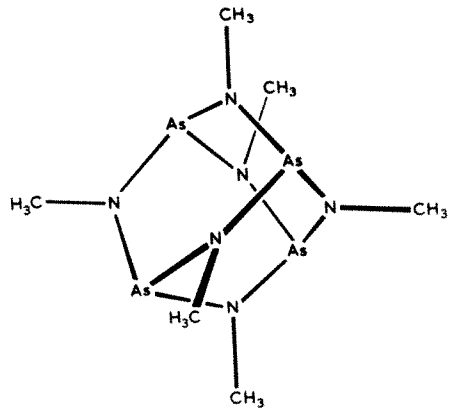


Fig. 11. Arsenic methylimide.

tion is obtained for the oxidation state III; if L is neutral as in CO, then X must be N or P. Reduction of symmetry requirements by allowing, as in the Mo compound, for different L groups gives greater flexibility. We can, for example, postulate $[Cl_2COIrCl]_4$, $[Cl(CO)_2IrO]_4$ or $[(CH_3)_2COIrI]_4$.

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The Earliest Known Record of a Solar Eclipse

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One of the Ugaritic texts of the Second Millennium BC describes a total eclipse of the Sun. The date is established as 1375 BC, May 3.

THE excavations of ancient Ugarit, begun in 1928, have revealed numerous clay tablets of great literary interest. Their discovery has been as revolutionary in the field of biblical studies as the discovery of the Dead Sea Scrolls. From archaeological evidence the texts date from the period 1450–1200 BC. They are written in a cuneiform script which is the earliest alphabetic script as yet deciphered.

Only one of the texts so far published is of an astronomical nature; this describes a solar phenomenon. A photograph of one side of the tablet, called side *A*, is shown in Fig. 1. A recent translation of the inscription on both sides of the tablet is given by Sawyer[1] as follows:

Side A: The day of the new Moon in the month of Hiyar was put to shame. The Sun went down (in the daytime) with Rashap in attendance.

Side B: (This means that) the overlord will be attacked by his vassals.

The absence of any parallel to this text in the Ugaritic literature suggests that the phenomenon was rare and spectacular, and the description can hardly refer to anything other than a total eclipse of the Sun. Numerous accounts of total solar eclipses are to be found in mediaeval European and Far Eastern literature. These leave no doubt that the sudden and intense darkness, appearance of stars, and other phenomena which accompany the complete disappearance of the Sun produced a profound impression on the observers. In contrast, annular and large partial eclipses attracted little interest. Few descriptions of total eclipses survive from before the Christian era, but we should expect such records to be found in areas where a sufficiently representative bloc of literature is extant.

The archaeological evidence allows the date of the event (hereafter regarded as a solar eclipse) to be fixed only between about 1450 and 1200 BC. It cannot have occurred later than this, for the city was destroyed by invaders about the year 1200 and was never rebuilt. The inscription states that the phenomenon occurred on the day of the new Moon in the month of Hiyar. Hiyar, the second month of the year, corresponds to about April–May. Total eclipses of the Sun are rare at a given place, occurring on average about three times in a millennium; this suggests that a definite date for the event can probably be established.

Accurate computation of an ancient solar eclipse for a given place is limited by the non-uniformity of the Earth's rotation and the tidal recession of the Moon from the Earth. Irregularities in the Earth's rotation are chiefly the result of tides, but other causes are changes in sea level and electromagnetic coupling between the core and mantle of the Earth. A computation of a solar eclipse which ignores these effects may be in error by up to about 4 h in time and 50 per cent in phase near 1300 BC. The most convenient method of analysis is to assume that the Earth rotates uniformly and to make corrections to the mean longitudes of the Sun and Moon from a study of

reliable records of astronomical events in history. These observations are themselves mainly of total eclipses of the Sun, for which the production of an accurate record makes the minimum of demands on the observer. The earliest of these records dates from 709 BC (China). By extrapolation, it is expected that errors in the computation of the Ugaritic eclipse do not exceed 1 per cent in the magnitude and 5 min in the time of occurrence.

The four dates in the table were selected from a computer analysis of about 500 solar eclipses which occurred on the Earth's surface during the period covered by the texts. The program required only the input of the geographical coordinates of the city of Ugarit (35° 37′ N, 35° 47′ E) and the Julian day number for each eclipse. Apart from the four eclipses in the table, no generally total eclipse reached a phase of greater than 88 per cent at the capital. The few annular eclipses during this time would not produce appreciable darkening of the daylight.

It is clear that only the eclipse of 1375 BC May 3 could have occurred in the month of Ḫiyar. This was total near the northern boundary of the Kingdom of Ugarit (see Fig. 2). There appear to be no records of the other eclipses in the table. The loss of daylight in 1340 BC and 1223 BC would probably be too small in Ugarit to attract attention there, but the eclipse of 1406 BC must have been very spectacular. Much of Ugarit has still be excavated, and it seems possible that a reference to this may yet turn up.

The period close to 1375 BC was one of great literary activity in Ugarit and it is not surprising to find a description of the eclipse of 1375 BC. Although the estimated uncertainty in the position of the belt of totality (about 30 km) does not make the eclipse quite complete at the capital, it would have been extremely striking in the northern part of the kingdom and may well have been total there. The reference to Rashap (= Mars) is interesting. Calculation shows that on the morning of May 3, 1375, Mars was below the horizon. Both Aldebaran and Capella, however (the latter of which was called "red" by Ptolemy and other early astronomers), were near the darkened Sun and either star may have been mistaken for Mars.

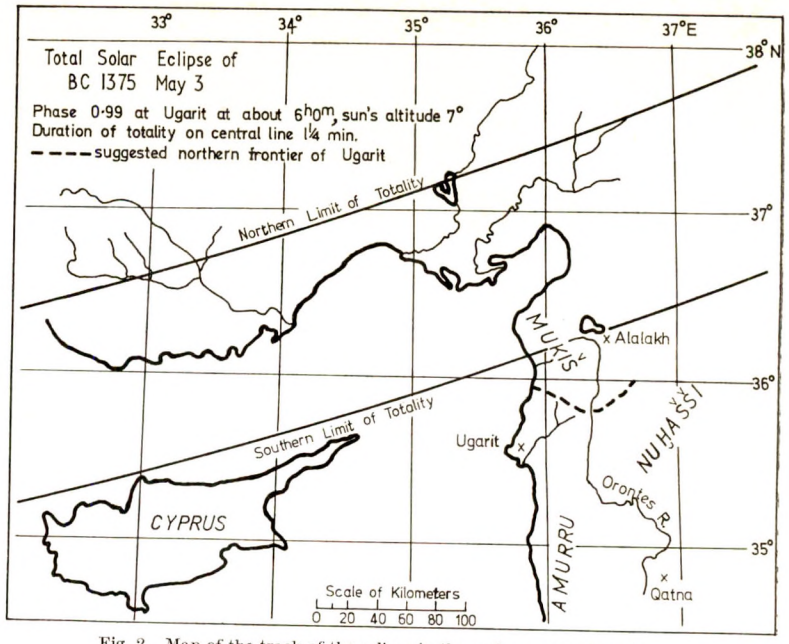


Fig. 2. Map of the track of the eclipse in the region around Ugarit.

Table 1. COMPUTED CIRCUMSTANCES FOR THE TOTAL SOLAR ECLIPSES VISIBLE NEAR UGARIT FROM 1450 TO 1200 BC

Date BC	Magnitude at Ugarit	Local time h	Local time min	Sun's altitude degrees	Zone width km	Total time min	Total time s	Position of central zone
1406 July 14	>100	15	30	43	180	3	30	Total at Ugarit
1375 May 3	99	6	00	7	130	1	15	55 km N of Ugarit
1340 Jan. 8	96·5	11	05	29	320	3	30	240 km N of Ugarit
1223 Mar. 5	96	13	25	40	170	3	30	165 km N of Ugarit

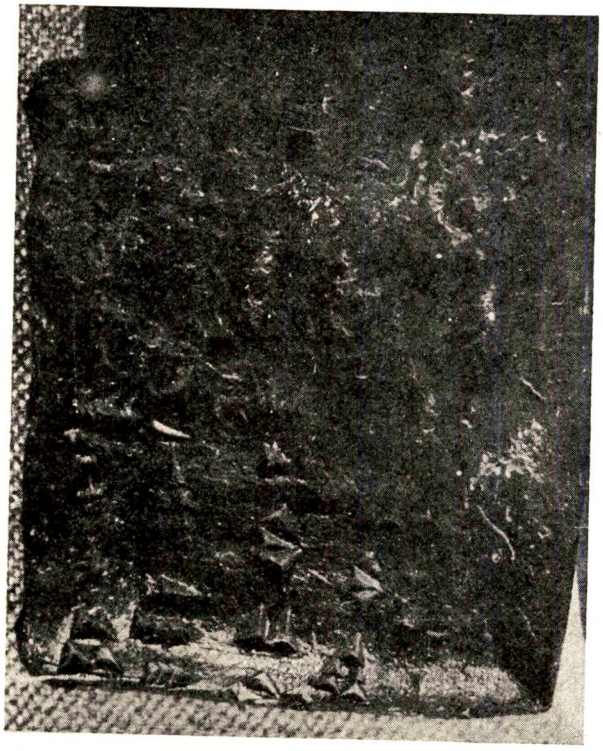


Fig. 1. One side of the tablet with text describing a solar phenomenon.

The importance of the eclipse of 1375 BC is two-fold. It is of considerable value in Ugaritic chronology, for its date is the first definite date to be established. The record probably implies that the eclipse was total within the boundaries of the Kingdom of Ugarit. As these can be fixed with a fair degree of confidence, the eclipse could prove useful in the study of the Earth's rotation.

The only known observation of a solar eclipse of comparable age to that made in Ugarit is the one recorded on one of the oracle bones found in Anyang, N. China[2,3]. It has been deciphered as reading: "Three flames ate the Sun and a great star was seen". Liu Chao-yang has put the date of the event as the late fourteenth or early thirteenth century BC. Computation shows that between 1400 BC and 1200 solar eclipses were total near Anyang only in 1370, 1330, 1304, 1250 and 1230. If the record is authentic, and indeed describes a complete eclipse of the Sun, the observation should have been made in one of these years. Precise identification of the eclipse, however, must await further work on Shang dynasty chronology. The solar eclipse observed in Ugarit on the morning of May 3, 1375 BC, would appear to be the earliest for which there is a record.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Ionospheric Electron Concentration Enhancement during a Solar Flare

DURING solar flares, sudden increases of extreme ultraviolet (EUV) and X-ray flux produce abrupt increases of electron concentration (N_e) in the sunlit part of the ionosphere. Because of their relatively short duration and the accompanying blackout in h.f. propagation, these enhancements of N_e have hitherto been difficult to investigate, especially in the E region. We report here some results of applying a new technique, incoherent scatter radar, to this problem. The observations were made at Malvern (52·1° N, 2·3° W) during a solar flare on February 20, 1970.

The equipment used was a pulsed vertically pointing radar operating at a frequency of 400·5 MHz, with a transmitted pulse length of 33 μs (5 km radar length). Scattered signal power was measured in ninety consecutive range sampling intervals, each 3 km wide, spanning the altitude range from 50 km upwards. Adequate precision was achieved by integrating the signals for periods of 5 min. The measurements were converted from scattered power to profiles of N_e against height by normalizing at a height of 110 km to N_mE measured at Slough (130 km from Malvern). The assumption that the scattering cross-section per electron is independent of height is valid except below 95 km, where corrections for a finite plasma Debye length have been applied, and above 140 km, where the cross-section becomes a function of τ, the electron to ion temperature ratio. Because the mode of operation does not allow τ to be measured, the latter correction has not been applied; this does not, however, affect the conclusions of this communication. Below 90 km, the signals are contaminated with intermittent coherent echoes from aircraft in the aerial sidelobes; only uncontaminated data have been used, but the results are less reliable than at greater heights. Further details of the equipment and data processing are given in refs. 1–3.

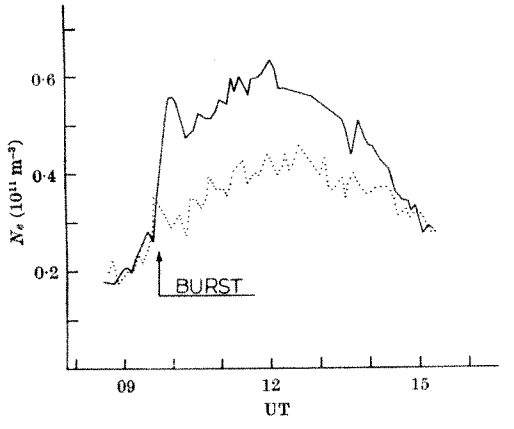


Fig. 1. Variations of Ne with time at height 96 km, on February 19 and 20, 1970. Time of start of radio burst is shown. · · · · , February 19; ———, February 20.

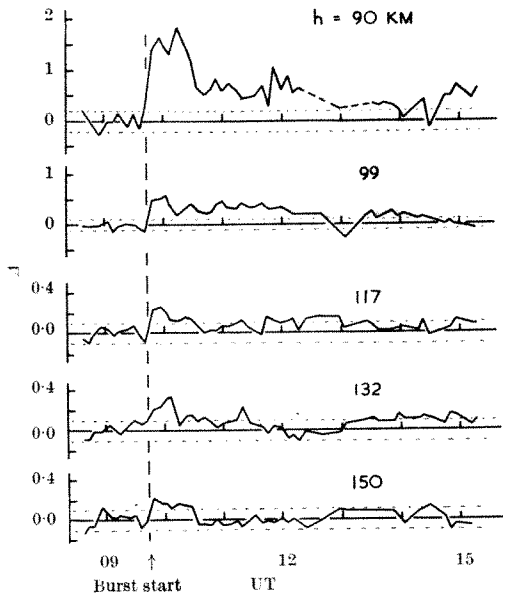


Fig. 2. Temporal variations of enhancement in N_e at five heights on February 20. Dashed lines are a conservative estimate of the baseline uncertainty.

The Hα flare on February 20, 1970, had an importance of at least 1*b*, with heliographic coordinates about 18° S, 32° E (McMath plage region 10584) and lasted at least 1 h[4]. The associated 10·7 cm radio burst observed at Slough[6] started at 0943 UT and peaked at 0944 UT. It was classified as a "great" burst, having a peak flux density of more than 500 flux units. There was an increase of flux density after the burst lasting about 3 h. A strong increase of X-ray flux was observed by the Explorer 37 satellite[5], which peaked in the 50–300 pm band at about the same time as the radio burst, and somewhat later at longer wavelengths. The X-ray flux was above normal until at least 1320 UT. The resulting sudden ionospheric disturbance, which was rated with importance 2+, was most intense at 1000 UT and ended at 1350 UT[5].

At all heights up to 150 km, the incoherent scatter measurements showed a rapid increase of N_e at the start of the disturbance, reaching a maximum between 0950 and 1000 UT. The magnitude and duration of the enhancement varied with height. As an example, plots of N_e against time for a height of 96 km on the flare day, February 20, and the previous day, February 19, are shown in Fig. 1. The problem of measuring the magnitude of a change in N_e is basically that of knowing what N_e would have been in the absence of the flare. On February 19, N_e at 96 km shows a regular diurnal variation with quasiperiodic fluctuations superimposed. The regular variation repeats well from one undisturbed day to the next, but the fluctuations are unpredictable. We have calculated the fractional deviation, Δ, of N_e on February 20, where

$$\Delta = (N_e - N_{eo})/N_{eo}$$

and N_{eo} is a smoothed reference value measured at the same time on February 19. Thus plots of Δ against time show the effects of irregular variations on both days, and also systematic changes due to the flare.

Fig. 2 shows the variations of Δ at five different heights ranging from 90 to 150 km. Dashed lines indicate a

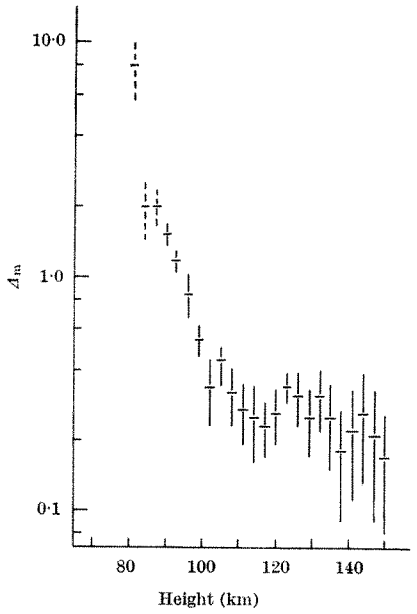


Fig. 3. Variation of the peak enhancement, Δ_m, with height. Values at the lowest heights are somewhat less reliable than the remainder. Baseline uncertainties are included in the error bars.

conservative estimate of the maximum uncertainty in the zero level obtained from measurements taken on two other consecutive pairs of undisturbed days in winter. At 90 km there is a large enhancement of N_e, lasting for at least 3 h, whereas at 132 and 150 km there are only marginally significant enhancements, which probably do not last longer than about an hour. It should be noted that the measurements are effectively 5 min running averages, so that variations on a time scale of a minute or so, such as may have occurred at the start of the disturbance, are inadequately depicted.

Fig. 3 shows the variation with height of the maximum enhancement, Δ_m, measured about 10 min after the start of the event. Values of Δ_m below 90 km are somewhat uncertain, partly because of the coherent echo problem, and partly because N_e on the control day was near the noise level. It is probable that enhancements approaching an order of magnitude are found at the bottom of the E region, and thus large increases in nondeviative absorption of h.f. waves must occur.

Somewhere below 100 km, solar X-rays become the chief source of photoionization in quiet conditions, with an uncertain contribution from HLyα[7]. It is thus significant that both the agreement between the time histories of the ionospheric and X-ray events and the height variation of the N_e enhancement point to X-rays as the main cause of the terrestrial flare effects. It is also interesting to see that the decimetric radio flux was a better indicator of the magnitude and duration of the ionospheric effects than were the Hα flare data.

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Six Minute Periodicity during X-ray Flares

The satellite OSO-IV, launched on October 18, 1967, carried a group of detectors to observe the soft X-ray emission from the Sun[1], Fig. 1 shows sample time profiles of the emission during flares as observed with a detector sensitive in the wavelength band 0·1 to 0·3 nm. In each case the time scale is the same but the intensity scale has been adjusted to show the full range of values during each event. During the visible class 1N flare represented in Fig. 1*a*, the X-radiation reached two peaks, the maxima being 6 min apart. Further evidence of such a time period is shown in Fig. 1*b* where the initial activity is considerably smaller than the second peak of the flare. Flares of this type have been noted by Teske[2] and by Culhane and Phillips[3]; the initial event has been called the precursor to the main event. It is suggested here that the precursor is really a part of the main flare activity, but that unlike the increase in Fig 1*a* the initial development is relatively small compared with the second increase. Indeed, further evidence that this is the case seems to be provided by Fig. 1*c*. In this case there is a minor increase in X-ray flux about 6 min after the main increase. If the relative intensities of the two increases had been different, the first peak might have been called the precursor.

Because the X-ray detector monitored the flux from the whole solar disk, there was no indication of the location of each flare event. Information has been obtained from solar geophysical data on the location of

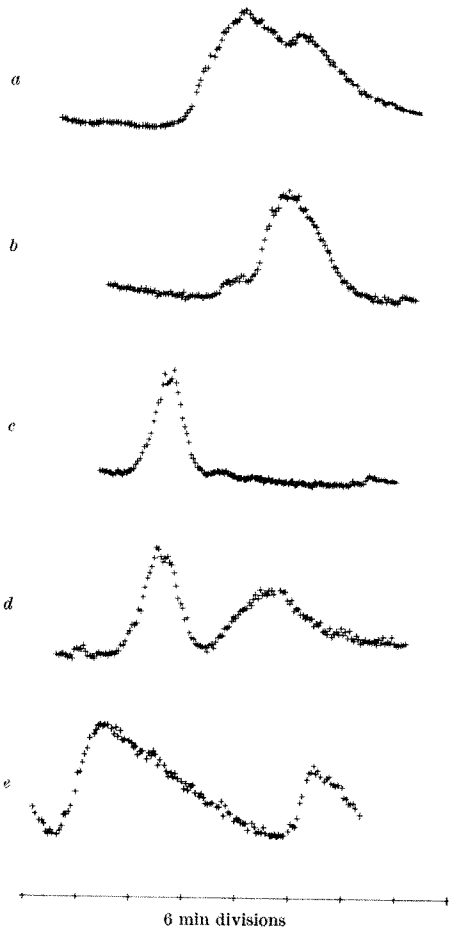


Fig. 1. Time profiles of X-radiation (0·1–0·3 nm) observed during selected flares. The linear intensity scale in each case has been chosen so that the complete profile can be presented. The time scale intervals are 6 min. (Time periods should be measured between points at which the rate of flux increase becomes relatively small.)

activity, and it seems fairly certain that increases shown in Figs. 1*a*, *b* and *c* came from the same active regions. It is uncertain whether the pairs of increases in Figs. 1*d* and *e* are associated in the same way, but the time separations between the peaks are very close to 12 and 24 min respectively. There is also a small increase 6 min after the first main peak in Fig. 1*e*.

An examination has been carried out of the time profiles of a number of flares which have more than one peak in intensity. Fig. 2 is a histogram showing the time period between peaks. (Because these maxima are often broad, timing has generally been carried out from the points where the rate of flux increase becomes relatively insignificant. In a preliminary study the timing was taken between the points at which the flare increases commenced, but there appeared to be no marked periodicity.) There is strong evidence that the time separations between related peaks are multiples of about 6 min.

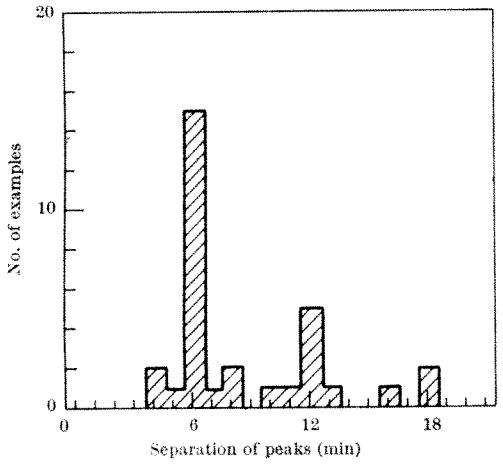


Fig. 2. Histogram of time separation between peaks of X-ray intensity. The data were collected over an observing period of about two weeks.

A possible explanation of the periodicity is that an oscillation of the X-ray emitting plasma volume occurs. There are, however, two reasons why this explanation does not seem satisfactory. (1) There seem to be examples of flare peaks 12 min apart with no intermediate large X-ray increases. If an oscillation of the flare volume occurred, each cycle would be expected to be observed until damping became significant. (2) An examination of Hα spectroheliograms has shown brightness changes at the times of precursor and main X-ray events which are up to one arc minute apart. If these separate Hα regions are indeed the ones corresponding to the individual X-ray peaks, then there is no single volume associated with the periodicity.

Because the 6 min time period is not greatly different from the 300 s oscillation observed at lower levels, it is worth speculating that the longer period arises as a result of the differences of height and magnetic field. In that case the flare increases would seem to be triggered by the arrival of energy in the solar atmosphere from the photosphere. I have already suggested (manuscript in preparation) that it is necessary to consider the plasma to be low in the chromosphere before the flare erupts. The material is then heated as it rises through the chromosphere in the vicinity of a magnetic instability or other heating mechanism. If this is the case it seems possible that the way in which the quiet corona is heated differs from the manner in which hot material is produced in flares only in the intensity of the event. Thus during times when no flare is observed, material rising from the photosphere is heated by hydromagnetic interaction with the magnetic field. If the magnetic field configuration is suitable, a magnetic instability might be produced and the material will be heated more effectively as a result. Unless this magnetic field configuration changes drastically during a flare it should be suitable to produce further heating of material rising about 6 min later. If, on the other hand, a change of field takes place so that no such heating can occur 6 min after the initial instability, it might still be possible for the field to have reverted to a suitable flare configuration by some multiple of 6 min later and further flare heating might occur.

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Gravitational Radiation and the Oblique Rotator Model

It has been known for some time that gravitational radiation can play an important part in the energetic consideration of neutron stars[1,2] and its effects have been included in some of the recent investigations of pulsar models[3–5]. In all of this, however, only the energy loss has been considered, and the angular momentum loss has been completely ignored. We have recently looked into the problem for the case of an oblique rotator, and we obtained expressions for both of these losses. We have also listed out clearly the conditions when all these become comparable with effects from the magnetic dipole mechanism, and a comparison with conclusions reported by others[3,5] is also made.

Following Melosh[5] and Pacini[6], we take the oblique rotator model to be one in which pulsars are rotating neutron stars with a frozen in magnetic field, the axis of which is inclined at an angle with the rotation axis. We assume furthermore that the mass distribution is not spherically symmetric and we describe the distortion by a flattening of the star in the region of the magnetic poles. The flattening is thus also asymmetric with respect to the axis of rotation, and we therefore have a time dependent mass quadrupole moment which leads to gravitational radiation.

The energy loss in the weak field approximation to general relativity is given by[7]:

$$P_G = \frac{dE}{dt} = \frac{-G}{45c^5}\left(\frac{\partial^3 D_{ij}}{\partial t^3}\right)^2 = \frac{-G}{45c^5}(\dddot{D}_{ij})^2 \qquad (1)$$

say, where D_{ij} is the quadrupole moment tensor.

Angular momentum can also be lost through gravitational radiation at a rate given by[8]:

$$N_i = \frac{dL_i}{dt} = \frac{-2G}{45c^5}\,\varepsilon_{ijk}\,\dddot{D}_{jm}\,\dddot{D}_{km} \qquad (2)$$

where L_i is the ith component of the angular momentum vector **L** and ε_{ijk} is a completely antisymmetric unit pseudotensor (repeated indices are summed).

We take the pulsar to be a spheroid of mass M and semiaxes (a_1, a_2, a_3) with $a_1 = a_2$ and the axis of mass symmetry **m** (taken to be the same as the magnetic axis in our model) making an angle θ with the direction of

angular velocity $\boldsymbol{\Omega}$. In a reference coordinate system with the z axis along $\boldsymbol{\Omega}$ and the x–z plane containing $\mathbf{m}$, D_{ij} can be easily calculated and we then obtain the following for the energy and angular momentum loss:

$$P_G = \frac{-2G}{5c^5} I^2 \Omega^6 \varepsilon^2 \sin^2\theta (1+15\sin^2\theta) \quad (3)$$

$$N_x = \frac{12G}{5c^5} I^2 \Omega^5 \varepsilon^2 \sin^3\theta \cos\theta \quad (4a)$$

$$N_y = 0 \quad (4b)$$

$$N_z = \frac{-2G}{5c^5} I^2 \Omega^5 \varepsilon^2 \sin^2\theta (1+15\sin^2\theta) \quad (4c)$$

Chau[2] and Chin[9] have considered special cases of the problem.

In the above equations, ε is the ellipticity defined to be

$$\varepsilon = \tfrac{1}{2} e^2 \quad (5)$$

where the eccentricity e^2 is given by:

$$e^2 = 1 - \frac{a_3^2}{a_1^2} \quad (6)$$

and I is the moment of inertia:

$$I = \frac{2}{5} Ma^2 \quad (7)$$

with $a = (a_1 a_2 a_3)^{1/3}$.

The corresponding effects arising from the magnetic dipole mechanism are now familiar and one can readily obtain from Pacini[6] the energy loss expressions P_{EM} and from Michel[10] the radiation torques. Thus, for example:

$$P_{EM} = \frac{-2\pi}{3c^3} \Omega^4 H_s^2 a^6 \sin^2\theta \quad (8)$$

The ratio of P_G to P_{EM} can then be written as:

$$\frac{P_G}{P_{EM}} = \frac{3GI^2\Omega^2\varepsilon^2(1+15\sin^2\theta)}{5\pi c^2 H_s^2 a^6} \quad (9)$$

where H_s is the surface value of the magnetic field. For an order of magnitude estimate, we can take $\theta = \pi/2$ and the following data for a typical neutron star[3]:

$$M = 1{\cdot}4\ M_\odot = 2{\cdot}8\times10^{33}\ \text{g}$$
$$a = 1{\cdot}2\times10^6\ \text{cm}$$

With this, equation 9 becomes:

$$\frac{P_G}{P_{EM}} \sim 1{\cdot}5\times10^{26}\frac{\Omega^2\varepsilon^2}{H_s^2} \quad (10)$$

We see that with sufficiently high rotation frequency ($\Omega\sim10^4$ Hz) and an $H_s\sim10^{12}$ G, P_G would become comparable with P_{EM} for an ε as small as $\sim10^{-5}$.

If we now assume that the ellipticity is caused only by the presence of the magnetic field, then from Ferraro[11]:

$$e^2 = 2\varepsilon = \frac{H_i^2 a^8}{GI^2} \sim 3\times10^{-35} H_i^2 \quad (11)$$

where H_i is the interior field responsible for the distortion. Equation 11 can now be written as:

$$\frac{P_G}{P_{EM}} \sim 3\times10^{-44}\,\Omega^2\left(\frac{H_i}{H_s}\right)^2 H_i^2 \quad (12)$$

With the help of equations 10–12, we can therefore see clearly the effects of rotation and field strength on the relative importance of the gravitational and magnetic mechanisms. The conclusions reached are consistent with those of Ostriker and Gunn[3], but they differ substantially from Melosh's[5], where the result is

$$\left(\frac{P_G}{P_{EM}}\right) \sim 3\left(\frac{\Omega a}{c}\right)^2$$

The difference arises, we believe, from the fact that the energy loss expression in the latter's work contains an e^2 whereas it should be $\varepsilon^2(=\frac{1}{4}e^4)$ as shown in equation 4. Our main result then is that the angular momentum loss and the torques arising would become comparable with those from magnetic dipole radiation under conditions discussed above. They should therefore be taken into consideration in the alignment calculations recently reported[10,12,13]. This calculation will be reported in a separate communication.

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Convection Currents and the Constant Q-Mechanism

Jeffreys has raised an interesting objection[1] to the possibility of convection or steady-state creep occurring in the upper mantle. Lomnitz has shown[2] that the logarithmic stress–strain relationship observed in igneous rocks in the laboratory may be used to derive an attenuation coefficient for stress waves that is directly proportional to frequency (constant-Q) over a limited range of frequency. Jeffreys argues that as the logarithmic creep behaviour is associated with the constant Q behaviour of stress waves, and experimental data indicate that stress waves in the mantle exhibit constant Q, the mantle material cannot undergo steady state creep under a constant stress.

Press[3] and Anderson and Kovach[4] have established that Q is constant, over the period range 11 to 25 s, as an average for the mantle as a whole. Calculations of the detailed variation of Q with depth from surface wave studies[5] assume, however, that Q is constant with frequency. If this assumption is accepted, does it necessarily preclude steady state creep ?

The logarithmic stress–strain relationship for igneous rocks is

$$\varepsilon = \frac{P}{\mu}[1 + q\log(1 + at)] \quad (1)$$

where ε is the strain, P the stress, μ the rigidity, q and a are experimentally determined constants, a being of the order of 10^3 s^{-1}. The wave equation based on (1) (ref. 2) gives a constant value of Q in the frequency range $10^{-8} < f < 1$Hz, provided that Q (which is proportional to q^{-1}) is greater than 600.

It has been established experimentally[6] that the values of Q for most rocks are constant over the frequency range $10^{-4} < f < 10^6$ Hz, the absolute values being about 200. Thus Q is constant over a range of frequencies which is not explicable in terms of the logarithmic stress–strain relationship and it must therefore be presumed that there are other mechanisms that can give rise to this behaviour.

Futterman has shown[7] that the constant Q behaviour cannot extend to zero frequency if the causative mechanism is linear. Experimental evidence for this linearity has been provided for metals by Savage and Hasegawa[8] and for sediments by myself[9] and shows that the superposition theorem is valid for stress waves showing constant Q propagation in these materials. Hence the constant Q mechanism cannot manifest itself at zero frequency as a particular stress–strain relationship and its presence cannot be taken as evidence for or against such a relationship.

An analogous situation arises in the study of the damping of electromagnetic waves in dielectrics. The values of Q of many dielectrics are independent of frequency[10] and cannot be derived from the d.c. conductivities. But the presence of the constant Q mechanism cannot be taken as evidence that a steady state, unidirectional flow of charge cannot occur through dielectrics, because it is known experimentally that they have measurable d.c. conductivities.

If the material of the mantle can undergo steady state creep it would be expected that a "viscous" attenuation mechanism with a characteristic relaxation time would be observed. McConnell has estimated[11] the viscosity of the upper mantle as 10^{20} to 10^{22} poise from the rate of uplift of Fennoscandia. The implied relaxation time is between 5×10^7 and 5×10^9 s. Bhatia shows[12] that stress waves with periods considerably less than the relaxation time are not attenuated or dispersed by the viscous mechanism. Thus there will be no evidence for this damping mechanism from stress waves of "ordinary" seismic periods. Jeffreys indicates[1] that the free nutation of the Earth's axis (an oscillation with a period of about 4×10^7 s) shows a viscous damping with a time constant of about 10^8 s. The agreement between the two relaxation times lends support to the idea that the upper mantle material can exhibit steady state creep.

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Plate-tectonic Regulation of Faunal Diversity and Sea Level: a Model

A growing body of evidence and theory supports the idea that processes of plate tectonics have been operating for the past 3×10^9 years[1]. The hypothesis of ocean-driven plates implies that certain mountain systems represent the remains of former large ocean basins. An important corollary of this notion is that the history of continental assembly and fragmentation can be inferred from the geological record of the various mountain systems. We show here how the diversity of marine fauna and fluctuations in sea level can be related to patterns of continental fragmentation and reassembly. It seems that changes in the diversity of fauna predicted from these patterns can account for a great deal of the variation in the diversification and extinction of marine biota that has long puzzled palaeontologists. The timing of major transgressions and regressions predicted from these patterns also agrees well with the observed record.

Among the factors that regulate species diversity are environmental stability[2,3], food supply[2,4,5] and provinciality[6,7]. Consider a large land mass composed of an assembly of all the presently dispersed continents. Such a continental state favours great seasonality of marine climate, partly as a result of excess summer heating and winter cooling of the interior compared with the surrounding oceans. Off-shore winter and spring winds cause upwelling which results in relatively low surface temperatures and high supplies of nutrients in coastal waters; on-shore summer and autumn winds favour the on-shore drift of relatively warm surface waters and low levels of nutrients in coastal waters. Barriers to migration are few, and although some endemism would occur in epicontinental oceanic arms and along topographically distinctive coastal stretches, species would tend to be widespread, especially if climatic differences around the continental margin were not too extreme.

If such a supercontinent were to fragment, the marine climates around the small daughter continents would have a different character. In general, the climates of these smaller land masses would be more equable, with less distinct seasonality arising from monsoon effects and with less vertical mixing and therefore fewer nutrients in shelf waters. In this more stable, more "oligotrophic" environment, increasing species diversity within communities is favoured[2,5,8]. Furthermore, there is now growing isolation of the biotas of the daughter continents, leading to growing endemism and the eventual appearance of distinctive biotas on each continent, increasing the total biotic diversity. Total species diversity has thus increased as a result of the increased stabilization of the shelf environment and the accompanying oligotrophic tendency, and the increased provincialization.

Subsequent reassembly of smaller continents into larger ones leads to an increase in seasonality in shelf waters and to increased supplies of nutrients, and also eventually brings endemic biotas into association. A significant reduction in diversity would thus occur. Extinction would be greatest among groups that were least pre-adapted to fluctuating conditions (usually the deeper shelf forms[9,10] and very specialized forms in general), and among groups that were unable to meet the competition or to withstand the predation pressures from invading members of the other biotas. Diversity would tend to be lowest when all continental fragments were reassembled into a single supercontinent, and highest when continents are most fragmented. Climatic zonation is, however, another major cause of provincialization; if continental configurations are such that latitudinal temperature gradients are high, then the biota of each north–south continental coast will be subdivided into climatic provinces and diversity levels will be multiplied by this factor. This situation partly accounts for the unusually high number of species at present[11,12].

A model of continental breakup and reassembly can be inferred from existing mountain chains[1]. Phanerozoic events were probably directly affected by the breakup of an early supercontinent (Pangaea I, Fig. 1, event *A*) which by the time of the upper Cambrian had given rise to a pre-Appalachian and pre-Hercynian and pre-Uralian ocean[13–17] (Fig. 1, event *B*). Judging from the emplacement of ophiolites[18,19], the pre-Caledonide–Acadian ocean began closing by the mid-Ordovician and was sutured in the late Silurian to late Devonian (Fig. 1, event *C*). The pre-Hercynian and pre-Appalachian oceans closed in the late Pennsylvanian or early Permian (Fig. 1, event *D*). Finally, the closing of the pre-Uralian ocean near the end of Permian time completed the reconstruction of a supercontinent (Pangaea II, Fig. 1, event *E*).

seem to correlate well with the events described. The correspondence seems close enough to support one of our principal theses, that a major part of the regulation of species diversity on continental shelves has been accomplished through changes in environmental stability, nutrient supply and provinciality which act in concert and are correlated with changes in continental configurations. The continuing rediversification during the Mesozoic and Cainozoic is partly the result of the widening latitudinal spread of continents and of the development of latitudinal chains of provinces as shelf waters have cooled in high latitudes[9,10].

The patterns of continental assembly and fragmentation inferred earlier also suggest a model which may explain a long standing enigma: the timing and correla-

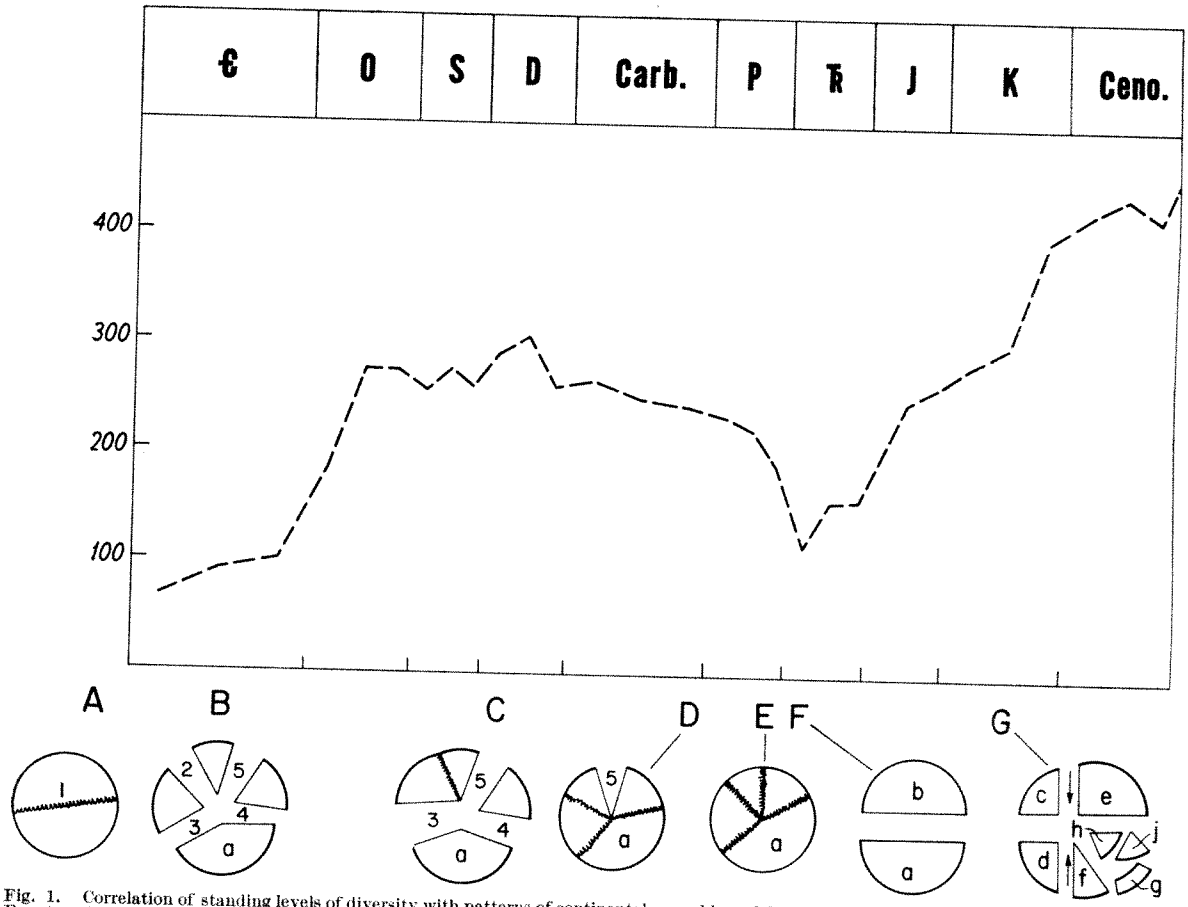


Fig. 1. Correlation of standing levels of diversity with patterns of continental assembly and fragmentation. Broken line is number of families. Events: *A*, Eocambrian suturing of Pan-African-Baikalian (1) system—formation of Pangaea 1; *B*, fragmentation of *A* in Cambro-Ordovician time to give pre-Caledonian–Acadian (2), pre-Appalachian (3), pre-Hercynian (4), and pre-Uralian (5) oceans; *C*, Siluro-Devonian suturing of Caledonian–Acadian orogenic system; *D*, Pennsylvanian–Permian suturing of Appalachian–Hercynian orogenic system; *E*, Permian–Triassic suturing of Urals—formation of Pangaea II; *F*, Triassic–lower Jurassic extension of Tethyan seaway; *G*, Cretaceous–Recent closing of Tethys, opening of Atlantic, fragmentation of Gondwana. *a*, Gondwana; *b*, Laurasia; *c*, North America; *d*, South America; *e*, Eurasia; *f*, Africa; *g*, Antarctica; *h*, India; *j*, Australia.

This Permo-Triassic supercontinent was short-lived, however, and separated into Laurasia and Gondwana as a result of the extension of the Tethyan ocean (Fig. 1, event *F*). Subsequent breakup of both these continental masses has been accompanied by a partial closure of Tethys, probably commencing in the late Jurassic to early Cretaceous (judging by the emplacement of ophiolites[20–23]) and may have been accelerated by the opening of the North Atlantic and fragmentation of Gondwana in mid-Cretaceous times (Fig. 1, event *G*).

The diversity of skeletonized benthonic shelf families of nine major invertebrate phyla is shown in Fig. 1[11,12]. The number of families present (family diversity) is represented by epoch; the major trends in standing diversity tion of major transgressions and regressions on presently separated continents.

Our model involves an expansion and application of a principle suggested by Russell[24]. Continental fragmentation occurs at ridges where ocean floor spreading is in progress. These ridges stand above the general abyssal plains to form broad topographic highs which, even on the scale of the world ocean, contain significant volume. A rough calculation based on published data[25–28] indicates that the volume of present ridges is about $2{\cdot}5 \times 10^8$ km^3, which would be sufficient to lower the ocean level by about 0·5 km across the entire surface of the Earth, or by about 0·65 km over the present ocean basins, were they to subside.

The lateral displacement of continents involves at least partial destruction in subduction zones[23] of either the plate on which they are borne or of another plate; these zones are expressed as topographic lows. As the continents move apart they displace ocean water from deeper regions bordering their outer edges, while the sea floor appearing between them, partly composed as it is of a ridge, stands relatively high. The total volume of the ocean basins is therefore reduced and water tends to rise relative to the continental platforms, which undergo transgressions.

If, on the other hand, continents are being joined, their suturing prevents further relative motion between the plates on which they ride. In this event, the component sea floor spreading of the world rift system which accounted for the relative motion between the now-sutured plates must eventually decay or new subduction zones must develop. There is evidence that if the spreading stops, the ridges subside[30–33], perhaps resulting from cooling and downward growth of the lithosphere. If new subduction zones develop, they would be associated with trenches. In either case, there would be an increase in volume of the ocean basins, and therefore an epicontinental regression.

According to this model, assembly of continents should be accompanied by regressions, and fragmentation by transgressions. In so far as the record is amenable to interpretation in these terms, these relations seem to hold. The emergent Eocambrian supercontinent (or continents; Fig. 1, event *A*) fragmented and was then transgressed during Cambrian time (Fig. 1, event *B*). Caledonian–Acadian, Hercynian and Appalachian suturings (Fig. 1, events *C*, *D*) were accompanied by regressions. Permo-Triassic continental assembly (Fig. 1, event *E*) was accompanied by a profound regression. Subsequent transgressions may mark episodes of fragmentation (Fig. 1, events *F*, *G*), as is suggested by the correlation of major continental breakup near the mid-Cretaceous with a widespread transgression. Post-Cretaceous sea level changes may have involved such events as movements on the "Darwin Rise"[27], suturing and continental underthrusting in the Alpine–Himalayan belt, and the well known eustatic effect of ice and snow storage.

The expected effects of transgressions and regressions tend to reinforce the effects of the ecological regulators of diversity. Widespread transgressions would tend to moderate the seasonal climates, while widespread regressions would tend to enhance continentality. Furthermore, the species-area effects of expanding and shrinking of the epicontinental seas, which may have less influence on marine biotic diversity than these other factors[5], would nevertheless be of some significance. According to the present hypothesis, all these factors operate in concert.

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Molecular and Crystal Structure of Nonachloroclovononaborane, B_9Cl_9

In a recent publication[1] we described the isolation and characterization of a yellow–orange boron subchloride, B_9Cl_9. The structure of this compound has now been determined from three-dimensional X-ray data collected by counter methods.

Attempts to grow single crystals by sublimation were unsuccessful, but well formed orange rhombs were obtained by crystallization from methylene chloride in high vacuum conditions. The compound crystallizes with four molecular units in the space group A2/m of the monoclinic system. Crystal data: $a = 9{\cdot}25$ Å, $b = 12{\cdot}66$ Å, $c = 13{\cdot}50$ Å; $\beta = 95{\cdot}1°$. Volume of unit cell = 1549 Å^3. Measured density (flotation in mixtures of organic solvents) was 1·7 g/cm^3, calculated density 1·78 g/cm^3.

Diffraction data to $2\theta = 50°$ (Mo-K_α radiation) were collected on a manual General Electric XRD-6 diffractometer. The structure was solved by Patterson, Fourier and least-squares refinement techniques. The coordinates and anisotropic thermal parameters of all atoms were refined to the present discrepancy factor of $R = 6{\cdot}5$ per cent using 1,305 independent reflexions above background. The relevant details of the parameters of the molecule are summarized in Table 1.

Table 1. Interatomic distances in (Å)

Atoms		Distances	Standard deviation
B_1	B_4	1·729	0·009
B_1	B_5	1·733	0·010
B_2	B_5	1·806	0·009
B_2	B_6	1·751	0·009
B_3	B_4	1·812	0·009
B_3	B_6	0·739	0·009
B_4	B_6	1·735	0·009
B_5	B_6	1·766	0·009
B_1	Cl_1	1·729	0·008
B_2	Cl_2	1·748	0·008
B_3	Cl_3	1·738	0·008
B_4	Cl_4	1·741	0·006
B_5	Cl_5	1·746	0·006
B_6	Cl_6	1·721	0·007

As shown in Fig. 1, B_9Cl_9 has a boron-cage structure with terminal chlorine atoms on each boron. The cage has the shape of a tri-capped trigonal prism with three boron atoms and their associated chlorines sitting on the crystallographic mirror plane; within experimental error the molecule has a three-fold axis although this is not a space group requirement. The B–B and B–Cl bond lengths are in close agreement with those found by Lipscomb for the related compound[2,3], B_8Cl_8. Both B_8Cl_8 (G. F.

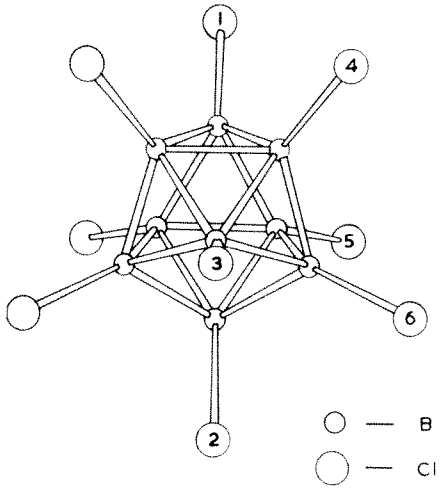


Fig. 1.

Lanthier, J. K., and A. G. M., to be published) and B_9Cl_9[1] are formed during the thermal decomposition of diboron tetrachloride.

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Plastic Deformation and Anisotropy in the Hardness of Diamond

A number of methods may be used to determine the hardness of solids. The most reproducible and most widely used for crystalline solids are those in which the size of an indentation is measured after the surface of the specimen has been subjected to deformation by an indenter under a known normal load. Ideally, the indenter material should be considerably harder than the specimen in order to avoid inaccuracies due to deformation of the indenter tip, but this implies that the hardness of diamond cannot be measured, for it is the hardest indenter material available. A limited amount of data concerning the hardness of diamond, using the static indentation technique, has nevertheless been published. More than thirty years ago the Knoop indenter was developed and used to measure the hardness of a wide range of ductile and brittle materials. This indenter produces an indentation in the form of a parallelogram with one diagonal at least seven times longer than the shorter diagonal. In that early work, the hardness of diamond was quoted as 8,000–8,500 kg mm^{-2} (ref. 1). No details were given concerning the normal load used, the type of deformation associated with the indentation or the crystallographic relationships between the indenter and the indented diamond surface. It was reported subsequently that the hardness of a (001) diamond surface, again using the Knoop indenter and a normal load of 500 g, was 6,000–6,500 kg mm^{-2}. Apparently, anisotropy in the hardness could not be detected at that time[2]. Bernhardt[3] gave a limiting value of 10,000 kg mm^{-2} on the (111) surface. He used a conventional square based pyramidal indenter and a normal load of 200 g. Kruschov and Berkovich[4], using an indenter in the form of a three sided pyramid with an angle of 65° between its geometrical axis and the side faces, derived a hardness of 10,060 kg mm^{-2} from one indentation also on a (111) plane and with a load of 200 g. On the basis of a very limited number of indentations, therefore, it seems that the hardness of diamond lies between 6,000 and 10,400 kg mm^{-2} with no evidence of anisotropy. On the other hand, studies of abrasion on diamond surfaces have established marked anisotropy in wear resistance. These observations have been used to suggest that the directions of high and low wear resistance represent the hard and soft directions respectively on a given diamond surface. For example, it has been proposed[5] that, on the (001) plane of diamond, the hard directions are ⟨110⟩ and the soft directions are ⟨100⟩. It is now clear that directions of high wear resistance, on a given crystal plane, are not necessarily the hard directions[6].

Table 1. KNOOP HARDNESS MEASUREMENTS ON A (001) DIAMOND SURFACE

Chronological order of indentation	Direction	Hardness (kg mm^{-2})	Deformation
1	[010]	9,400	No cracking
4	[100]	9,400	No cracking
6	[010]	9,600	No cracking
10	[100]	10,400	No cracking
12	[010]	9,400	No cracking
14	[100]	9,400	No cracking
16	[010]	8,900	No cracking
2	[110]	7,700	No cracking
3	[1$\bar{1}$0]	6,600	No cracking
5	[110]	7,000	No cracking
7	[110]	5,700	Cracking
8	[110]	7,600	Slight cracking
9	[110]	6,400	Slight cracking
11	[1$\bar{1}$0]	7,700	No cracking
13	[110]	—	Extensive cracking
15	[1$\bar{1}$0]	7,000	No cracking
17	[110]	6,000	No cracking

Anisotropy in the hardness of crystals can be most effectively studied by Knoop indentation measurements where the indenter is aligned so that the long diagonal in the indentation corresponds to a specific crystallographic direction on a crystal plane. The length of the longer diagonal is then used to calculate the hardness. The purpose of the present work was to use the Knoop indenter to investigate anisotropy in the hardness of diamond. The indentations were made on the same (001) diamond surface as that used in earlier work on the frictional anisotropy of diamond by Bowden *et al.*[7] and Bowden and Brookes[8]. Measurements were carried out on this (100) surface in ⟨100⟩ and ⟨110⟩ directions using normal loads of 500 g, 1 kg and 2 kg. There is a good deal of elastic recovery in diamond and the indentations at 500 g were difficult to measure with sufficient accuracy to establish anisotropy effects. Also a significant amount of fracture was observed around the indentation at 2 kg in the ⟨110⟩ directions although this was never observed in the ⟨100⟩ directions at any load. Most of the measurements were therefore made with a normal load of 1 kg. Hardness values are given in Table 1 for all these measurements and typical indentations produced by a 1 kg load for the two principal directions on the (001) plane are shown in Fig. 1. A further single indentation in the [120] direction gave a hardness value of 8,900 kg mm^{-2}.

For such a hard material, there is surprisingly little scatter in the hardness for the ⟨100⟩ directions, and the average value is 9,600 kg mm^{-2}. The scatter is greater for the indentations in ⟨110⟩ directions, but the average value is approximately 6,900 kg mm^{-2} whether based on all the measurements or just on those where cracking was not observed. Clearly the hard directions on a cube diamond surface are ⟨100⟩ and the soft directions are ⟨110⟩. This is in direct contradiction to the earlier conclusions based on abrasion resistance and confirms the suggestion that different mechanisms control the two processes[6].

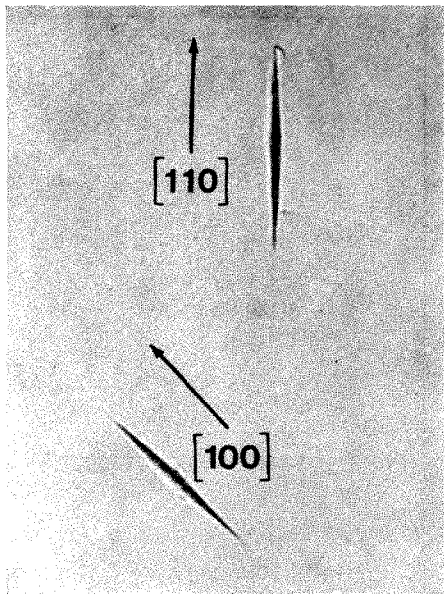


Fig. 1. Knoop indentations in the [$\bar{1}$10] and [$\bar{1}$00] directions on the (001) plane of diamond. (×750.)

The observations made in this study also serve to resolve the controversy concerning the possibility of plastic flow in diamond at room temperature. Recently, Gane and Cox[9] used a scanning electron microscope to observe the mutual indentation of crossed diamond knife edges. They concluded that plastic deformation in diamond was not possible at room temperature except perhaps in very small amounts not resolvable in the scanning electron microscope. There can be very little doubt, however, that the indentations obtained in this work, such as those shown in Fig. 1, were formed by plastic flow. Furthermore, the observed anisotropy was predictable on the basis of a study by C. A. B., J. B. O'Neill and B. R. W. Redfern on anisotropy in the hardness of crystals using the Knoop indenter (to be published). They have established a clear relationship between the effective resolved shear stresses developed in the bulk of the crystal beneath the indenter and the primary slip system in a given crystal structure. Their analysis was shown to be equally applicable to a wide range of metallic and nonmetallic crystals, covering hardness values from 13 to 3,000 kg mm^{-2}, but is valid only when dislocations are mobile in the given experimental conditions. When this analysis is applied to the diamond cubic structure, assuming slip is possible on {111} ⟨1$\bar{1}$0⟩ systems[10], the effective resolved shear stress calculations confirm that the hard and soft directions should be ⟨100⟩ and ⟨110⟩ respectively on low index planes. It has also been shown that cracking is most likely in the softer directions on such crystals as magnesium oxide and aluminium oxide and does not significantly influence the hardness measurements (C. A. B., O'Neill and Redfern). The results obtained in this study are therefore entirely consistent with those in the earlier work with the Knoop indenter and confirm that plastic flow is possible in diamond at room temperature.

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BIOLOGICAL SCIENCES

Tissue-specific Factor required for the Translation of a Mammalian Viral RNA

RNA from several mammalian viruses promotes protein synthesis in cell-free systems from certain mammalian and avian cells[1–5]. We have recently investigated a cell-free preparation derived from mouse Krebs II ascites cells which responds to RNA from encephalomyocarditis virus (EMC) (M. B. M. and A. Korner, unpublished). The EMC RNA-directed product is virus-specific, which confirms that the viral RNA is acting as a messenger RNA. We have found that cell-free systems prepared from certain other mammalian cells fail to respond with amino-acid incorporation, and that the unfractionated rabbit reticulocyte system evinces an inhibitory response. This inhibition is caused by an interference with the normal process of globin chain initiation (M. B. M. and A. Korner, manuscript in preparation), comparable with that produced by the synthetic polynucleotides poly U and poly A in this system[7,8]. No detectable amino-acid incorporation, however, is directed by the viral RNA. We show here that reticulocyte ribosomes can translate EMC RNA but that reticulocytes lack at least two soluble components necessary for the response.

Viral RNA and protein-synthesizing extracts were prepared from rabbit reticulocytes and mouse Krebs II ascites cells by the methods previously described (unpublished results of M. B. M. and A. Korner). Where applicable, the unfractionated systems were preincubated for 40 min at 37° C with the cofactors of protein synthesis to reduce the endogenous incorporation. Ribosomes and cell sap were separated by centrifugation for 2·5 h at 165,000*g* (average). Ribosomes were spun through 1 M sucrose in incubation medium. Incubations were carried out in a medium containing 5 mM magnesium acetate, 125 mM KCl, 30 mM Tris-HCl (*p*H 7·5) and 6 mM 2-mercaptoethanol.

To clarify the difference between the reticulocyte and ascites cell systems in their response to EMC RNA, ribosomes and cell sap were exchanged between the systems. A preliminary experiment (Table 1) indicated that the endogenous incorporation of non-preincubated ribosomes could be maintained by heterologous sap. This

Table 1. ENDOGENOUS AND POLY U-PRIMED SYNTHESIS IN MIXED SYSTEMS

Ribosomes	Sap	^{14}C-Phenylalanine incorporation (c.p.m.) Control	+Poly U	Relative stimulation
Reticulocyte	Reticulocyte	5,160	35,584	7·0
Reticulocyte	Ascites	5,076	10,516	2·1
Ascites	Reticulocyte	2,109	27,176	13·3
Ascites	Ascites	1,921	4,389	2·3

Reactions (0·3 ml.) contained 0·15 ml. sap, 100 μg reticulocyte or 140 μg ascites ribosomes (non-preincubated), 1 mM ATP, 0·1 mM creatine phosphate, creatine phosphokinase 0·2 mg/ml., naturally occurring amino-acids except phenylalanine 20 μM each, 0·5 μCi/ml. ^{14}C-phenylalanine (450 mCi/mmole), 100 μg poly U where indicated, and salts as in the text. After 45 min at 37° C, 0·5 ml. 1 M NaOH containing 2 per cent casamino acids was added and incubation was continued for 15 min. Protein was precipitated with 2 ml. 20 per cent trichloroacetic acid, collected on glass-fibre filters and washed. Radioactivity was estimated with a Nuclear-Chicago gas-flow counter at an efficiency of 25 per cent.

Table 2. RESPONSE OF MIXED SYSTEMS TO EMC RNA

Ribosomes	^{14}C-Phenylalanine incorporation (c.p.m.) Ascites sap		Reticulocyte sap	
	Control	+EMC RNA	Control	+EMC RNA
Ascites	86	1,475	96	14
Reticulocyte	200	1,619	188	203

Reactions (0·1 ml.) contained 0·03 ml. sap, 25 μg preincubated ribosomes and, where indicated, 2 μg EMC RNA. Other conditions were similar to those of Table 1. Identical observations have been made with non-preincubated ribosomes. The absence of an inhibitory response in the homologous reticulocyte system is probably due to the decreased extent of globin chain initiation after fractionation of the S-10 into ribosomes and sap[6].

shows that the supernatant factors—sRNA and transfer enzymes—are compatible. The heterologous systems also responded to poly U, though ascites cell sap permits less stimulation—possibly because it contains ribonuclease (unpublished results of M. B. M. and A. Korner). Stimulation by EMC RNA unequivocally requires ascites cell sap, reticulocyte sap being totally ineffective (Table 2), even when its concentration is increased (data not shown). The viral RNA can stimulate protein synthesis from ribosomes of either source in the presence of ascites cell sap. To characterize the products of the cell-free incorporations, incubations were carried out in the presence of ascites cell sap and ^{35}S-methionine and the proteins were digested with trypsin and subjected to electrophoresis. The peptide patterns obtained from preincubated reticulocyte and ascites cell ribosomes

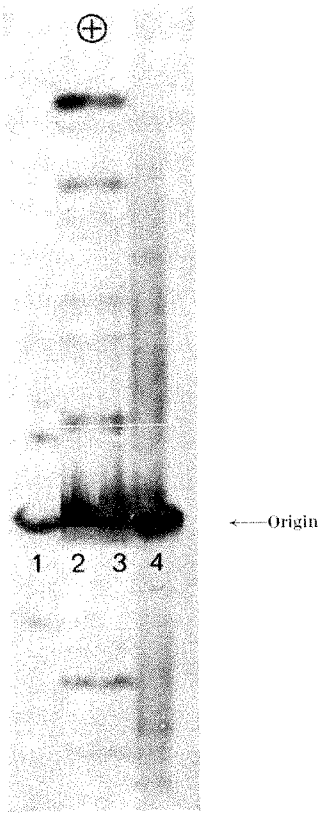


Fig. 1. Tryptic digestion of ^{35}S-methionine labelled cell-free products. (1) Reticulocyte ribosomes, endogenous; (2) reticulocyte ribosomes, EMC RNA-directed; (3) ascites ribosomes, EMC RNA-directed; (4) ascites ribosomes, endogenous. Incubations (50 μl.) contained 30 μl. ascites sap, 25 μg ribosomes, 1 μCi ^{35}S-methionine (20 Ci/mmole), nineteen labelled amino-acids, except methionine, 20 μM each, and other components as detailed in Table 1. Reactions 1 and 2 contained reticulocyte ribosomes and 3 and 4 ascites ribosomes; reactions 1 and 4 used non-preincubated ribosomes, and 2 and 3 preincubated ribosomes + 3 μg EMC RNA. After 45 min, EDTA was added to 50 mM and incubation was continued for 5 min with 1 μg pancreatic ribonuclease. Proteins were precipitated with 5 per cent trichloroacetic acid containing 2 per cent casamino acids, washed three times with trichloroacetic acid and then with ethanol, ethanol: ether and ether. The dried precipitate was oxidized with performic acid, dissolved in 1 per cent ammonium bicarbonate and digested with trypsin for 4 h at 37°. After lyophilization, the digest was applied to Whatman '3MM' paper and electrophoresed at pH 6·5 for 75 min at 3 kV. The dried paper was autoradiographed for 10 days. Material remaining at the origin is attributable to underdigestion.

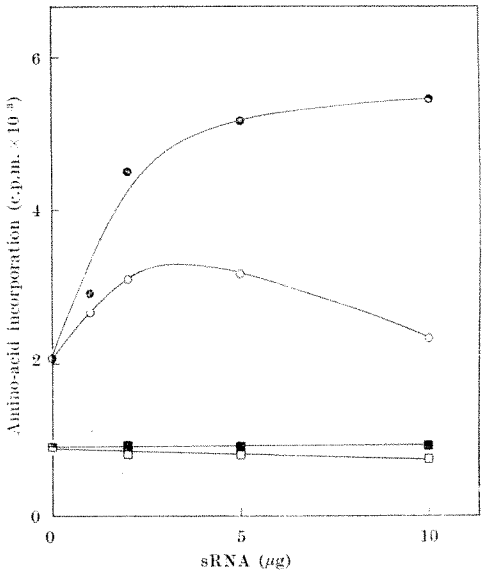


Fig. 2. Dependence of response to EMC RNA on added sRNA. Reactions (50 μl.) contained 10 μg preincubated ascites ribosomes, 25 μl. reticulocyte sap, 15 μl. DEAE-cellulose treated ascites sap and sRNA and EMC RNA (2 μg) where indicated; ●, ascites sRNA + EMC RNA; ○, reticulocyte sRNA + EMC RNA; ■, ascites sRNA, no EMC RNA; □, reticulocyte sRNA, no EMC RNA. The small stimulation obtained in the absence of added sRNA is attributable to slight contamination of the ascites ribosomes with sap, and is not seen if reticulocyte ribosomes are used. A similar partial replacement of the requirement for ascites sRNA by reticulocyte sRNA is observed in the reticulocyte S-10 supplemented with treated ascites sap.

under the influence of EMC RNA are identical (Fig. 1), and similar to those previously demonstrated to be virus specific[6]. Reticulocyte ribosomes therefore translate the viral RNA faithfully in the presence of ascites cell sap. The EMC RNA-directed peptides are quite distinct from those produced by non-preincubated ribosomes from either source.

The addition of reticulocyte sap to incubations containing ribosomes and ascites cell sap does not suppress the stimulation of protein synthesis by EMC RNA (Table 3). Furthermore, supplementation of the reticulocyte S-10 (the unfractionated 10,000g supernatant) with ascites sap dramatically transforms its inhibitory response to the viral RNA into a stimulation (Table 3). This shows that the reticulocyte sap does not contain components which inhibit the translation of EMC RNA, and implies that the ascites sap contains factors, absent from reticulocyte sap, which are necessary for the translation of the viral RNA. Fractions of ascites sap were therefore added to incubations containing preincubated ribosomes and reticulocyte sap in the presence and absence of EMC RNA. If ascites sap is warmed to 55° C for 2 min, it no longer permits the stimulation, suggesting that a protein is involved. Treating the ascites sap with DEAE-cellulose at 0·125 M KCl, which removes the sRNA and some of the proteins, virtually abolishes its activity (Table 4). Raising the KCl concentration to 0·3 M to prevent the adsorption by DEAE-cellulose of almost all the proteins does not restore the activity. If, however,

Table 3. EFFECT OF EXTRA SAP ON THE RESPONSE TO EMC RNA

Experiment	Ribosomes	Reticulocyte sap	Ascites sap	Incorporation (c.p.m.) Control	+EMC RNA
1	Ascites	30	0	96	14
	Ascites	0	30	86	1,475
	Ascites	30	30	50	1,770
	Ascites	60	30	79	1,464
2	Reticulocyte S-10		0	11,653	6,142
	Reticulocyte S-10		10	10,518	47,407

The conditions for experiment 1 were identical to those of Table 2. In experiment 2, reactions (25 μl.) contained 10 μl. reticulocyte S-10, 1 μCi ^{35}S-methionine (20 Ci/mmole), unlabelled amino-acids except methionine 20 μM each, and 5 μg EMC RNA where indicated. Other details are as under Table 1.

Table 4. EFFECT OF ASCITES SAP FRACTIONS ON THE RESPONSE TO EMC RNA

DEAE-cellulose treated ascites sap (μl.)	Ascites sRNA (μg)	Incorporation (c.p.m.) Control	+ EMC RNA
0	0	43	232
0	14	41	466
15	0	76	513
15	14	81	2,105
Unfractionated ascites sap (15 μl.)		114	2,773

Reactions (50 μl.) contained 25 μl. reticulocyte sap, 5 μg preincubated ascites ribosomes and ascites sap fractions and EMC RNA (2 μg) as indicated. Other details were similar to those of Table 1. Increasing the amounts of treated sap or sRNA did not increase the response observed. The ascites sRNA was prepared by phenol extraction of ascites cell sap, followed by alcohol precipitation and ether washing. It was discharged by incubation in 1·8 M Tris-HCl (*p*H 8·5) for 1 h. Ascites sap was treated with DEAE-cellulose by passing it through a column of Whatman 'DE 52' equilibrated with incubation medium. Treatment at 50 mM KCl resulted in complete loss of the stimulatory activity.

the treated sap is supplemented with ascites sRNA (obtained by phenol extraction of untreated sap) the activity returns: the sRNA alone is ineffective (Table 4). Likewise, both the protein and sRNA fractions are required to overcome the inhibitory action of EMC RNA in the reticulocyte S-10. Fig. 2 shows that reticulocyte sRNA can substitute for the ascites sRNA, but is less efficient.

The requirement for sRNA may signify that EMC RNA contains one or more codons which are rare in globin and for which the reticulocytes have little tRNA. They may also lack the cognate amino-acid activating enzyme, which would explain the need for a protein factor. In this case, charged ascites cell sRNA might be sufficient to permit stimulation by EMC RNA in the absence of the protein component, but experiments have not supported this hypothesis. Nor have any short EMC RNA induced peptides been found in the reticulocyte system, which indicates that the block in the translation of the viral RNA occurs at initiation rather than at an early step of chain elongation. The DEAE-cellulose treated sap, supplemented with ascites sRNA, can support the translation of poly U by ascites ribosomes, so it is unlikely that the protein factor is one of the transfer factors. These experiments are not conclusive and further fractionation of both the protein and RNA components is being undertaken.

The phenomenon described here may be general: mouse brain and mouse reticulocyte systems are also incapable of responding to EMC RNA because of sap inadequacy, although mouse liver systems can respond (unpublished results). These factors may perform a role similar to that of the messenger-specific initiation factors of bacteria[9–11], and are perhaps concerned in the regulation of protein synthesis *in vivo*.

I thank Dr A. T. H. Burness and Dr L. V. Crawford for help in growing the virus; Dr K. A. Marcker for his advice and suggestions; Professor A. Korner for his interest in the early part of this work; and several colleagues for reading the manuscript. I am a Beit Memorial Research fellow.

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Absolute Stereochemistry of the Second Step of Ribonuclease Action

PANCREATIC ribonuclease catalyses the decomposition of a ribonucleic acid by a two-step mechanism: a transesterification followed by hydrolysis of the resulting cyclic phosphate[1]. The various mechanisms suggested can be classified as either "in-line" or "adjacent" depending on the relative geometry of the incoming and outgoing groups during the displacement reaction[2] (Fig. 1). In particular, any simple mechanism in which it is assumed that the same group acts first as a general base toward the attacking nucleophile, then as a general acid toward the leaving group, implies an adjacent geometry. The alternative, in which there is a considerable movement of either the catalytic group or the substrate, is unlikely. The two steps may, however, have different mechanisms according to this criterion without violating microscopic reversibility, for a nucleoside is obviously not necessarily equivalent to a water molecule[3].

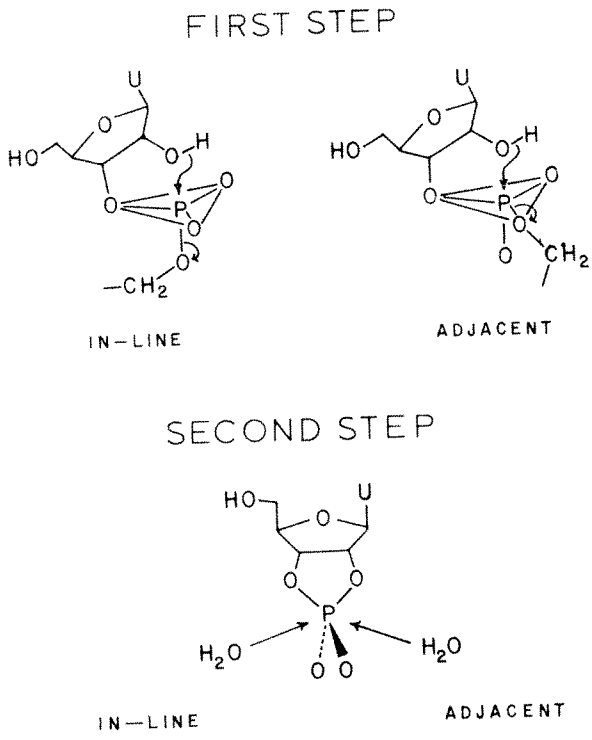


Fig. 1. The in-line and adjacent mechanisms for the two steps of ribonuclease action. In the adjacent mechanisms, the molecule would undergo a pseudorotation before product formation[2].

The earliest suggested mechanism was in-line[4], but it was later amended[5] to an adjacent form requiring the five-membered cyclic phosphorane intermediate or transition state to span two basal positions of a trigonal bipyramid, and therefore it is unlikely to be correct[3,6]. A number of other adjacent mechanisms have been suggested that use as a general base either a group on the enzyme[2,7,8], the 2-oxygen of the nucleoside[9], or both[10]. More recently, another in-line mechanism has been proposed[11], but although it is based on X-ray and NMR evidence of the mode of binding of dianionic inhibitors, the arguments, particularly for the second step, are not compelling. We report here an unambiguous determination of the geometry of the second step that enables us to identify the correct mechanism.

The key to the successful resolution of the geometry was the isolation by Eckstein[12,13] of the two diastereoisomers of uridine-2′,3′-cyclic phosphorothioate shown in Fig. 2. The cyclic thiophosphate (isomer a) was hydro-

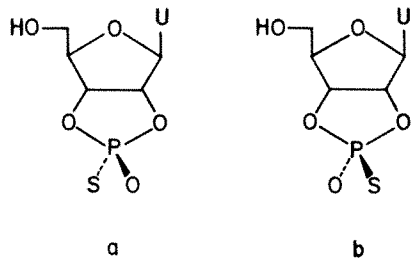


Fig. 2. Two diastereoisomers of uridine-2′,3′-cyclic phosphorothioate[12,13]. Isomer a shows[12,13] the same K_m towards ribonuclease as uridine cyclic phosphate, but v_{max} is lower by a factor of 5. We assume that enzymic hydrolysis gives only the 3′-monoester, as found for the related phosphate.

lysed at *p*H 7 by pancreatic ribonuclease A in water enriched in ^{18}O, and the resulting monoester was re-cyclized by treatment with diethyl phosphorochloridate in pyridine solution to give a mixture of the two isomers a and b (see Fig. 3). The preference rules predict[6] that the ring closure would be in-line, because in these basic conditions the relatively electropositive anionic groups would preferentially remain in basal positions (see Fig. 4) and pseudorotation would be inhibited[2]. If the geometry of the enzymic ring opening is also in-line, then the isomer a that is produced should contain no excess ^{18}O, while isomer b should show the incorporation of one equivalent of ^{18}O from the water. A detailed analysis of the possible effects of pseudorotation on the enzymic reaction reveals that scrambling of the isotopic purity can occur if both of the ring oxygens are allowed to occupy basal positions at the same time, or if free cyclic ester is reformed from pseudorotamers below the dotted line (see Fig. 5). Both possibilities are considered unlikely, the first on grounds of ring strain[6] and the second on the basis of the following argument. There are three pseudorotamers below the dotted line: loss of sulphur from A would not give starting material and does not occur[12,13]; loss of ^{18}O from B would be the reverse of an adjacent mechanism for the addition of water and yet this diagram is for an in-line mechanism; loss of ^{16}O from C would imply that more than one oxygen from the solvent could be incorporated into the ring opened material and this can be excluded[14]. It should be noted that product formation from any of the rotamers always gives the same geometry in the resulting 3′-monoester.

As a check on the arguments given above, a control

Table 1. HYDROLYSIS OF URIDINE-2′,3′-CYCLIC PHOSPHOROTHIOATE (ISOMER a) BY RIBONUCLEASE A

Isomer isolated after ring closure (and purity)	Percentage incorporation: Expected if ring opening was adjacent (per cent)	Expected if ring opening was in-line (per cent)	Found (per cent)
a (about 90 per cent)	86	14	6
b (about 75 per cent)	28	72	70

The isomeric purity of the starting material was assumed to be 95 per cent but may have been higher. Water used in the hydrolyses contained between 4 and 5 per cent ^{18}O; analysis of the thiophosphate for ^{18}O incorporation was carried out by pyrolysis with mercuric chloride and cyanide[15], followed by mass spectrometric determination of the carbon dioxide produced on an isotope ratio machine (Consolidated 401). The identity of the peaks was checked at high resolution on an MS-902 spectrometer.

experiment was done using hydroxide ion (enriched in ^{18}O) for the initial ring opening. (The probable formation of a mixture of 2′ and 3′-monoesters does not invalidate the arguments presented here.) In this case the ring opening and ring closure should both be in-line, and the incorporation of ^{18}O in the product was indeed near zero for isomer a and about 85 per cent of the theoretical value for isomer b. Allowance was made for the presence of about 10 per cent isomer b in the starting material and in the isomer a isolated after ring closure.

The results, given in Table 1, show that for this compound the enzymic hydrolysis and the ring closure proceed with the same geometry. We conclude that the second step in the action of ribonuclease A is in-line, and that all mechanisms so far proposed for the ring opening that require one group to act first as a general base toward

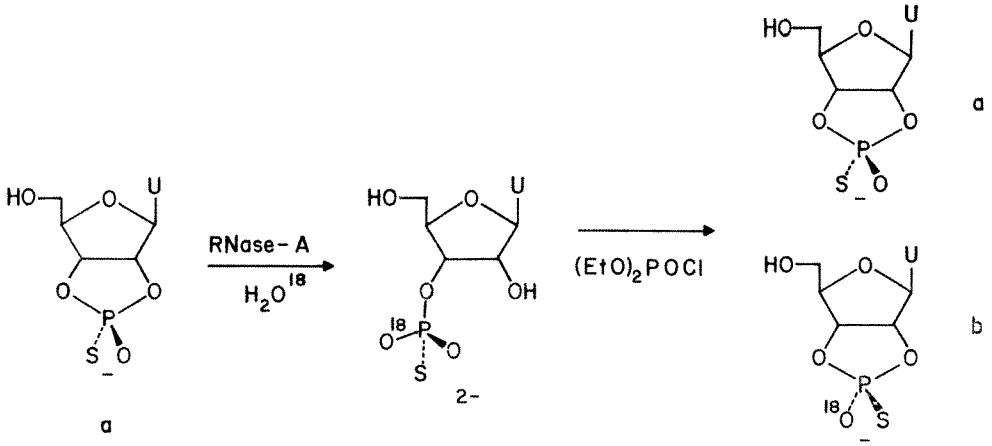


Fig. 3. The method used to determine the geometry of the ring opening step. This diagram shows an in-line mechanism.

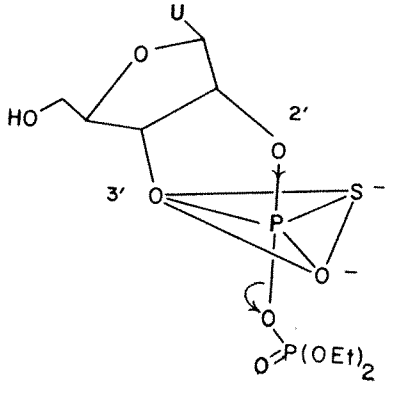
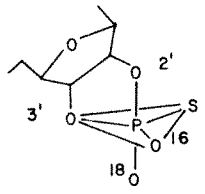


Fig. 4. Loss of diethyl phosphate gives uridine cyclic phosphorothioate by an in-line meachnism[2,6]. Pseudorotation is inhibited.



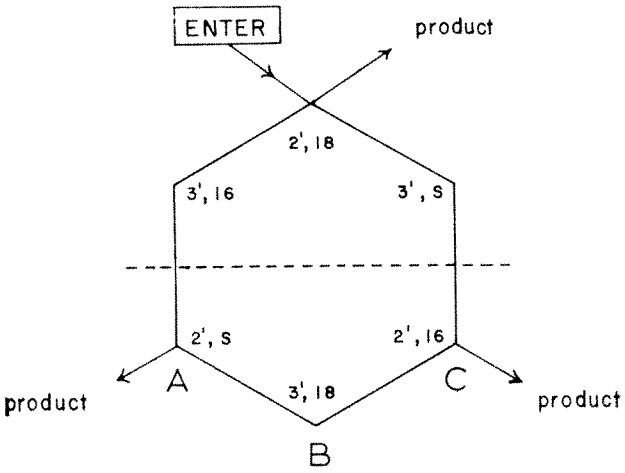


Fig. 5. A pseudorotation diagram[16] for the enzymic hydrolysis of uridine cyclic phosphorothioate. This diagram assumes an in-line mechanism, but a similar diagram can be drawn for an adjacent geometry. The apices of the hexagon represent individual pseudorotamers, the structures of which are denoted by the apical groups[16]. The structure shown is thus (2',18).

the attacking water molecule and then as a general acid toward the 2'-oxygen are unlikely to be correct.

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In vivo Cortisol Action on RNA Synthesis in Rat Liver Nuclei and Mitochondria

Various mechanisms have been proposed for the action of hormones in cells. One is a cytoplasmic mechanism involving mitochondria[1,2]; a second implies that cytoplasmic protein receptors act as sites[3] for the hormone action; a third proposes that the nuclei act as a primary site for hormone action[4,5]. We now demonstrate that cortisol, when used for brief periods and in physiological doses, increased the RNA polymerase activity of rat liver mitochondria. In these conditions nuclear RNA synthesis was not affected. Hormone treatment for longer periods or treatment with large doses for short time periods increased the nuclear RNA polymerase activity and suppressed the mitochondrial enzyme.

Charles River female rats (140–160 g) were bilaterally adrenalectomized and ovariectomized and will be referred to in the text as adrenalectomized rats. Animals were kept on 1 per cent NaCl and fox chow *ad libitum* in a light and temperature controlled room. All autopsies were performed at 0900 h. Livers were immediately excised and homogenized in cold 0·25 M sucrose.

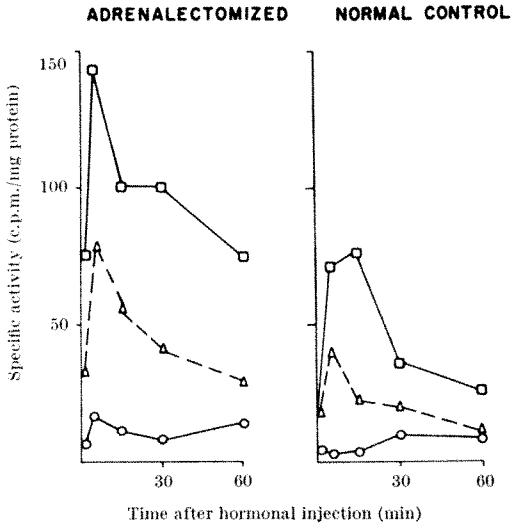


Fig. 1. 6 days after operation, rats were injected (0·25 mg/100 g with 1,2-^{3}H-hydrocortisone (specific activity 18·2 Ci/mmole, NEN, diluted with unlabelled cortisol to a final concentration of 1·25 mg/ml.) and autopsied at the indicated times. Nuclear mitochondrial and ribosomal pellets were extracted and counted as described[10]. Sham operated rats were used as controls. Measurements from at least two rats were made after each time interval. ○—○, Nuclear fraction (2,500*g*); △- - -△, mitochondrial fraction (9,000*g*); □—□, ribosomal fraction (105,000*g*).

1,2-^{3}H-Hydrocortisone (specific activity 18·2 Ci/mmole, New England Nuclear, diluted with unlabelled hydrocortisone to a final concentration of 1·25 mg/ml.) was used in the hormone incorporation experiments. Animals were injected once intraperitoneally with 0·5 μCi/100 g body weight with a hydrocortisone concentration of 0·25 mg/100 g. In all other experiments, cortisol (11β,17α,20-trihydroxypregn-4-ene-3,20-dione, Sigma) was dissolved in 95 per cent ethanol and diluted with an equal volume of distilled water. Animals were injected intraperitoneally with the specified dose or vehicle.

Mitochondria were separated by centrifugation[6] and washed three times with 0·25 M sucrose. The protein content of the various fractions was estimated[7] and adjusted to 2–4 mg protein/50 μl. *In vitro* RNA polymerase was assayed[8]. At various intervals aliquots were pipetted on disk filter paper (Whatman No. 3) and processed as described by Bollum[9].

Fig. 1 represents the specific activity (c.p.m./mg protein) of cortisol binding to the subcellular components.

A rapid incorporation is found in every fraction and this reached a peak at 5 min. The decline of incorporation after 5 min was rapid in the mitochondrial fraction and more gradual in the nuclear fraction. *In vitro* studies of the effect of cortisol on rat liver nuclei gave similar results[11]. Other studies have also shown an association of cortisol[2], as well as oestradiol[10,12], progesterone[13] and testosterone[14] with the mitochondria. There are reports, however, that show only a small degree of cortisol binding to the mitochondrial fraction[15,16]: data were reported as an absolute percentage of incorporation rather than on a protein or nucleic acid basis. Nevertheless, these differences might be due to various factors such as the dosage of hormone, the sex of the animals, or the time after hormone injection. Other reports have dealt with these variables[16–18]. For example, high hormone dosage has been shown to suppress steroidal incorporation into the mitochondrial fraction of guinea-pig liver and kidney[17]. The dosage used by us minimized this effect.

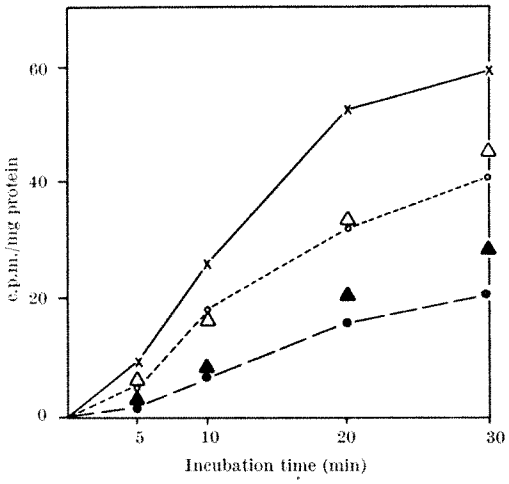


Fig. 2. Adrenalectomized ovariectomized rats were injected intraperitoneally 7 days after operation with 0·5 mg cortisol/100 g and autopsied 15 min, 1, 4 or 8 h later. The ability of nuclei and mitochondria to incorporate acid soluble ^{14}C-GTP into the insoluble fraction was tested *in vitro* at 37° C. Results are from mitochondrial fractions and are expressed as c.p.m./mg protein (2–4 mg mitochondrial protein per assay were used as the enzyme source). Each point represents the mean of two separately run assays. There was no significant difference between controls and the nuclear fractions. ● – – ●, RNA polymerase activity of liver mitochondria from non-treated controls; ▲ – – ▲, from rats 8 h after cortisol injection; △ - - - △, from rats 4 h after injection; × — ×, from rats 1 h after injection; ○ - - - ○, from rats 15 min after injection.

The *in vitro* RNA polymerase activity of the adrenalectomized rat liver mitochondria was studied after a single injection of 0·5 mg cortisol/100 g body weight. Fig. 2 represents a time course study of animals killed at various times after injection. After an incubation of 30 min, the increase in activity ranged from 40 to 200 per cent depending on the length of the initial hormone treatment. Mitochondrial RNA polymerase activity increased 200 per cent if the animals were injected with 0·5 mg cortisol 1 h before autopsy. The enzyme activity of mitochondria isolated from livers 15 min or 4 h after hormone injection was twice that of the controls, whereas 8 h after the hormone injection there was only a 40 per cent increase. At this dosage no effect on nuclear RNA polymerase activity was detected.

The effect of varying the dosage of cortisol while maintaining the time between injection and autopsy constant (at 60 min) on mitochondrial RNA polymerase activity from adrenalectomized rats is shown in Fig. 3. The activity was about twice that of the controls when 0·5 mg of cortisol was injected, whereas a 67 per cent increase was found after injection of 2 to 4 mg. Injection of 8 mg/100 g caused no significant increase in activity. By contrast,

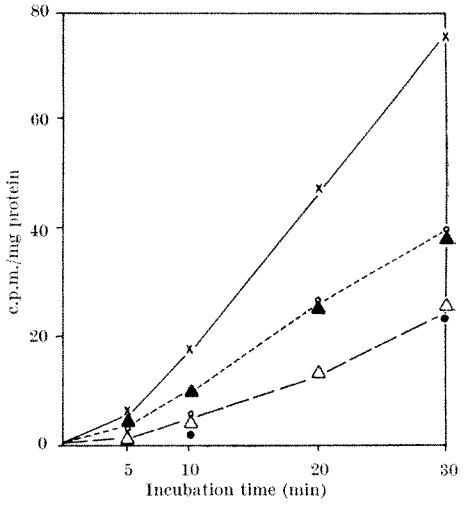


Fig. 3. 0·5, 2, 4 and 8 mg cortisol/100 g were injected intraperitoneally into adrenalectomized ovariectomized rats. Autopsy was performed 1 h after injection and RNA polymerase activity of liver nuclei and mitochondria was tested *in vitro* (see legend to Fig. 2). Results are from the mitochondrial fraction. There was no significant difference between the non-injected controls and the nuclear fraction. ● – – ●, Non-injected controls; △ – – △, 8 mg cortisol/100 g.; ○ - - - ○, 4 mg cortisol/100 g; ▲ - - - ▲, 2 mg cortisol/100 g; × — ×, 0·5 mg cortisol/100 g.

no dose in this range (0·5–8·0 mg/100 g) had any effect on nuclear RNA polymerase activity 60 min after injection of cortisol.

When cortisol (0·5 mg/100 g) was injected 4 h before autopsy (Fig. 4) a 50 per cent increase in the mitochondrial RNA polymerase activity was observed. Larger doses had no appreciable effect. On the other hand, Fig. 5 shows a 45 per cent increase in activity in nuclei isolated from adrenalectomized rat liver 4 h after injection of 4 mg cortisol/100 g, in agreement with the findings of Barnabei *et al.*[19]. Others[20] have reported a higher percentage increase in nuclear RNA polymerase activity, possibly because of the use of rats which had been both adrenalectomized and ovariectomized, rather than rats that were only adrenalectomized[20]. Another explanation is that we used whole nuclei in 0·25 M sucrose rather than the purer nuclear fraction that others have used[19,20].

Our previous work[21,22] has shown that mitochondrial DNA synthesis is stimulated earlier and to a greater

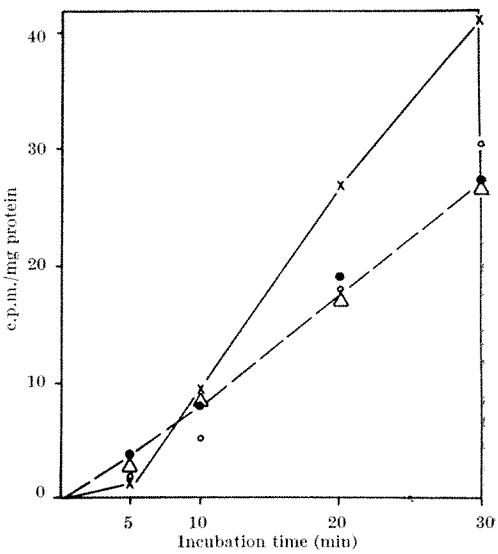


Fig. 4. 0·5, 4 and 8 mg cortisol/100 g were injected intraperitoneally into adrenalectomized ovariectomized rats. Autopsy was done 4 h after injection. Mitochondrial RNA polymerase activity, see legend to Fig. 2. ● - - - ●, Non-injected controls; △ - - - △, 8 mg cortisol/100 g; ○ - - - ○, 4 mg cortisol/100 g; × — ×, 0·5 mg cortisol/100 g.

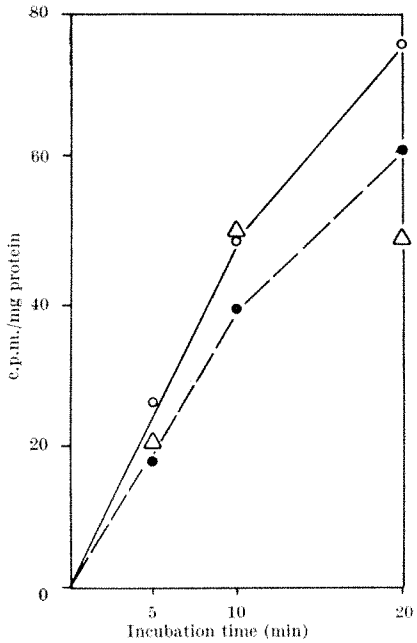


Fig. 5. RNA polymerase activity of rat liver nuclei 4 h after cortisol injection with 4 and 8 mg cortisol/100 g. ●- - -●, Non-treated controls; △, 8 mg cortisol/100 g; ○—○, 4 mg cortisol/100 g.

extent than is nuclear DNA synthesis after partial hepatectomy. This study indicates that mitochondrial RNA synthesis responds earlier and at lower doses of cortisol than does nuclear RNA synthesis. Consideration of these results in relation to specific structural changes in mitochondrial DNA of certain malignant cells[23] leads us to suggest that the study of mitochondrial nucleic acid metabolism will throw light on the regulation of nuclear nucleic acid metabolism[24].

Note added in proof. A recent report by Fu Li Yu and P. Feigelson (*Biochim. Biophys. Acta*, **213**, 134; 1970) confirms our results that, after hormone treatment, rat liver mitochondrial RNA synthesis increases at a faster rate than nuclear RNA synthesis.

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Strontium-90 and Infant Mortality in Canada

Sternglass and his associates[1-4] have claimed that strontium-90 in fallout from atomic tests has produced and continues to produce great numbers of foetal and infant deaths. We have now tested on Canadian data the reported finding[5,6] that infant mortality in various states of the US closely paralleled the level of strontium-90 in cows' milk from that same state.

In this analysis, both strontium-90 level and infant mortality are expressed in a special way. Strontium-90 is given as a four year moving average. Why the basis of this should be four years is not clear, but if, as Sternglass believes, strontium-90 acts by producing lethal mutations in the germ line of parents some kind of moving average or cumulative dose is appropriate.

Infant mortality is expressed as "per cent excess infant mortality". This variable originates with an observation by Sternglass (and no doubt others) on the manner in which infant mortality has declined in the United States. When plotted on a logarithmic scale, infant mortality can be seen to have decreased in nearly linear fashion between 1935 and 1950 (see curve for US in Fig. 3). If a linear least-squares line is fitted to the data for this period and extrapolated to recent years, the observed mortality for every year since 1950 lies above the line. Percentage excess infant mortality is defined as the difference, expressed as percentage of total mortality, between the least-squares line calculated from 1935 to 1950 and the observed mortality.

Sternglass computed the percentage excess infant mortality for every state and year in which measurements of strontium-90 in milk are available. He gives plots for six states, said to be "typical", in which strontium-90 and excess mortality increase together in striking fashion.

We have retained Sternglass's definitions of both variables and have made a computer program to compute the 1935–50 least-squares line and from that percentage excess infant mortality. Another program calculates the four year moving average of strontium-90. The program for excess mortality was checked on a set of data supplied by Dr Sternglass and produced the same result as he had found.

In Canada, there are nine provinces for which adequate data on both infant mortality and strontium-90 are available. For each of these we have calculated the least-squares line best fitting the mortality from 1935 to 1950 and, from this, the percentage excess infant mortality. The percentage excess infant mortality, together with the four-year moving average of strontium-90, is shown for each province in Fig. 1.

The strontium-90 levels in Canada are somewhat higher than those in the US, yet it will be seen that strontium-90 appears to have had no effect on mortality. We have noticed that in Canada use of the period 1945–55 as the interval for which to calculate the least-squares line would give a result more in accord with the plots Sternglass has published. At the urging of Dr Sternglass, we have pursued this line of endeavour, and the conclusion is unfavourable to his hypothesis. He tells us that recently he has discovered that the base period 1946–55 is the most appropriate one, not only for Canada but for other countries, and he is re-doing many of his calculations and plots for various countries on this basis. We have, accordingly, recalculated our result.

When we re-do the calculation using 1946–55 as the base period, excess infant mortality in some provinces does appear to rise during the period of atomic testing. There is, however, no real association between the two variables (Fig. 2). There are six provinces for which data are available from 1960 to 1965, with 1965 corresponding roughly to the peak of strontium-90 level. It is evident from the plot that, among these provinces, a large rise in strontium-90 does not tend to go with a large rise in excess mortality.

If anything, the reverse is true. There is a slight negative association between the amount of change in the two variables over this period. That is, the provinces with the most fallout have the least increase in excess mortality.

Is there reason to think that the association asserted by Sternglass does, nevertheless, hold for the US? An analysis of the US data by the method just used for Canada is very much to be desired. Unfortunately, we have been unable to obtain a full set of data on strontium-90 of the sort necessary to make a plot like that in Fig. 2. The only material we have is a set of data sent to us by Dr Sternglass covering the years 1960–67. If we take 1966 as the peak year for strontium-90, we are able to calculate the four-year moving average of strontium-90 for only the years 1963–66. The result is nonetheless informative. The percentage excess mortality for these same years has been calculated using Sternglass's original period of 1935–50 as the basis for the least-squares line; and the result is as before. The strontium-90 and the mortality variables in the various States do not rise together. If anything they show a slight negative association (regression coefficient $b = -0{\cdot}81$).

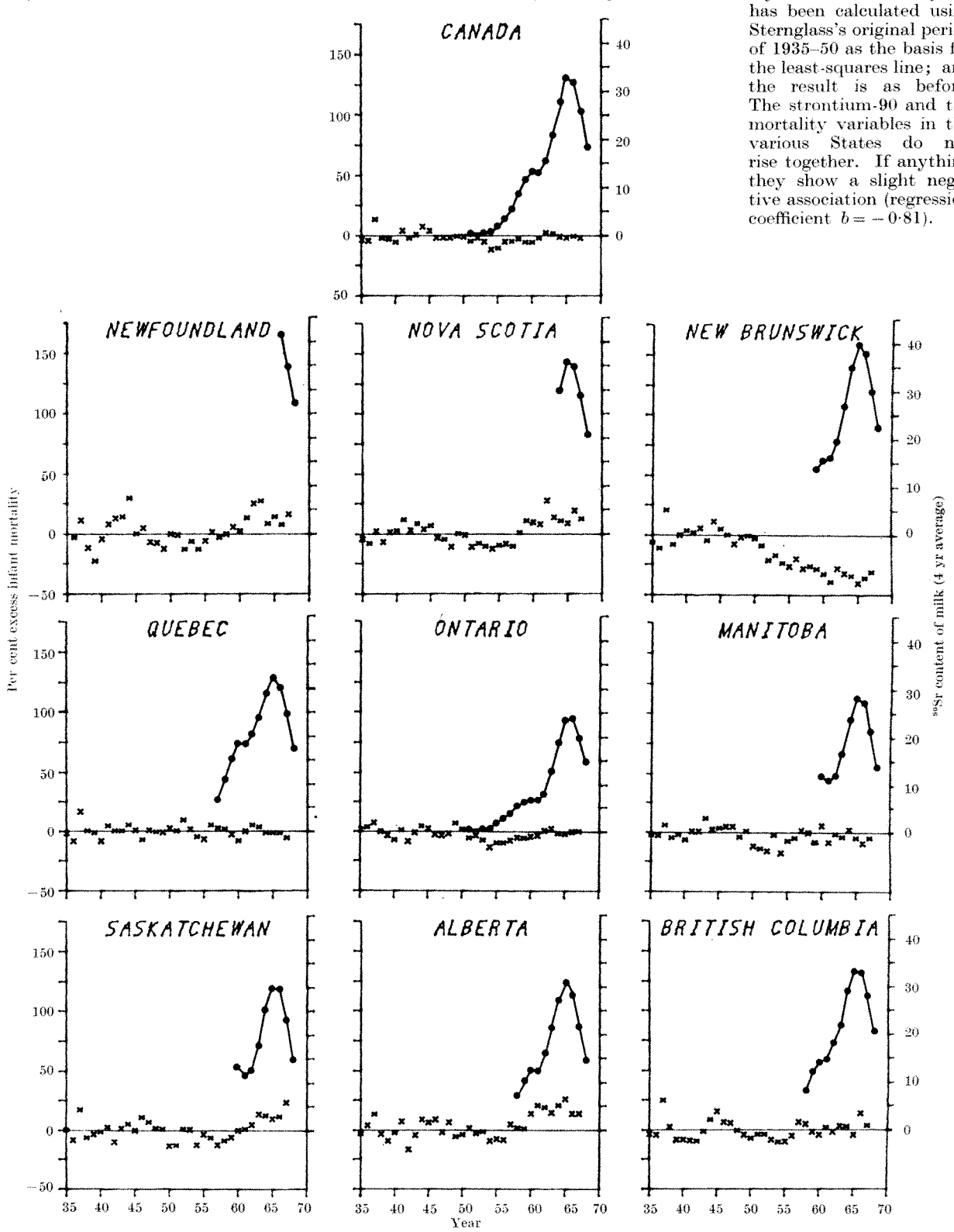


Fig. 1. Canadian infant mortality as related to strontium-90 in cows' milk. For definition of "per cent excess mortality" see text. Units of strontium-90 are picocuries per litre. ×, Excess infant mortality; ●, strontium-90 level in milk.

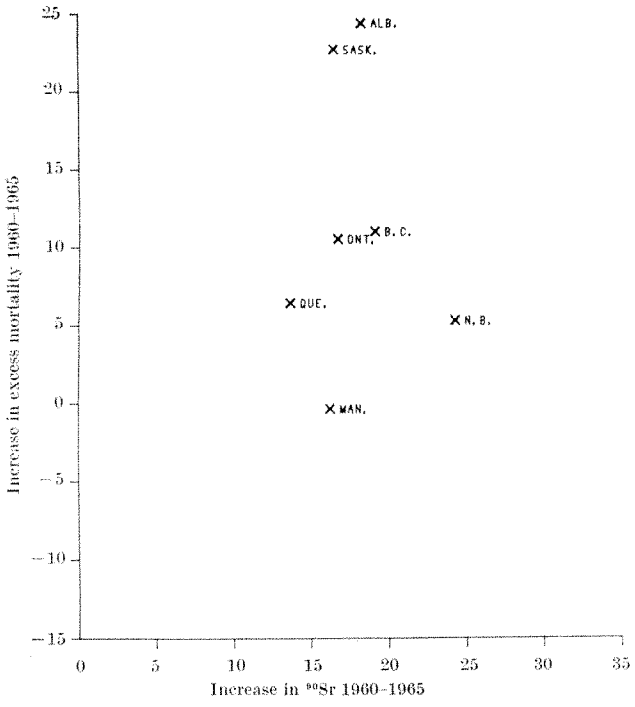


Fig. 2. Change in infant mortality and change in strontium-90 level, calculated using 1946–55 as the basis for the least-squares line. (Least-squares estimate of slope is $b = -0{\cdot}12$.)

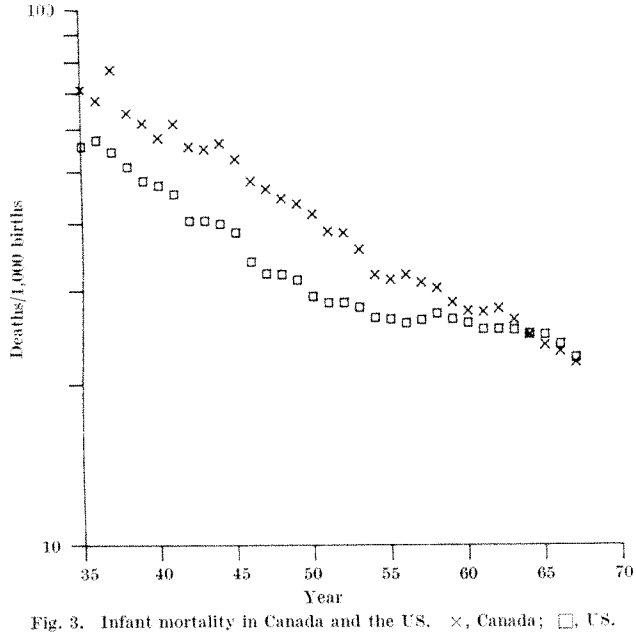


Fig. 3. Infant mortality in Canada and the US. ×, Canada; □, US.

We thank Mr R. G. McGregor for providing the Canadian data on strontium-90 and Dr A. Irwin for assistance in locating and interpreting both these and mortality data.

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Influence of Hydrostatic Pressure on the Reversible Polymerization of Fibrin Monomers

The conversion of fibrinogen to fibrin, which is the last stage of the complex process of blood coagulation, is composed of three successive reactions[1]: an enzymatic hydrolysis by thrombin of four arginyl-lysyl bonds per molecule of fibrinogen, yielding a fibrin monomer; a spontaneous polymerization of monomers to form a gel or fibrin clot; and an enzymatic transamidation by fibrin stabilizing factor (factor XIII or fibrinase) between ε-amino groups of lysyl and glutaminyl side chains. The mechanism of the reversible polymerization of fibrin monomers remains largely unknown, although it can be studied separately because fibrin monomers can be stabilized in acidic media of high ionic strength such as 1 M NaBr, *p*H 5·3[2].

The hypothesis of hydrogen bonding between ionizable groups, in which histidyl functions as acceptor and tyrosyl or lysyl as donors, as proposed by Sturtevant *et al.*[3], accounts for the depolymerizing effect of dilution, of slight temperature increase and of salts and urea. It also explains the *p*H range of polymerization (between 5·5 and 10·5), the *p*H evolution during the reaction (proton liberation below *p*H 7·5) and the inhibiting effect of chemical blocking of histidyl and tyrosyl residues. The only real shortcoming of this hypothesis is its failure to account for the magnitude of the heat evolution during polymerization[4]. It seems that additional heat evolving reactions such as hydrogen bonding between non-ionizable groups, ion pair bonding, hydrophobic bonds or conformational changes should also be involved. Up to now, however, no sufficient independent evidence for either of those additional reactions has been presented.

The origin of the heat evolution in reversible fibrin polymerization has become an interesting problem since a number of other cases of exothermic protein polymerizations, including flagellin, β-lactoglobulin and lactic dehydrogenase, have been discovered.

A study of the influence of hydrostatic pressure on the polymerization equilibria gives the sign and magnitude of the volume increment, which is dependent on the nature and the number of the interactions involved in polymerization. Volume changes during polymerization have been noted before, for example in tobacco mosaic virus protein[5] and in myosin[6].

Bovine fibrinogen was prepared according to the method of Blombäck and Blombäck[7]; fibrin monomers were prepared according to Donnelly[2] as modified by Endres[4].

A technique for observing association and aggregation reactions of proteins by means of light scattering measurements under high pressure has recently been developed by Putzeys and Vaneghem (unpublished thesis). We were able to use the apparatus and technique in the Laboratory of Biochemistry I. Light scattering measurements were taken at 90° and under pressure up to 3,200 kg/cm². Temperature variations resulting from application of pressure were prevented by a water jacket, maintained at 25° C.

Fibrinogen and fibrin monomer solutions showed no change in light scattering under pressure up to 3,200 kg/cm² except for a small stepwise change during the application of pressure, caused by deformation of the windows in the optical path (Fig. 1A).

Intermediately polymerized fibrin monomer solutions of 10 mg/ml. in 1 M NaBr, 0·05 M acetate buffer, with a *p*H varying from 5·75 to 6·15, showed a large decrease in light scattering under pressure (Fig. 1B–E) and were apparently already completely depolymerized under a pressure of 2,500 kg/cm², for after equilibration a further increase did not change the amount of scattered light. The polymerization–depolymerization reaction was always completely reversible. The influence of the concentration

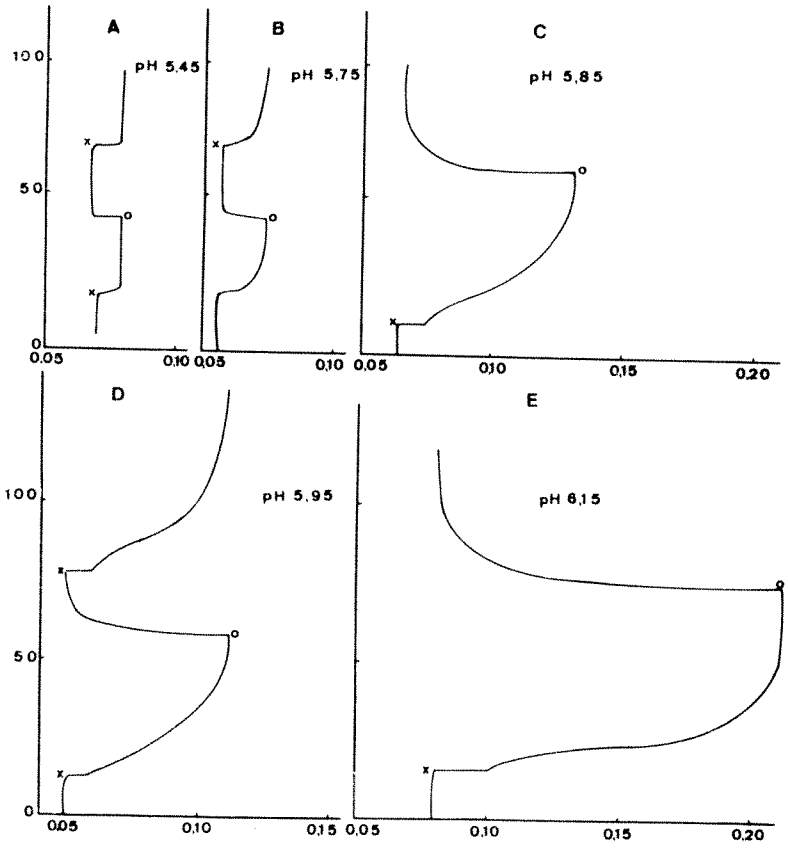


Fig. 1. Influence of pH on the polymerization of fibrin monomers in 1 M NaBr; abscissa, photomultiplier current in μA; ordinate, time in min; ○, application of pressure of 2,500 kg/cm²; ×, release of pressure. The solutions were initially depolymerized by application of pressure.

of protein on the light scattering changes under pressure is shown in Fig. 2. Turbid clots[8], prepared by 20 fold dilution of 10 mg/ml. fibrin monomer solutions in phosphate buffer ($I = 0{\cdot}2$, $p\mathrm{H} = 6{\cdot}0$), depolymerized equally well under a pressure of 2,500 kg/cm².

Because the polymerization is very pH sensitive and the pH of the acetate buffer decreases under pressure (about 0·4 pH units at 3,000 kg/cm²) it was tested whether the depolymerizing effect of pressure could be ascribed to pH changes. The experiments were therefore repeated in a 0·05 M ammonium acetate buffer (decrease of about 0·1 pH units at 3,000 kg/cm²) and in 0·05 M MES (N-morpholino - ethane - sulphonic acid) buffer (increase of about 0·25 pH units at 3,000 kg/cm²). In all cases an apparently complete depolymerization was obtained at 2,500 kg/cm².

Finally, it was tested whether the pressure effect could be ascribed to an alteration of the protein during the preparation of monomers. Native plasma, diluted with buffer to a concentration of 1 mg fibrinogen/ml. and clotted with thrombin, behaved identically under pressure.

On the basis of the complete reversibility of the depolymerization and polymerization reactions and in view of the fact that the phenomenon is the same in buffers with different pH dependence on pressure, we conclude that the polymerization is accompanied by an increase in volume. This volume increase is not explained by the hypothesis of hydrogen bonding between histidyl and tyrosyl or ε-amino groups as the only polymerization mechanism for this reaction in the pH range investigated should be accompanied by a volume decrease (resulting from hydrogen bonding and from electrostriction around the liberated protons).

In fact, our results indicate that during polymerization an additional interaction occurs with a significant volume increase sufficient to overcome the volume decrease due to hydrogen bonding and electrostriction. This seems to point in the direction of the existence of additional hydrophobic bonding or ion pair bonds (salt linkages)[9]. The role of hydrophobic bonding seems to be small in view of the depolymerizing effect of temperature and electrolyte concentration. Attempts to show the presence of hydrophobic sites on the surface by binding the ligand

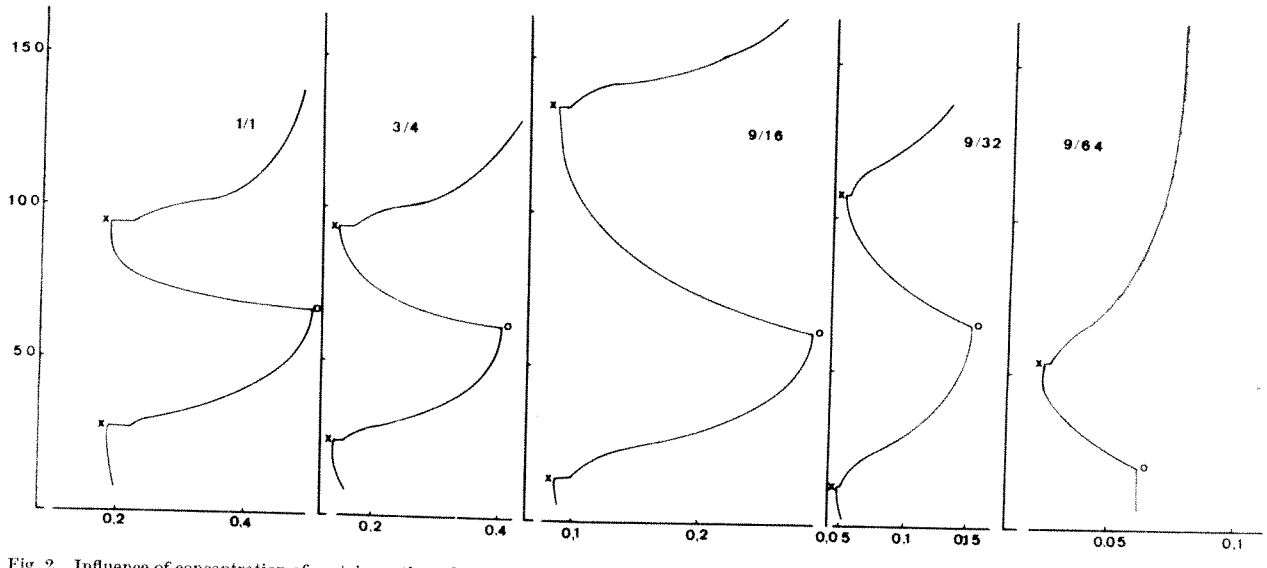


Fig. 2. Influence of concentration of protein on the polymerization in 1 M NaBr, pH 6·1. Abscissa, photomultiplier current in μA; ordinate, time in min; ○, application of pressure of 2,500 kg/cm²; ×, release of pressure. The solutions were initially depolarized by application of pressure. Dilution 1/1 = 10 mg fibrin monomers per ml.

ANS (1-anilo-8-naphthalene sulphonate)[10,11] were unsuccessful. Further studies to estimate the volume increase per link by quantitative determination of the effect of pressure on the successive polymerization constants will be undertaken.

We thank Professor P. Putzeys for the use of his special equipment and Dr A. Persoons and Dr K. Heremans for their advice. We also thank Mr F. Decock for his technical assistance.

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In vitro Production of Nucleolar Channel System by Progesterone in Human Endometrium

Electron microscope examination of normal human endometrium during the midsecretory phase of the menstrual cycle demonstrates that the nucleolus of the epithelial cell differentiates into an organelle variously termed a nucleolar canalicular system[1], a nucleolar basket[2] or a nucleolar channel system[3]. Such a structure appears if ovulation has occurred. Nucleolar baskets have been seen as early as the sixteenth day of a normal 28 day cycle. After this they may increase in number, reaching a maximum around the twentieth day and then disappear, not being found later in the cycle. They have not been seen in the proliferative phase of the cycle or in early pregnancy.

This system seems to be a unique form of nucleolar differentiation having been observed in human glandular endometrial cells and in no other tissue. It consists of an involved labyrinth of channels coursing through a more dense homogeneous matrix (Fig. 1). Electron opaque 150 Å particles are arranged in a regular fashion around the periphery of the total channel mass but may also surround the individual channels (our unpublished results).

Previous attempts to demonstrate the specific physiological effect of progesterone by means of organ culture were frustrated by the induction of secretory changes in the endometrial cell in the control culture[4]. Electron microscope examination of such organ cultures failed to show nucleolar channel systems[5]. Using organ culture of proliferative human endometrium it has recently been shown[6,7] that progesterone does act directly on the epithelium to convert proliferative to secretory endometrium. Thus the effects observed *in vivo* could be mimicked *in vitro*. This conversion was specifically due to progesterone and was not produced in the control cultures, in cultures with oestrogen alone or with cortisol. We undertook this study to find out whether nucleolar differentiation can be induced by progesterone in organ cultures of proliferative endometrium.

Specimens were obtained aseptically from normal women by endometrial biopsy on the tenth day of the menstrual cycle. A small piece was processed for electron microscopy and another was examined histologically. The remainder of the endometrium was cut into pieces 1–2 mm³. Each of six pieces was placed on 'Millipore' filter paper resting on agar enriched with TC 199 medium to which 10 per cent calf serum and 10 μg/ml. insulin had been added. In addition to control cultures, cultures containing progesterone (1 μg/ml. and 10 μg/ml.), medroxyprogesterone acetate (1 μg/ml. and 10 μg/ml.), oestradiol (10 μg/ml.) or oestradiol (10 μg/ml.) plus progesterone (10 μg/ml.) were prepared. These were incubated at 37° C in a moist atmosphere of 95 per cent oxygen and 5 per cent CO_2. Specimens were examined both histologically and by electron microscopy after 48 and 96 h. For electron microscopy, a small portion of the tissue was fixed in 3 per cent glutaraldehyde in phosphate buffer (*p*H 7·0) at room temperature for 1 or 2 h. After re-fixation for 1 h in 1 per cent veronal buffered osmium tetroxide and subsequent dehydration, the specimens were embedded in 'Epon 812'. Thin sections were double stained with uranyl acetate and lead citrate and examined in an Hitachi HU-11B electron microscope. At least two tissue blocks containing representative samples of endometrium were examined from each specimen.

The histological appearance of the control cultures resembled that of the original proliferative endometrium. Oestrogen produced no significant change. The addition of progesterone produced the changes characteristic of secretory endometrium, first subnuclear deposition of glycogen, then secretion of the glycogen into the lumen of the glands.

Electron microscope examination of the original specimen showed the characteristic appearance of proliferative endometrium (ref. 8 and our unpublished data). No nucleolar channel systems were seen. Extensive search failed to reveal nucleolar channel systems in the control cultures and none were found in the cultures exposed to oestradiol. Characteristic nucleolar channel systems were produced in the cultures containing progesterone (Fig. 2). They were found after 48 and 96 h at both progesterone concentrations but it was not possible to quantitate the results in terms of length of exposure to, or concentration of, progesterone. Although our results suggest that differentiation may be accelerated *in vitro*, such a conclusion is not justified without further study. Nucleolar baskets were also produced in the culture

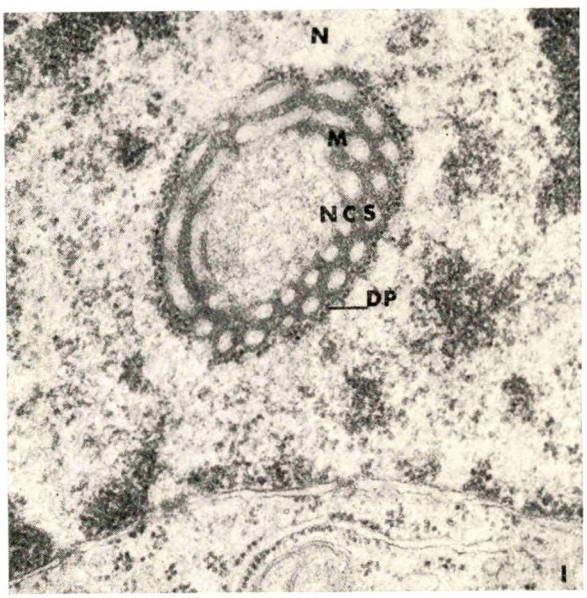


Fig. 1. Electron micrograph of endometrial epithelial cell from a biopsy specimen taken on the eighteenth day of a normal 28 day cycle. A nucleolar channel system (NCS), characteristic of secretory stage endometrium, is seen within the nucleus (N). The channels course through a dense matrix (M). Dense particles (DP) are seen at the periphery of the nucleolus. (×24,375.)

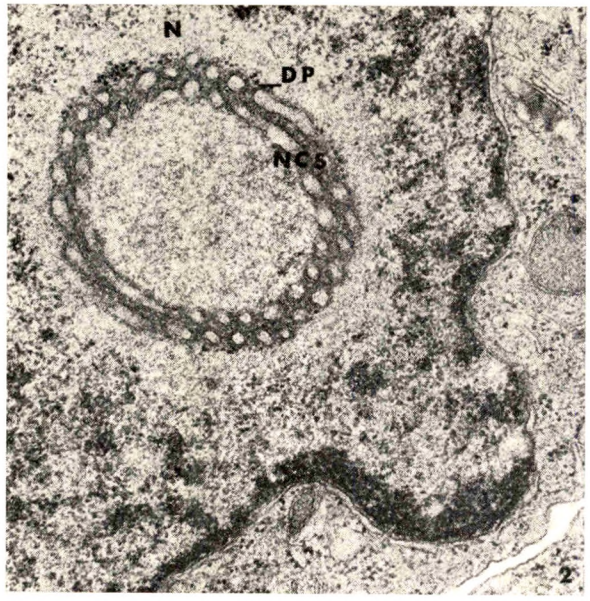


Fig. 2. Electron micrograph of epithelial cell from 10 day normal human endometrium cultured for 96 h in a medium containing 10 μg/ml. progesterone. Comparison with Fig. 1 demonstrates that a similar nucleolar channel system (NCS) is present within the nucleus (N) although the biopsy specimen was in the proliferative stage. Dense particles (DP) are at the periphery of the nucleolus. (×24,375.)

containing oestrogen and progesterone, demonstrating that oestrogen is not inhibitory to nucleolar differentiation. It would be of interest to determine whether greater concentrations of oestrogen would counteract the influence of progesterone. Cultures containing 1 μg/ml. medroxyprogesterone acetate also showed baskets, confirming the physiological action of this compound.

These results illustrate a close relationship between progesterone and the development of nucleolar channel systems in glandular cells of human endometrium. The combined technique of organ culture and electron microscopy offers a means of further exploring the effects of progestational steroids to help elucidate their mode of action more precisely.

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'Tuftsin': a Natural Phagocytosis Stimulating Peptide

WE have shown in this laboratory that a specific cell bound leucophilic γ-globulin fraction, leucokinin, is essential for maximal stimulation of the phagocytic activity of the blood neutrophilic leucocyte[1,2]. We now report that the whole stimulatory effect of leucokinin can be ascribed to a single peptide fragment liberated by a specific enzyme in the outer membrane of the neutrophile. We shall refer to this peptide as 'tuftsin' (after Tufts University) and to the enzyme as leucokininase. The peptide 'tuftsin' has been produced *in vitro* from a γ-globulin fraction, PC IV, by treatment with leucokininase. PC IV is prepared by chromatography on phosphocellulose columns[3] and contains all the leucokinin activity of serum γ-globulin[2].

To prepare leucokininase, polymorphonuclear leucocytes from 100 ml. of human or dog blood were isolated on glass columns[4]. These were lysed in 5 ml. of 0·34 M sucrose and the cytoplasmic granules separated out in the supernatant by centrifugation[5]. The sedimented membranes and nuclei, about 0·3 ml., were washed four times in 5 ml. of 0·1 M phosphate buffer (*p*H 6·7). This was followed by homogenization in a 'Teflon'–glass homogenizer, after which the membrane was floated in 2·1 M sucrose at 30,000 r.p.m. for 16 h in a Spinco 'SW39' rotor. The membrane portion so isolated was then washed in phosphate buffer and broken further by high frequency sound (Biosonic, microtip; setting 20; 2 min). The preparation of neutrophilic leucocytes should be practically free of erythrocytes. Red cell membranes similarly treated exhibit strong peptidase activity towards 'tuftsin', thereby destroying its biological effect.

The enzyme has a sharp *p*H optimum at about 6·7; its activity becomes negligible at *p*H 6 and 7·8 and its optimum temperature is around 37° C. At 22° C it has very low activity and at 45° C it is destroyed. Thoroughly washed membrane preparations retain full activity. It does not dissociate readily on standing at 4°–6° C for a week, but is partly solubilized after repeated freeze–thawing. It is presumably present on the outer surface of the membrane, for the intact thoroughly washed cell shows highly active leucokininase.

To prepare the peptide 2–4 μg of membrane protein (leucokininase), 1·0 mg (approximately 6·5 nmoles) of γ-globulin fraction PC IV, are mixed in a final volume of 0·5 ml. of 0·1 M phosphate buffer (*p*H 6·7). This is incubated at 37° C for 60 min. The reaction is stopped by the addition of 4 volumes of 95 per cent ethanol and placed in a water bath at 80° C for 8 min, after which it is cooled. The supernatant containing 'tuftsin' is separated from the precipitate and dried by an air stream. The residue is then washed twice with ethyl ether and dried *in vacuo*. 0·5 ml. of distilled deionized water is added and 0·05 ml., equivalent to 0·1 mg of the original protein, is used for each phagocytosis test. These determinations are carried out in Hanks medium or in buffered isotonic sucrose medium[2]. The stimulatory effect of 'tuftsin' is equivalent to the amount of PC IV from which it is derived. About 0·1 mg of PC IV and its 'tuftsin' equivalent constitute the minimal amounts necessary for maximal *in vitro* stimulation in a total volume of 0·4 ml.[2,6].

PC IV was prepared from commercially available Cohn fraction II γ-globulin and its leucokinin activity was completely precipitable with specific anti-γG serum[2]. PC IV shows only a γG band with immunoelectrophoresis and diffusion against anti-human serum[2,3]. It sediments as a 7S γ-globulin with a symmetrical peak. It shows one band in disc electrophoresis[3]. PC IV so purified was subjected to papain digestion and the Fc and Fab fragments were isolated[7] and tested directly *in vitro*[6]. The phagocyte activity usually expressed as percentage of cells containing bacteria[1,2] was shown to reside only in the Fab fragment. Representative values of several determinations (between three and ten) showed that with 0·1 mg equivalent of PC IV, Fab gave an average value of 48 per cent compared with 18 per cent obtained with Fc and control values of 20 per cent given by the standard reaction mixture alone. Similarly, the heavy and light chains of the parent molecule were studied. These were prepared by reduction and alkylation[8]. Following

leucokininase treatment, 'tuftsin' was extracted with ethanol and assayed for its phagocytic stimulation. All activity resided in the heavy chain which yielded a value of 50 per cent while the light chain gave values similar to the controls. Thus 'tuftsin' seems to be in the amino-terminal portion of the heavy chain in the Fd fragment; it is therefore unlikely that it contains carbohydrate moiety.

The stimulatory effect of 'tuftsin' on phagocytosis is destroyed by incubation for 3 h at 30° C with pronase, subtilisin, carboxypeptidase B and leucinaminopeptidase, using 10 μg protein/ml. in optimal conditions of enzyme action[10]. Similarly, it is destroyed by incubation with soluble supernatants of disrupted leucocytes and membrane preparations from human erythrocytes and rat liver at *p*H 7·0 in phosphate buffer. In all these, phagocytosis was at the level of the controls. 'Tuftsin' is not destroyed by exposure to aeration in the presence or absence of cysteine, treatment at 80° C for 10 min in 80 per cent ethanol, trypsin, chymotrypsin, clostridiopeptidase B, phosphatase, deoxyribonuclease. Phagocytosis values obtained after such treatment were 46 per cent to 50 per cent, about 2·5 times those of the controls. 'Tuftsin' is presumably present only in a small fraction of leucophilic γ-globulin and its *in vitro* activity can be elicited with hormone like quantities. The yield of biologically active alcohol extractable and ninhydrin positive material per mg of PC IV protein is very small. More than 1,000 phagocytosis determinations can be carried out on samples that yield a moderately detectable ninhydrin colour. Purification has therefore been cumbersome and simple contamination from glassware and chemicals makes analysis difficult. It seems likely, however, that 'tuftsin' does not contain cysteine, methionine, tyrosine, phenylalanine or tryptophan. Its great susceptibility to carboxypeptidase B indicates the presence of a basic amino-acid as the carboxyl-terminal residue. 'Tuftsin' has other characteristics in common with polypeptide hormones: it is a highly active and readily dialysable polypeptide; and it is a part of a larger carrier molecule.

'Tuftsin' has been found deficient or absent in one male and three female patients with recurring infections. No stimulation of phagocytosis above the usual control values could be elicited despite repeated testing. Should it turn out that 'tuftsin' can stimulate phagocytosis *in vivo* as it does *in vitro*, it might have some therapeutic application in diseases in which phagocytosis is important.

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Interaction of Atropine with the Muscarinic Receptor

WE report here the crystal structures of two muscarinic antagonists of acetylcholine, (−)-(*S*)-hyoscine hydrobromide[1] and (−)-(*S*)-hyoscyamine hydrobromide (atropine). Correlation of these two structures and that of quinuclidinyl benzilate hydrobromide[2] shows marked similarities among the three molecules although they are subjected to very different crystal packing forces. This suggests that intramolecular forces impose a preferred conformation on many such antagonist molecules. The structural correlations may be used to derive a set of rules for predicting the conformation relevant to muscarinic antagonism of acetylcholine for many molecules which at first sight are rather dissimilar to atropine (T. J. P., in preparation).

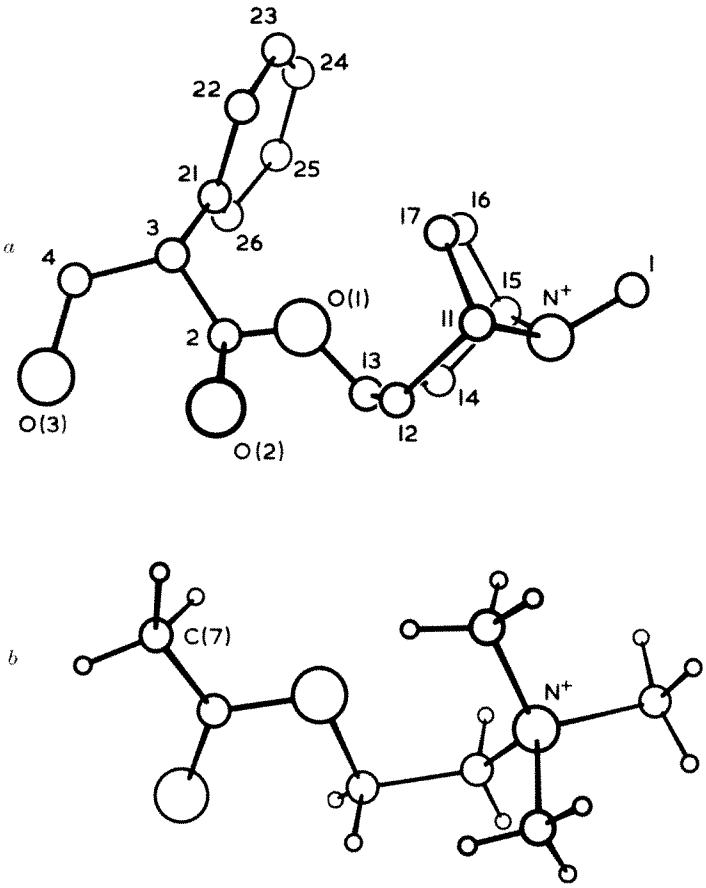


Fig. 1. Molecules of atropine (*a*) and acetylcholine (*b*) in the conformations relevant to interaction with the muscarinic receptor.

The crystal structure of (−)-(*S*)-hyoscyamine hydrobromide was determined by standard methods from diffractometer data and refined by full-matrix least-squares to a present *R* of 0·09. The molecule is shown (Fig. 1*a*) in the absolute configuration corresponding to that of the more potent enantiometer and with an intramolecular hydrogen bond O(3)–H . . . O(2). This bond is not present in the crystal, but the conformation shown is that which correlates best with the other crystal structures. The hydrogen bond has been shown by spectroscopic measurements[3] to exist in solution. This is the only change from the conformation observed in the crystal, which otherwise coincides exactly with the observed conformation of hyoscine and is very similar to that of quinuclidinyl benzilate. The molecule is best described by three planes: that of the benzene ring, of the planar acetoxy group, and of the mirror plane bisecting the tropane fused ring system through N^+ and C(13). The

angle between these last two planes is 35°, which is exactly the mean value found in a survey of secondary esters (P. J. P., in preparation) and the plane of the benzene ring makes an angle of 86° with the plane of the ester group. The corresponding angles in hyoscine are 45° and 89° respectively. The situation in quinuclidinyl benzilate is a little different, but the spatial arrangement of the nitrogen atom, one of the two benzene rings, and the hydroxy group is very similar to that in the other two molecules.

The acetylcholine molecule, in the preferred conformation relevant to interaction with the muscarinic receptor (C. H. Chothia, P. J. P., and T. J. P., in preparation), is shown (Fig. 1*b*) for comparison. The only groups believed to interact with the receptor are the $-N^+(CH_3)_3$ cationic head and the C(7) methyl group, the former by electrostatic and the latter by van der Waals forces. The relationship of the plane containing the C(7) hydrogen atoms to the cationic head is very similar to that of the benzene ring and the charged nitrogen atom in atropine (Fig. 1). The N^+-plane distances are, moreover, very similar (575 and 625 pm respectively). We therefore suggest that both atropine and acetylcholine interact with the muscarinic receptor in similar ways and at the same site. The van der Waals interaction of the C(7) methyl group in acetylcholine is paralleled by the rather stronger interaction of the flat benzene ring in atropine; the charged nitrogen atoms interact with the same anionic site. The criteria distinguishing antagonists from agonists, then, are (*a*) the presence of a lipophilic group capable of stronger van der Waals binding than a methyl group at a similar distance from a charged nitrogen atom, as is C(7) in acetylcholine (this explains the transition in properties from agonist to antagonist as acetylcholine is successively substituted at C(7) by larger alkyl groups); and (*b*) for efficient antagonism, the presence of an auxiliary electrophilic group, O(3)H in the case of atropine, in a suitable spatial relationship to the first two groups. Examination of space-filling models shows that practically all potent muscarinic antagonists satisfy these criteria.

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Bicuculline and Inhibition of Crayfish Stretch Receptor Neurones

THE recent reports[1,2] on the antagonistic effect of the alkaloid bicuculline both of the actions of γ-aminobutyric acid (GABA) electrophoretically applied to single neurones and of certain strychnine-resistant inhibitory mechanisms in the mammalian central nervous system have suggested that the effects of this substance should be examined in crustacea where GABA is likely to act as a transmitter of post-synaptic inhibition[3].

Action potentials developed by the slowly adapting stretch receptor neurones of the Murray River crayfish *Eustacus armatus* were recorded following the method of Florey[4]. When 10–30 μg/ml. of GABA (1×10^{-4}–3×10^{-4} M) was applied to these cells an immediate inhibition of the spike discharge was consistently produced followed by a slow recovery beginning 30–60 s after the application. Bicuculline in concentrations ranging from 3–30 μg/ml. had the effect either of reducing or of completely preventing the inhibitory action of GABA. The blocking effect was fully developed in 5–10 min and could be wholly reversed by washing the preparation for the same length of time. When comparisons were made on the same preparations, bicuculline was about ten times more potent as a GABA antagonist, weight for weight, than was picrotoxin, and three times more effective than picrotoxinin. These ratios are in accord with other reported actions of the substances: the convulsant dose of bicuculline in rabbits is 0·2 mg/kg (ref. 5); those for picrotoxin and picrotoxinin in mice 2·0 and 1·0 mg/kg respectively[6]. The inhibition of neurones of Deiters's nucleus following cerebellar stimulation is prevented by 0·2 mg/kg of bicuculline[2] and by 10 mg/kg of picrotoxin[7].

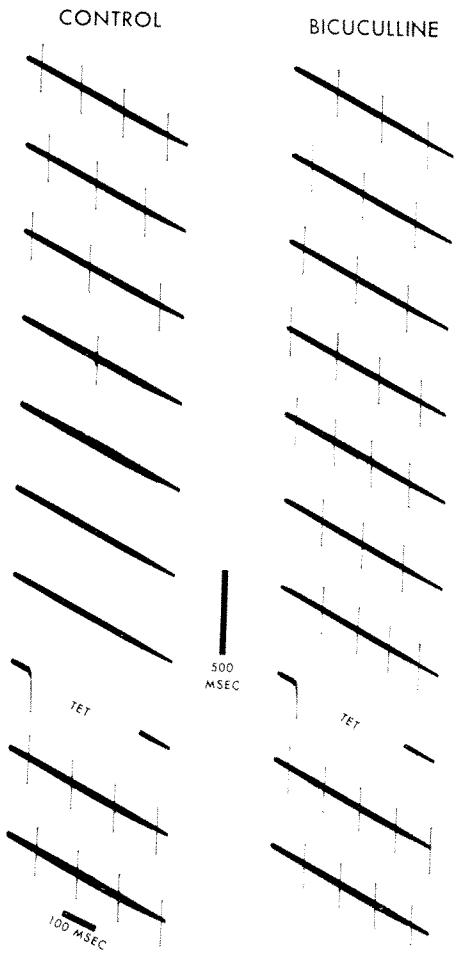


Fig. 1. The action of bicuculline (30 μg/ml.) on the inhibition of discharge of a crayfish stretch receptor neurone brought about by stimulation (TET) of its inhibitory nerve supply for 400 ms with 5 V, 0·1 ms pulses at 100/s. The traces in each column are continuous and read from below upwards; the 500 ms vertical bar indicates rate of film movement.

Bicuculline also antagonized the inhibition of discharge of the crayfish neurones which follows repetitive stimulation of their inhibitory nerve supply (Fig. 1); and again this substance was approximately 10 times more potent than picrotoxin. Because it is probable that this crustacean inhibitory mechanism is mediated through the release of GABA as a transmitter, it seems that in crustacea, as in the mammalian central nervous system, bicuculline is a powerful antagonist of the actions of GABA whether applied exogenously or released by some synaptic mecha-

nism; and that bicuculline is more potent as an antagonist than is picrotoxin.

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Doubtful Value of Bicuculline as a Specific Antagonist of GABA

In view of the recent report that bicuculline[1–3] antagonizes the action of γ-aminobutyric acid (GABA) at various sites in the central nervous system[4] we have systematically tested this alkaloid, applied by microiontophoresis to neurones in the cerebral cortex of four cats under 'Dial' anaesthesia. Two samples of bicuculline were used: the first was obtained from Pierce Chemicals; it was prepared either as a 0·1 M solution in dioxane, or by dissolving at a concentration of 0·05–0·1 M in dilute HCl (final *p*H 3). The second sample (bicuculline hydrochloride) was kindly given to us by Dr R. Manske (University of Waterloo): this was prepared as in the experiments of Curtis *et al.*[4] at a concentration of 10 mM in 165 mM NaCl (*p*H 4). One barrel of the multi-barrelled glass micropipettes was filled with these solutions 2–3 days before the experiments. The 0·1 M solutions did not readily pass iontophoretic currents > 30–50 nA (see ref. 4), but very large currents (≫100 nA) could be passed through the 10 mM solutions for several tens of minutes.

The tests were performed as follows. A given unit was excited in a regular manner by 4–6 s applications of L-glutamate (20–60 nA) at intervals of some 5 s (Figs. 1 and 2). The glutamate-evoked responses were then depressed to a various extent by different amounts of GABA applied for 30–40 s; in most cases, a total block of responses was achieved by doses of GABA in the range 7–28 nA. Bicuculline was then administered by an outward current (electrode tip relatively positive),

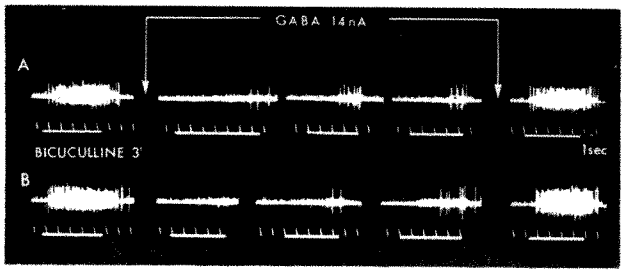


Fig. 1. Discharges of cortical neurone evoked by microiontophoretic release of L-glutamate (56 nA, during periods marked by white lines below oscilloscope trace); firing was almost completely blocked by release of GABA (14 nA: period between arrows) from another barrel of multiple micropipette. (*A*) Control; (*B*) 3 min after starting application of bicuculline (70 nA, from 0·05 M solution of hydrochloride).

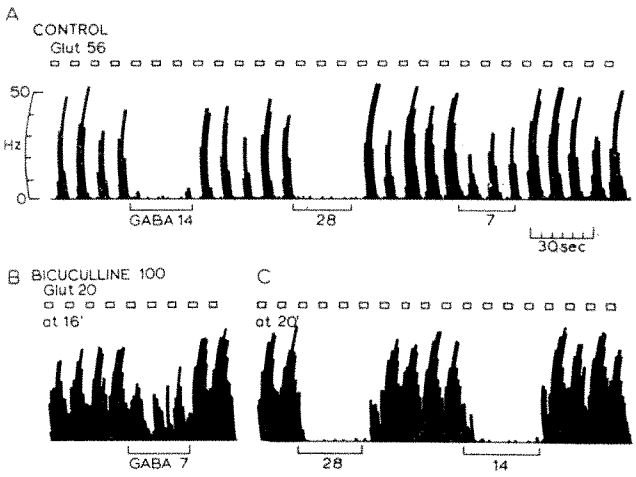


Fig. 2. Glutamate-evoked discharges of another cortical neurone, inhibited by three doses of GABA: paper trace records firing frequency. (*A*) Control period; (*B*) 16 min and (*C*) 20 min after starting release of bicuculline (100 nA, from 10 mM solution of hydrochloride in 165 mM NaCl). White squares indicate applications of L-glutamate; all doses are indicated in nA of iontophoretic current. Note sharply reduced doses of glutamate and spontaneous activity in *B* and *C*.

usually for periods of at least 5–10 min; during this time, GABA was reapplied to test again its depressant effects on the glutamate-evoked discharges. Whenever possible, dose–response curves for GABA were constructed (Fig. 3) by comparing the mean amplitude of three glutamate-evoked responses just before and during the release of GABA.

Of 32 units on which adequate tests could be made, 22 showed no clear change, six were more effectively depressed by GABA, and four somewhat less effectively, during the concurrent release of bicuculline. Very large doses of bicuculline (> 100 nA through 10 mM bicuculline-HCl in 165 mM NaCl) sometimes caused an increase in excitability, manifested by a gradual rise in "spontaneous" firing and greatly augmented responses to glutamate (Fig. 2). Not surprisingly, in these conditions GABA could not silence the cells so readily; but if the doses of glutamate were adjusted to yield a response at the control level, in all cases but one the effect produced by GABA was identical to that seen previously.

In Fig. 1, the oscilloscope traces show a very similar degree of inhibition produced by GABA during the initial control period (*A*) and 3 min after starting the release of bicuculline (*B*). In Fig. 2, paper traces recording the rate of firing of another cortical neurone show the glutamate-evoked discharge and its depression by three doses of GABA (*A*): the second set of traces demonstrates that even 20 min after the start of a large application of bicuculline (100 nA), there was no evidence of any diminished potency of GABA (*B*, *C*). Some GABA dose–response curves obtained from other neurones before and during the release of bicuculline are also illustrated (Fig. 3). Bicuculline was also tested four times on three acetylcholine-sensitive cells which were either firing rapidly "spontaneously" or were excited by regular applications of acetylcholine. The inhibition of these discharges by GABA was in no way diminished by bicuculline given for as long as 15 min.

Because of the failure to produce any clear effects, one might question whether the samples of bicuculline used had any biological activity. We tested aliquots of solutions taken from the micropipette, either by intravenous injection or by direct application to the cortical surface, and in this way confirmed the original observations[5] of pronounced convulsions following the systemic administration of bicuculline (5 μmole); further, we found that even weak solutions (1 mM) applied to the cortical surface rapidly evoke local paroxysmal activity. Clearly our

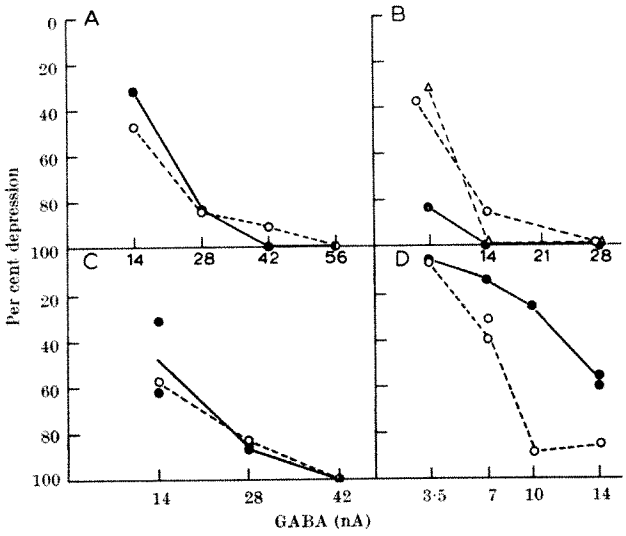


Fig. 3. Dose–response curves for GABA effect on glutamate-evoked firing of four other cortical neurones. ○—○, Control; ●—●, during iontophoretic release of bicuculline; △—△, control after. Doses of bicuculline and periods of release were respectively: (*A*) 25 nA, 6 min; (*B*) 25 nA, 6 min; (*C*) 50 nA, 14 min; (*D*) 70 nA, 20 min.

samples were not inactive, and bicuculline does have a convulsant action on the cerebral cortex. Paroxysmal activity of this type can, however, be elicited by a variety of procedures—such as local freezing, prolonged neural isolation, and the application of many substances, including strychnine and curare. There is no ground for thinking that these various procedures interfere specifically with the action of GABA. Whatever the mechanism of its convulsant action, we find it difficult to see how bicuculline could be useful for critical tests of GABA-mediated inhibition.

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Bicuculline and Central GABA Receptors

We are unable to accept the implication by Krnjević and his associates[1] that we erred in reporting[2] bicuculline to be a relatively specific antagonist of γ-aminobutyric acid (GABA) in the central nervous system of the cat.

The bicuculline (Fluka, AG) used in our investigation was identified on the basis of the following observations: melting point, 201°–205° C; elemental analysis (calculated from formula $C_{20}H_{17}NO_6$: C, 65·39; H, 4·66; N, 3·81 per cent. Found: C, 65·38; H, 4·73; N, 3·83 per cent); homogeneity by thin layer chromatography on silica gel HF_{254} in three solvent systems, located with iodine (*n*-butanol–acetic acid–water, 4 : 1 : 1; chloroform–methanol, 1 : 1; cyclohexane–chloroform–33 per cent ethanolic dimethylamine, 5 : 4 : 1); mass, infrared and NMR spectra in accordance with the proposed structure and previous findings[3–5]. When prepared without heating, solutions of the alkaloid in 165 mM NaCl at *p*H 3–3·2 contained approximately 5 mM bicuculline.

Our report[2] indicated that this bicuculline blocked the depression by GABA of both spontaneous and amino-acid (DL-homocysteate) induced firing of cortical neurones in cats anaesthetized with pentobarbitone; consistent observations had been made on twenty-two pyramidal tract neurones in ten preparations. This action has been confirmed in animals anaesthetized with 'Dial'–urethane, and with neurones excited by L-glutamate, as shown in Fig. 1. Enhancement of neuronal firing by bicuculline was not responsible for an apparent loss of GABA sensitivity, for rates of firing could be restored to pre-bicuculline levels by reducing the amount of amino-acid excitant ejected, and a control was provided by using another depressant such as glycine. In addition to the antagonism demonstrated between bicuculline and GABA in the cerebellum, cerebral cortex, and spinal cord[2], we have also found that this substance consistently blocks the inhibitory effect of both GABA and impulses in Purkinje cell axons on neurones of Deiters's nucleus[6], and is an antagonist of both the action of GABA on, and inhibition

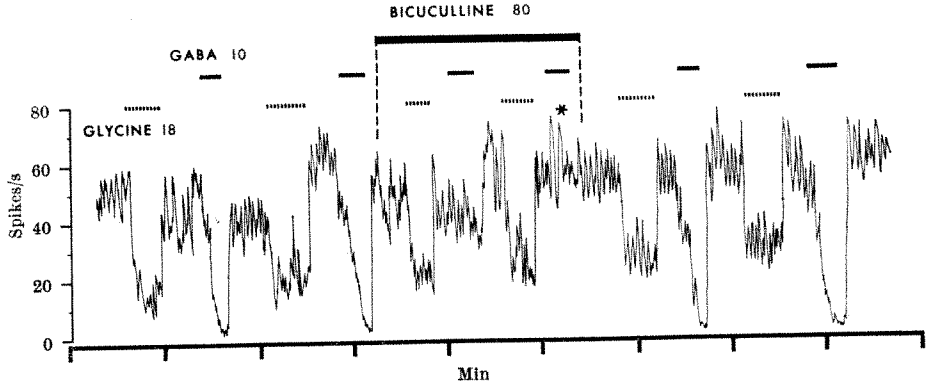


Fig. 1. Effect of bicuculline on the inhibition by glycine and GABA of an antidromically identified deep pyramidal cell in the postcruciate cerebral cortex. Cat anaesthetized with 'Dial'–urethane, rate of cell firing (ordinate) maintained by the continuous electrophoretic ejection of L-glutamate (12 nA) from one barrel of a seven barrel micropipette. Glycine (18 nA) and GABA (10 nA) were ejected at regular intervals for the periods indicated by the dotted and solid horizontal lines respectively. Bicuculline, ejected from a barrel containing 5 mM bicuculline hydrochloride in 165 mM NaCl with a current of 80 nA over the time indicated by the thick horizontal bar and vertical broken lines, rapidly and reversibly suppressed the effect of GABA (abolition of GABA action indicated by an asterisk) without reducing that of glycine. Abscissa, time (min).

of, neurones in the ventrobasal thalamus[7] and hippocampus[8]. Furthermore, bicuculline blocks the effect of GABA on stretch receptor neurones of the crayfish *Eustacus armatus*, and the synaptic inhibition of these neurones[9].

Identical pharmacological results in the feline central nervous system have been obtained with other samples of bicuculline, including that purchased from the Pierce Chemical Company, K and K Laboratories, and one kindly provided by Dr R. H. F. Manske.

We are therefore convinced that our results do indeed relate to bicuculline and that our findings provide significant evidence for the function of GABA as the transmitter released at certain inhibitory synapses in the feline cerebral cortex, cerebellum, brain stem, thalamus and hippocampus.

Because bicuculline is also an antagonist of the depressant action of imidazole acetic acid, further neurochemical evidence is required to determine whether this compound also has a role as a transmitter at bicuculline-sensitive inhibitory synapses.

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Temporal Characteristics of Amnesia induced by Protein Synthesis Inhibitor: Determination by Shock Level

It is widely accepted that cerebral protein synthesis is involved in "long term" but not "short term" memory. Evidence for this comes from experiments in which amnesia for a multiple-trial avoidance task is induced by administration of protein synthesis inhibitors before or shortly after training[1–3]. In mice[4–6], the amnesia, which is permanent, does not appear until 3 h or more after training, leading to the notion of a "short term" memory, and is not produced if the criterion for learning is too severe. We have given mice a different learning task and used the protein synthesis inhibitor cycloheximide to induce amnesia that has such different characteristics as to warrant a re-evaluation of the hypothesis.

Subjects were male mice weighing 25 g from the C57Bl/6J strain. Apparatus was a standard passive avoidance box with a small clear 'Plexiglas' compartment separated from a large darkened compartment by a guillotine door. The large compartment was illuminated by a flashing light which came on when the door was opened. After the mice had entered the large compartment, a single 2 s foot shock (either 0·16 or 1·6 mA) was automatically given. Latencies to enter the large compartment were recorded on the training trial and on the retention test trial.

Mice were injected subcutaneously with 3 mg of cycloheximide dissolved in 0·3 ml. 0·85 per cent saline or with saline alone. At this dose level less than 10 per cent were sick at retention test time. These were discarded. For determination of the extent of inhibition of cerebral protein synthesis mice were injected intraperitoneally at various times after receiving the drug with 25 μCi of L-4,5-^{3}H-leucine. Exactly 30 min later, mice were decapitated and brains removed for determination of amino-acid incorporation by a method similar to that of Barondes and Cohen[7] and to be described elsewhere (Randt, C. T., Barnett, C. T., B. S. M., and D. Q., in preparation). Fig. 1 shows that maximum inhibition of amino-acid incorporation was attained at around 30 min after cycloheximide injection and that the inhibition disappeared over the subsequent 6 h. Two measures of incorporation are presented: specific activity of the protein (c.p.m./mg) and the ratio of total incorporated radioactivity to TCA-soluble radioactivity, which is presumably free amino-acid.

In our first experiment we injected groups of mice with cycloheximide at various times both before and after the one-trial learning task. Half the subjects were trained with high (1·6 mA) foot shock and half with low (0·16 mA) foot shock. Half of these groups were tested for retention 6 h after training and the other half 24 h after. Animals tested 6 h after training were divided into five groups ($n = 40$) and injected with cycloheximide at the

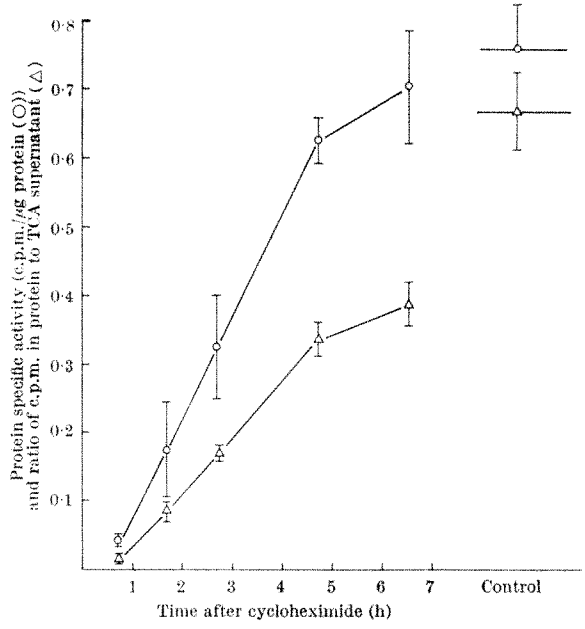


Fig. 1. Incorporation of intraperitoneally injected ^{3}H-leucine into total brain protein during a 30 min period as a function of time after subcutaneous administration of 3 mg cycloheximide.

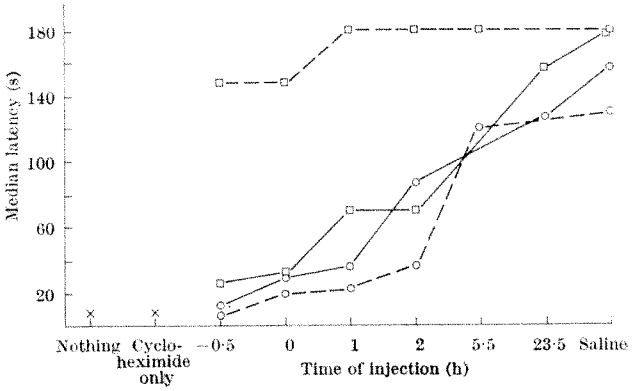


Fig. 2. Median latency to enter the large compartment on the retention test trial as a function of time interval between cycloheximide injection and training. □—□, High shock, test 24 h; ○—○, low shock, test 24 h; □ - - - □, high shock, test 6 h; ○ - - - ○, low shock, test 6 h.

following times: 30 min before training, immediately after, 1, 2, and 5·5 h after training. A sixth group ($n = 40$) was injected with saline. Half of each group ($n = 20$) was trained with high and the other half with low foot shock. The same injection times were used for mice tested 24 h after training, except that a group injected 23·5 h after training replaced the 5·5 h group. Again, half of each group was trained with high and the other half with low shock. Two further groups were trained: one group ($n = 20$) was injected with cycloheximide but not given foot shock, and a second group ($n = 20$) received neither foot shock nor cycloheximide.

Results are shown in Fig. 2. When memory is tested 24 h after learning, both the high and the low foot shock groups show a similar temporal gradient of amnesia, retention being poorest when cycloheximide is injected before and immediately after learning. When each of these groups is compared statistically with the appropriate saline control group (using Mann–Whitney U tests), significant amnesia occurs with injection delays as long as 1 h after training ($P < 0.05$) for both high and low foot shock groups. By contrast, there are marked differences between the two shock intensities when retention is tested 6 h after training. The low foot shock groups show a temporal gradient closely comparable with that seen in the groups tested at 24 h. In these conditions, amnesia can be produced when cycloheximide is injected as long as 2 h after training ($P < 0.05$). There is, however, no loss of memory evident in any of the high shock groups. Apparently, amnesia for the high shock takes some time to develop; it is present 24 h but not 6 h after training.

With low shock, amnesia is present 6 h after training, but memory may have been present at some earlier time as predicted by the two-stage hypothesis[2,3]. Habits learned under different shock levels may go into long-term storage at different rates. To investigate this we carried out a second experiment, in which we tested for retention of habits that had been in storage for different times. Five training-test intervals were chosen: 1 min, 5 min, 6 h, 24 h, and 48 h. Four groups ($n = 20$) were assigned to each interval; two of these groups were injected with saline and trained with either high or low shock, and two were injected with cycloheximide and trained with either high or low shock.

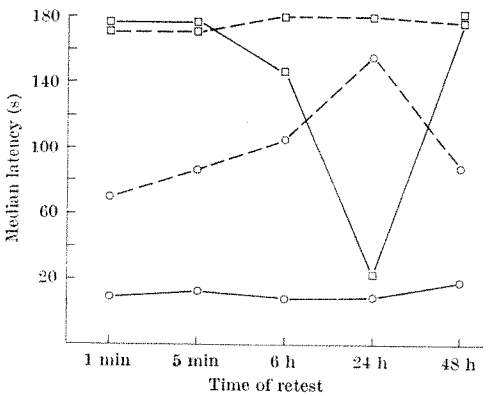


Fig. 3. Median latency to enter the large compartment on the test trial as a function of time between the training trial and the retention test. Cycloheximide and saline were injected 30 min before training for all groups. □, High shock; ○, low shock; - - - saline; ———, cycloheximide.

The results are shown in Fig. 3. The low-shock, saline-injected subjects show an improvement in retention with time. Comparison of these groups with the cycloheximide injected groups indicates that significant memory loss occurs at all intervals ($P < 0.001$ for 1 min, 5 min, 6 h, 24 h; $P < 0.01$ for 48 h), and no time can be found when memory is present. With the high shock condition, on the other hand, there is no significant difference in latencies between saline and cycloheximide-injected subjects until 24 h after testing ($P < 0.001$). At 48 h, however, the memory returns spontaneously. It therefore seems that the amnesia apparent at 24 h after training with high shock is temporary.

Two specific conclusions seem warranted by these data. First, the presence of a protein synthesis-resistant "short-term" memory is clearly dependent on whether the habit was learned under high or low shock. The amnesia for the high shock takes time to develop but amnesia for the low shock is apparent when retention is tested 1 min after training. This suggests that in certain conditions cycloheximide may prevent the retention of "short term" memories. It is improbable that this is simply the result of failure to learn. The reaction to the foot shock by the cycloheximide-injected mice was indistinguishable from the saline controls; furthermore, we have shown that the amnesia for the low foot shock is not permanent but can be recovered by giving the mice a "reminder shock"[8]. This could not occur if the mice had not learned the response. Second, the permanence of the amnesia is also dependent on the level of foot shock used to punish the response. The memory for the high shock returns spontaneously 48 h after training, but memory for the low shock did not return within the time intervals we studied.

These findings raise the possibility that protein synthesis inhibitors may be interfering with mechanisms necessary for information retrieval rather than exclusively preventing consolidation of long-term memory.

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Extremely Small Motions of the Basilar Membrane in the Inner Ear

THERE is a great deal of speculation about the amplitudes of cochlear membrane motion calculated by various authors for the lower threshold of hearing, which indicate an order of magnitude of motion smaller than the diameter of a hydrogen atom. These deflexions are detected and converted by the hair cells into nerve impulses which are recognized by the brain. Because these motions are too small to be observed physically, the various published values are theoretical extensions of experiments performed at much higher amplitudes. For example, von Békésy[1,2] has linearly extrapolated his measurements to give 10^{-11} cm r.m.s. basilar membrane motion for 1,000 Hz at the lower threshold. Because the behaviour of the auditory system is nonlinear, the calculations based on a linear model are questionable.

In order to appreciate the magnitude of these excursions one should consider that the amplitude of corresponding air particle motion in the sound pressure waves, just barely audible to the ear, is of the order of 10^{-9} cm at 1,000 Hz. The thermal fluctuations of air particles at room temperature and pressure are of the order of 10^{-10} cm, that is, the signal to noise ratio is 10.

The model used in our investigation to calculate the motion of the basilar membrane at low intensities is based on the anatomical descriptions of von Békésy[1] and Iurato[3]. These authors describe the basilar membrane as a long, narrow tissue membrane with no initial tension. Bending stresses in such a membrane with thickness h dominate all other stresses when the deflexions of the membrane are small compared with h. Axial tension was not observed experimentally in the long and narrow basilar membrane, so the idealized model chosen is that of a series of independent tissue beams which are transverse to the longitudinal axis of the unrolled cochlea. A clear picture of the model may be obtained by cutting transverse slits in the basilar membrane and then treating each strip as a beam fixed at both ends. Thus the small deflexions of the basilar membrane may be described by the bending theory of beams. Von Békésy's hydrostatic measurements of cochlear duct flexibility are used to compute a Young's modulus of near 10^7 dynes/cm^2 for the membrane tissue which is in the same range as Young's modulus for other tissues such as muscle.

When the membrane deflexions are of the same order of magnitude as h, a nonlinear term must be added to the beam equation due to the stretching of the middle plane of the beam. This added term is very similar to the nonlinear resistance term of a string with no initial tension. Such a term becomes important in the upper hearing range where the observed displacements are of the same order as h. Because of this nonlinearity, the model chosen thus predicts saturation and presence of overtones at high intensity levels which is in accord with observed auditory behaviour.

On the basis of the model described, the calculated values of the motion of the basilar membrane are between one and two orders of magnitude larger than those based on von Békésy's linear model. At 3,000 Hz von Békésy's calculated basilar membrane motion is 8×10^{-11} cm r.m.s. at the lower threshold, but our model predicts a value of 2×10^{-9} cm r.m.s. at that frequency. Rhode and Geisler[4] have measured basilar membrane motion in the mid amplitude range of the squirrel monkey cochlea using the Mössbauer effect. Linear extrapolation of their data to the lower threshold gives about 5×10^{-10} to 10^{-9} cm at 5,000 Hz. The calculated values for our model are in agreement. Although these recent calculations are larger than the ones von Békésy predicted, they are still on a subatomic scale at the lower hearing threshold.

Harris[5] has computed the Brownian motion of the basilar membrane based on both a weakly coupled and a strongly coupled model of the cochlear hair cells. According to Spoendlin[6] the hair cells are tightly fixed in the reticular membrane, resulting in strong coupling between adjacent hair cells. For the strongly coupled model at 3,000 Hz the thermal fluctuations have an amplitude of $4{\cdot}8 \times 10^{-11}$ cm r.m.s. or $6{\cdot}8 \times 10^{-11}$ cm peak value. Using the above value as a measure of the noise level, von Békésy's calculated basilar membrane motion gives a signal to noise ratio just above unity while calculations from our model give a signal to noise ratio an order of magnitude higher. The latter value seems to be more reasonable.

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Demonstration of Intention in the Reaching Behaviour of Neonate Humans

Neonate infants extend their hands towards seen objects, touch, and occasionally grasp them[1] (see Fig. 1). To some observers this behaviour looks like true reaching, but others[2,3] dismiss it as excited threshing. It will therefore be useful to define reaching according to two characteristics: that it is oriented, and that it expresses a distal aim or intention.

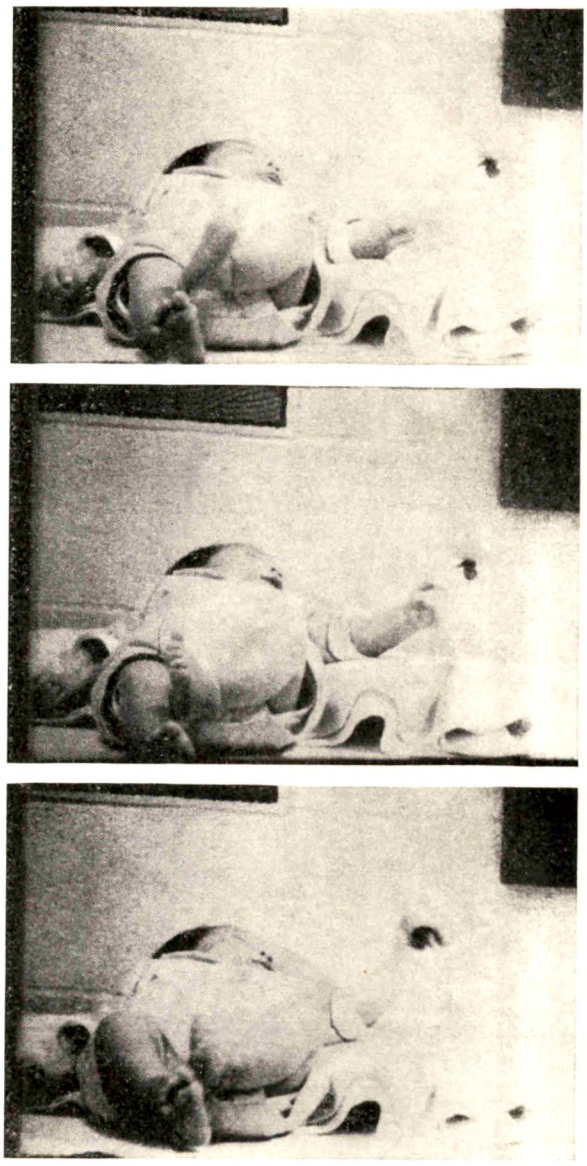


Fig. 1. A sample of neonate reaching taken from a film of a 10-day-old infant, in position for experiment 1. Top, 0 ms; middle, 324 ms; bottom, 756 ms.

The degree of orientation in the behaviour was assessed by presenting the infants with an object in one of five positions: midline 30° right, 60° right, 30° left and 60° left. Five alert infants served as subjects, usually having just been fed, each being observed—and filmed—for each position, each starting with a different position and proceeding through in counterbalanced order. The object was presented for 3 min continuously and there was a one-day interval between each presentation. The ages of the infants (which had been delivered without medica-

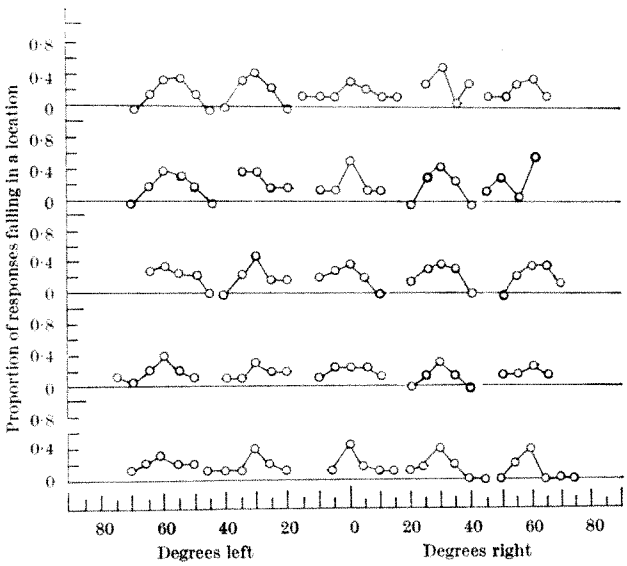


Fig. 2. The results of experiment 1. Each row represents the results for a single infant.

tion) at the beginning of the experiment were 6, 8, 9, 10 and 11 days. The object was an orange foam rubber sphere, 3·5 cm in diameter, placed 19 cm from the infant; the infant, loosely held in position by an arrangement of pillows (Fig. 1), lay under the object.

The films were analysed by two observers who recorded the location of the infant's hands on a protractor centred on the infant's midline and placed in the film plane, at the zenith of each arm extension. Seventy per cent of hand positions for each object position were within 5°—1·5 cm—of the object (Fig. 2). The behaviour was therefore considered oriented.

The assessment of intention was more complicated. We used a virtual object presentation (Fig. 3), which depends on the fact that neonate humans have stereoscopic vision[4–7]. Such objects look like real objects and so should elicit the same amount of reaching or excited threshing. If, however, the infant intends to contact the seen object when he extends his hand towards it, the virtual object will frustrate him, whereas frustration

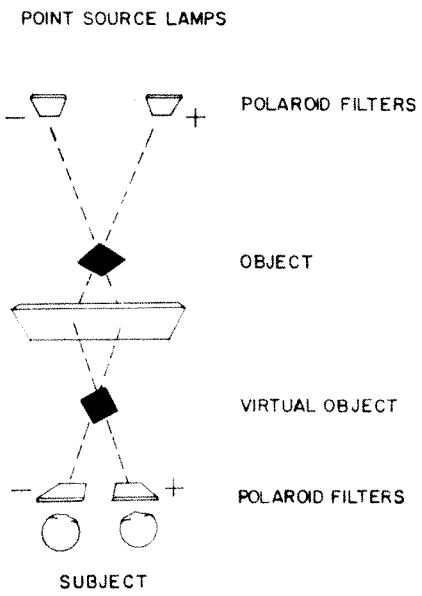


Fig. 3. A schematic diagram of a device to produce virtual objects.

should not result if the hand and arm behaviour is mere threshing, when there would be no intention to be violated. In this case one would also expect to see a lower rate of behaviour in the virtual object situation, for there can be no tactile input to stimulate further response. As measures of frustration, we used distressed crying and time before crying became chronic, compared with the same measures taken during real object presentations.

The infant was placed conjugate with the lamps 40 cm from the screen. Seven male and four female infants, whose ages were 8, 8, 10, 11, 12, 17, 18, 21, 24, 28 and 31 days at testing, served as subjects. They were full term and born without anaesthesia. Only infants whose preferred "reaching" posture allowed them to view the screen comfortably, and who were not upset by the goggles, could be used, so the first session was taken up with discovering the preferred posture and fitting the goggles. At the second session the infants were presented with one of the three virtual objects: a red transparent plastic tetrahedron, a red transparent plastic hexahedron, or a blue transparent plastic sphere, which dangled in the light beams. Initially it was placed against the screen on the opposite side from the baby, so that the virtual object was 40 cm away from him. After 1 min it was brought in slowly until reaching began. The average distance at which reaching began was 20 cm. The object was left as long as reaching persisted, or until the baby's crying was well established and upsetting to mother and experimenters, at which point the baby was soothed and then replaced in the apparatus. A virtual object was presented at 40 cm for as long as the infant had been in the situation before, and reaching behaviour was filmed. Other aspects of behaviour were recorded by an observer. A comparison group of forty infants in the same age range was run in a similar setup with real objects. These infants could assume any posture, were not wearing goggles and were reaching for palpable objects which provided tactile input on contact.

The results were that all the virtual-object infants cried, with a latency after the first reach of 15–75 s. None of the infants in the real object situation cried: the range of duration of presentation used with them without upset was 6–12 min. By the measures adopted here, therefore, the virtual object produced much more frustration. The differences between the real and virtual object groups could reflect the fact that the virtual object group was wearing goggles; but the group was preselected for its ability to wear goggles without crying for 3 min, much longer than any of them managed in the experiment; and in the post test, only two of the eleven infants cried, that crying not being associated with reaching. It therefore seems that it was not the situation which produced the frustration, but the consequences of attempting to reach for the virtual object. No crying at all occurred during the pretest, or during the first minute of the experiment, until the virtual object had been brought in to 20 cm and attempts to reach it had begun. During the post test, no reaching occurred and only two babies out of eleven cried. So the observed behaviour seems to have been a consequence of frustration produced by the experimental situation, and demonstrates "intention" in the observed behaviour.

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Maternal Thyroid Disease and Twinning

INTERRELATIONSHIPS suggested by various workers among thyroid function, twinning and chromosomal abnormalities[1-4] have prompted this study of thyroid disease in mothers of twins.

All Caucasian women who had delivered twins weighing 500 g or more were ascertained from the obstetrical files of the New York–Cornell Medical Center for the period 1955–64. There were 409 mothers of twins; using the same files, each was matched as closely as possible in age, race, colour, hospital service and delivery date, to a mother who had only singleton pregnancies. All information was obtained directly from the hospital medical record of each study mother.

Thyroid pathology was established on the basis of laboratory or pathology findings, thyroidectomy with final diagnosis, or repeated clinical description of thyroid "goitre" or "tumour" in nonpregnant women. Eight mothers of twins and two mothers of singletons with histories of taking thyroid pills have not been included in the pathological group, because it cannot be assumed that this information constitutes direct evidence of thyroid disease in all instances. Only data for maternal thyroid disease preceding the study pregnancies were included in this analysis. The data were analysed by the matched-pair method[5].

Table 1. THYROID DISEASE IN MOTHERS OF TWINS AND IN MOTHERS OF SINGLETONS

		Thyroid disease history Mothers of twins		
		Positive	Negative	Total
Mothers of singletons	Positive	0	4	4
	Negative	13	392	405
	Total	13	396	409

$t = 2{\cdot}18$, $P < 0{\cdot}05$.

The results appear in Table 1. There were thirteen pairings where the mother of twins had a positive history of thyroid disease and the respective mothers of singletons did not, and only four pairings in which the situation was reversed. The excess of mothers of twins with thyroid disease is statistically significant, $P < 0{\cdot}05$; using the exact method[6], $P = 0{\cdot}049$. Because the influences of age and race were controlled in these pairings, the heterogeneity in thyroid disease observed must be caused by other biological differences between mothers of twins and mothers of singletons. The data could not be analysed in relation to monozygous and dizygous twinning, because it is impossible accurately to determine the zygosity on all like-sexed twin pairs at birth.

An increased frequency of thyroid disease[1,7] and/or thyroid autoantibodies[2,8] has been found in mothers of patients with chromosomal aberrations, that is, Down's and Turner's syndromes. Understandably, thyroid disease and antibodies were determined in the latter mothers a considerable time after the pregnancy in question. That thyroid dysfunction may exist before such pregnancies is suggested by the result of this investigation and the relationships observed in the following studies.

The twinning rate is high among women who have had offspring with Turner's syndrome[9]. Although data are not available on a complete twin sample throughout development, reports suggest that this syndrome and other forms of aberrant sex development with abnormal karyotypes may not be unusual in twins[10-14]. Other lines of evidence indicate not only that mothers of twins had more offspring with abnormal genital development (F. V. D. G., unpublished), but also that gynaecological disease is associated with twinning[15]. It is therefore interesting that twin born individuals and their siblings seem often to have children whose development is defective, whether or not they have abnormal chromosomal complements[11,14,16,17].

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Elimination of the "*p*H Effect" in Cell Fragility Determinations

WHEN fish is stored frozen, the properties of the flesh slowly change so that the thawed material becomes tougher, with consequent problems for the food industry. Research in the field has been hindered by the lack of convenient methods for measuring the phenomenon. Love and Mackay[1,2] devised a simple technique in which 200 mg of muscle is subjected to a standard gentle homogenization in 20 ml. dilute formaldehyde (the "cell fragility" technique). Fresh fish is disintegrated to the myofibrillar level, but after freezing and thawing increasingly less disintegration occurs as the cold-storage period is increased; after about 12 weeks at −14° C, only intact cells are present. The extent of disintegration is assessed from the light-scattering characteristics of the homogenate, which are measured in a colorimeter. The soft tissue of fresh fish forms an opaque "soup" of high absorbance, whereas tissue which has toughened is shredded to a few strands, most of the suspending medium remaining clear and giving a low reading in the colorimeter. It was later discovered, however, that the readings were influenced by the *p*H of the muscle[3,4] in such a way that toughening attributable to low *p*H, without cold storage, gives rise to a high absorbance, whereas toughening in the frozen state gave a low absorbance reading.

This method was therefore useless for quality control of frozen fish unless the *p*H could be determined also. The routine determination of *p*H along with cell fragility has been suggested[5], but this would make quality control more tedious without guaranteeing accurate assessment of storage history. Though an experienced operator can distinguish by eye between homogenates of high *p*H–low absorbance density and cold storage–low absorbance, this requires skill and leaves room for doubt about values obtained in different laboratories.

By soaking pieces of muscle in weak buffers of varying *p*H we have shown that the effect lies in the *p*H itself, and not necessarily in the protein depletion that often characterizes fish of high post-mortem *p*H. A sample of fresh muscle can be made to yield almost any desired cell fragility value by a 4-day soaking in the appropriate buffer. Phase-contrast microscopy of "pale" homogenates from high *p*H fish showed a lack of definition compared with those of low *p*H fish, as though the formaldehyde in the suspending liquid has been insufficient to precipitate the cell proteins and partial dissolution was resulting in a more transparent suspension. Likewise, electron photomicrographs of homogenates revealed the presence of more ill-defined debris outside the myofibrils in the high *p*H specimens, as though protein had left the cell during homogenization. We have now tried stronger precipitants to counteract the dispersion of the proteins, and have found the most satisfactory combination to be 2 per cent trichloroacetic acid in 1·2 per cent formaldehyde. The absorbance after using such a medium is often above the sensitive range of the colorimeter, so we have reduced the sample size to 100 mg.

To assess this modified technique we dissected several anterior myotomes from each of forty cod (*Gadus morrhua* L.) which were in rigor mortis, freed them from connective tissue, and wrapped them separately in aluminium foil. Those from each individual fish were placed together in a numbered bottle, frozen at −30° C and stored at −14° C. A sample was thawed every 2 weeks and determined by both the original and the modified methods. The average *p*H of twenty of the cod was 7·02 (range 6·96–7·17) and that of the other twenty was 6·29 (6·06–6·39).

The superiority of the modified method is clear from Fig. 1. The range of *p*H is about as large as it is possible to obtain with this species; the ultimate values are lower because of the smaller size of the sample. Over a batch of 384 cod taken from various grounds it was found that the correlation coefficient between *p*H and cell fragility (original method) was −0·666 ($P < 0·01$) whereas with the modified method it was only +0·125 ($P < 0·05$). Readings from the modified method were also less scattered. They showed less effect of water content and length of fish, and were better correlated with the results of tasting tests for toughness and "cold storage flavour".

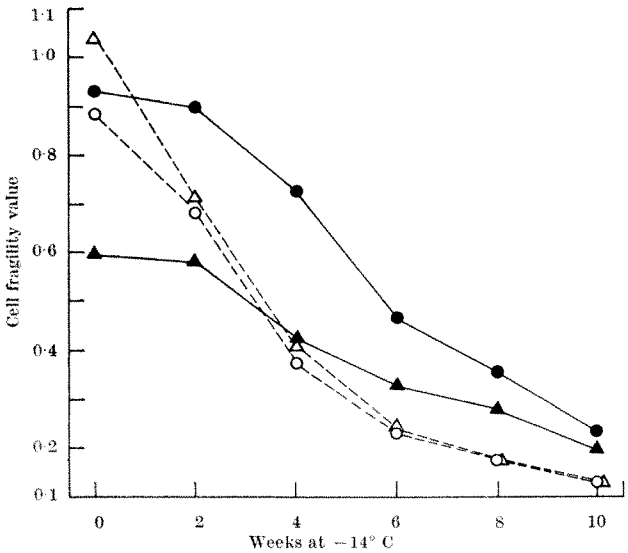


Fig. 1. Cell fragility values of cod muscle of high and low *p*H during storage at −14° C, as determined by the original and modified methods. The *p*H modifies the values of the original method but has almost no effect on those of the modified method. Original method: ▲, high and, ●, low *p*H; modified method: △, high and, ○, low *p*H.

A full account of this work will be published elsewhere.

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Nature of Acquired Resistance to Filarial Infection in Albino Rats

Albino rats infected with the filarial parasite *Litomosoides carinii* acquire resistance in 3–4 months as judged by the absence of microfilariae in the peripheral blood[1]. The rats at this stage have latent infection revealed by the presence of adult worms and microfilariae in the pleural cavity. *In vivo* studies on the factors involved suggested that circulating antibodies of these rats which include a homologous anaphylactic[2] and reaginic[3] types do not play a significant part[4]. Available data, however, indicated that the acquired resistance was related to a systemic cell-mediated immunity[3] that seems to be most active in the pleural cavity[4], where the adult worms and microfilariae were present. We report here the results of a study of the acquired resistance.

Infection was induced and monitored as described elsewhere[2,4]. Circulating antibodies of the rats with latent infection and the hyperimmune sera induced in rabbits against whole adult *L. carinii* did not affect the microfilariae or adult worms *in vitro*. Further, respiratory quotient of the adult worms (0·91) as measured by the Warburg apparatus[5] in Krebs–Ringer glucose medium was found unaffected in the presence of the antisera. Cortisone and radiation have been known to modify immune response[6], so the effect of such treatment has been studied in rats with latent infection. Cortisone was administered subcutaneously to five rats with latent infection at a dose of 50 mg/kg daily for 13 days. This resulted in microfilaraemia in the rats 4–7 days after the initiation of the treatment suggesting suppression of acquired immunity by the hormone. A similar effect was observed when five rats with latent infection were subjected to irradiation at a dose of about 800 rad from a ^{60}Co source. Further radiation treatment of ten infected rats or transplantation of about fifteen female worms into the thoracic cavity[4] of irradiated uninfected rats led to prolongation of the time required for the development of acquired immunity by 1–4 months.

To see whether adult worms or microfilariae were responsible for inducing the resistance, further experiments were conducted. In four groups of rats, each group containing five animals, one of the following types of worms was transplanted into the thoracic cavity: large

numbers of adult worms of both sexes; females alone; males alone; and worms killed with solid CO_2 immediately before transplant. When the infection in animals of the first two groups became latent as shown by the absence of microfilaraemia, each animal in the four groups was challenged with fresh female worms. In these conditions microfilaraemia was observed only in groups originally transplanted with live males and dead worms. The results therefore suggest development of acquired resistance to the infection arising from live adult female worms or microfilariae but not because of adult males. In a subsequent experiment it was found that female worms, exhausted of microfilariae by serial passages in rats till they no longer caused microfilaraemia, failed to induce acquired resistance, thus implicating microfilariae as the immunizing agent. Indeed, repeated injections of microfilariae, collected from the thoracic cavity of infected rats, into normal rats resulted in development of acquired resistance confirming these conclusions.

There are two possible reasons for the absence of microfilariae in the peripheral blood of rats with latent infection. The microfilariae may enter the blood from the thoracic cavity but be trapped in immune sites such as lymph nodes, spleen, lungs or bone marrow. Such evidence as could be collected did not, however, support this hypothesis: for example, splenectomy or partial pneumonectomy did not result in microfilaraemia in rats with latent infection. There was no histological evidence for the presence of microfilariae in bone marrow, lymph glands, spleen, liver or lungs of these rats. Further, transplantation of adult worms in splenectomized normal rats led to development of acquired resistance in these rats as in the controls. Similarly, splenectomized infected rats developed latent infection in the usual way.

The other possibility was that the microfilariae were prevented from penetrating the capillaries of the pleura of these rats because of cellular reactions. When the thoracic contents of the rats, taken soon after the disappearance of microfilariae in the peripheral blood, were examined under a cover slip, a number of living microfilariae were seen attached to cells. Examination of the fluid after staining with Giemsa revealed intense cellular infiltration which consisted of lymphocytes, macrophages, eosinophils and giant cells. Most of the microfilariae were found to adhere to the lymphocytes and macrophages and occasionally to eosinophils in the thorax. The cells were firmly attached to the sheath or body of microfilariae, thereby immobilizing them (Fig. 1) which might eventually have led to their destruction (Fig. 2).

Recently, antilymphocyte serum (ALS) has been reported[7] to be an effective suppressor of cell-mediated immunity. Rabbit ALS to rat thymus cells was prepared according to the method of Gray *et al.*[8]. The serum did free the microfilariae from the attached cells when tested *in vitro* and, further, two injections with 1 ml. of ALS on alternate days into the thoracic cavity of three rats with latent infection resulted in appearance of microfilariae in the peripheral blood. Similar suppression of immune response by ALS has been recently reported by Kassai *et al.*[9] in *Nippostrongylus* and Domingo and Warren[10] in *Schistosoma mansoni* infections.

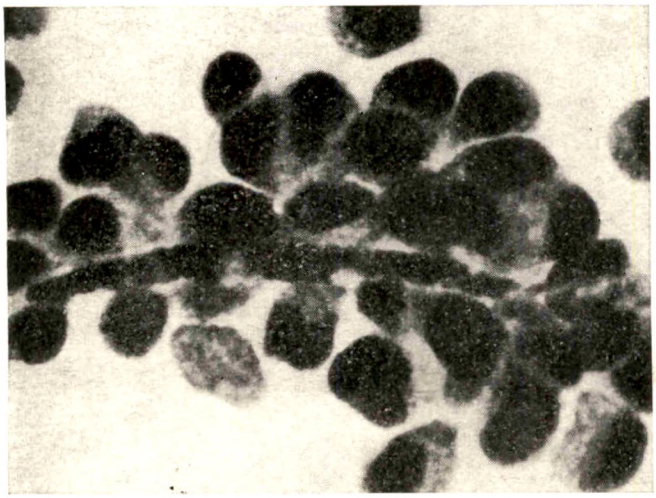


Fig. 1. Thoracic contents of a rat with latent infection, stained with Giemsa and showing adherence of microfilaria to lymphocytes and macrophages (×1,100).

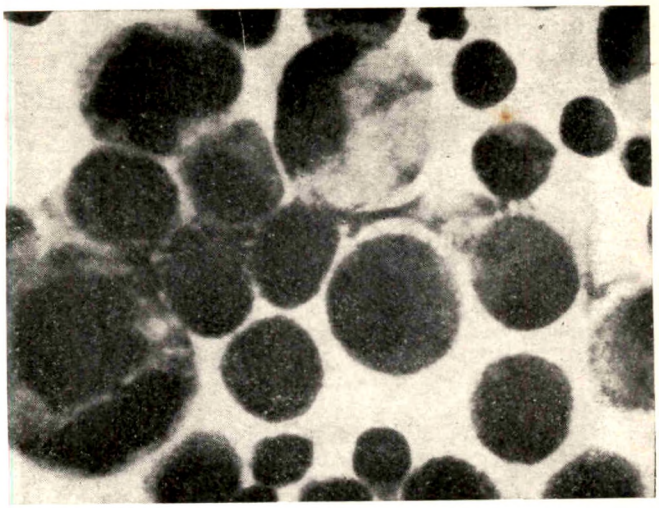


Fig. 2. Eventual destruction of microfilaria in rat thorax (×1,100).

Cell-mediated immunity has been recognized, in recent years, to be important in certain helminthic infections[11,12]. Our findings suggest that cell-mediated immunity possibly of the delayed hypersensitivity type is operative in albino rats with filarial infection. The ability of cortisone in inducing microfilaraemia in filarial patients[13] exhibiting no microfilariae in the peripheral blood prompts speculation that a similar type of cellular response, perhaps in lymphatics, exists in human infections.

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Mode of Action of the Anthelminthic Thiabendazole in *Haemonchus contortus*

THIABENDAZOLE, 2,4′-thiazolyl benzimidazole, is an efficient, non-toxic, broad spectrum anthelminthic[1] and antifungal agent[2]. Its mode of action is not known, although its properties as a chelating agent have been noted[3].

A fumarate reductase (NADH : fumarate oxidoreductase) has been shown to be an essential component of the fermentative pathways of the adult parasitic stages of the nematode *Ascaris lumbricoides*[4], the cestodes *Moniezia expansa*[5] and *Hymenolepis diminuta*[6], and the trematode *Fasciola hepatica*[7]. Many parasitic helminths, living in environments low in oxygen, excrete succinic and volatile fatty acids. It is therefore probable that the fumarate reductase mechanism is essential for the regeneration of NAD in these parasites. This is in marked contrast to the aerobic metabolism of the hosts. There is also considerable evidence[6,8–10] that ATP is generated anaerobically in the oxidation of NADH by fumarate in these organisms.

The specific activities of, and the effect of thiabendazole on, the NADH oxidase and fumarate reductase of a particulate preparation from adult *Haemonchus contortus* were determined. Worm-free Merino lambs, aged 3 months, were infected with 6,000 *H. contortus* third-stage larvae. The lambs were slaughtered 4–6 weeks after infection, the abomasum removed and adult *H. contortus* recovered. The worms were cleaned with several washes of warm physiological saline, and homogenized immediately in 0·25 M sucrose–0·005 M Tris–HCl buffer (*p*H 7·5) at 0°–4° C. The homogenate was spun at 4° C for 10 min at 700*g* and the supernatant recentrifuged for 15 min at 7,000*g*. The residue was suspended in the sucrose solution and centrifuged at 17,000*g* for 15 min. The residue (particulate fraction) was resuspended in sucrose solution and used for enzyme assays on the same day. Generally 1–2 g of worms were recovered which yielded approximately 2·5 mg protein in the particulate fraction.

The rate of NADH oxidation by endogenous oxygen (NADH : O_2 oxidoreductase) was determined with a Shimadzu recording spectrophotometer at 340 nm (Table 1). Fumarate was found to stimulate markedly the rate of NADH oxidation, even under air ($P < 0.01$).

Table 1. EFFECT OF FUMARATE AND THIABENDAZOLE ON NADH OXIDATION MEASURED AEROBICALLY

Addition	Rate of NADH oxidation nmole/min/mg protein	Percentage of NADH oxidase activity	Percentage of NADH oxidase and fumarate reductase activities
NADH	80·0 (14)	100	64·8
NADH, fumarate	123·4 (14)	154·3	100
NADH, fumarate, 10^{-5} M thiabendazole	88·7 (4)	110·9	71·9
NADH, fumarate, 10^{-4} M thiabendazole	77·6 (5)	97·0	62·9
NADH, fumarate, 10^{-3} M thiabendazole	25·4 (5)	31·8	20·6

The rate of NADH oxidation was measured spectrophotometrically at 340 nm. The assay contained, in 3·0 ml., 0·4 M potassium phosphate buffer (*p*H 7·0), particulate fraction (about 0·1 mg protein), 10 μmole $MgCl_2$, 0·3 μmole NADH and 10 μmole sodium fumarate as indicated. The thiabendazole was added in 0·1 ml. ethanol. The results are expressed as means, the number of observations being shown in parentheses.

Various concentrations of thiabendazole were found to inhibit the rate of NADH oxidation in the presence of fumarate (Table 1). The increase in rate of NADH oxidation caused by the addition of fumarate was completely eliminated in the presence of 10^{-4} M thiabendazole. Increasing the concentration of thiabendazole to 10^{-3} M further inhibited the rate of NADH oxidation, suggesting that the anthelminthic inhibited both the fumarate reductase and the NADH oxidase.

To determine whether thiabendazole reduced the rate of NADH oxidation by oxygen, NADH oxidase was assayed polarographically with a Gilson Medical Electronics 'Oxygraph' (Table 2). Thiabendazole was found to inhibit moderately the NADH oxidase activity from *H. contortus*, but much of the inhibitory effect of thiabendazole on NADH oxidase could be attributed to the small inhibitory effect caused by the ethanol in which the thiabendazole was dissolved. The inhibition was much less marked than the inhibition of the rate of NADH oxidation in the presence of fumarate (Table 1).

Table 2. EFFECT OF THIABENDAZOLE ON NADH OXIDASE

Addition	Rate of O_2 uptake nmole/min/mg protein	Percentage inhibition
None	56·3 (9)	—
NADH	487·6 (9)	0
NADH, $1·2 \times 10^{-3}$ M thiabendazole	294·5 (5)	39·6
NADH, 0·1 ml. ethanol	346·2 (4)	29·0

The rate of oxygen uptake was measured polarographically. The reaction vessel at 37° C contained, in 2·5 ml., 0·035 M potassium phosphate buffer (*p*H 7·4), particulate fraction (about 0·1 mg protein), and 5 μmole NADH as indicated. The thiabendazole was added in 0·1 ml. ethanol. The results are expressed as means, the number of observations being shown in parentheses.

These results suggest that the fumarate reductase system is much more susceptible to thiabendazole than is NADH oxidase. This conclusion was confirmed by measuring the rate of NADH oxidation by fumarate under anaerobic conditions (Table 3). NADH was not oxidized, except after the addition of fumarate. This fumarate reductase activity was completely inhibited by 10^{-3} M thiabendazole. Ethanol had little effect on the fumarate reductase activity. The inhibitory effect of thiabendazole was found to be partially relieved by high concentrations of cysteine, which suggests that thiabendazole may be reacting with a sulphydryl group involved in the fumarate reductase mechanism.

Table 3. EFFECT OF THIABENDAZOLE ON FUMARATE REDUCTASE

Addition	Rate NADH oxidation nmole/min/mg protein	Percentage inhibition
None	0 (11)	—
Fumarate	100·1 (11)	0
Fumarate, 10^{-3} M thiabendazole	0 (5)	100
Fumarate, 10^{-3} M thiabendazole, $3·3 \times 10^{-3}$ M cysteine	55·9 (5)	55·8
Fumarate, 10^{-3} M thiabendazole, $3·3 \times 10^{-4}$ M cysteine	0 (4)	100
Fumarate, 10^{-3} M thiabendazole, $3·3 \times 10^{-5}$ M cysteine	0 (2)	100

The assay conditions are as described in Table 1, except that the compartments of the spectrophotometer containing the samples were gassed with oxygen-free nitrogen (100 l. min) before and during the determination. The results are expressed as means, the number of observations being shown in parentheses.

The role of fumarate reductase in the overall metabolism of *H. contortus* has not been determined, but it is likely to be as important in the re-oxidation of NADH and in the production of ATP as it is in many other helminth parasites. It is a mechanism which is confined to the parasite in the host–parasite relationship and therefore ideally placed for anthelminthic action.

Another anthelminthic, tetramisole, has also been shown to inhibit the fumarate reductase mechanism in helminths (ref. 11 and my unpublished results). New anthelminthics which specifically act on the fumarate reductase system of parasitic helminths could be developed on a rational basis. In addition, potential anthelminthics could be screened by observing their effects, *in vitro*, on the fumarate reductase system of helminths. This would require only small amounts of the chemical under investigation, leading to substantial economies in the production of chemicals for screening.

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Species Specific Aggregation of Dissociated Sponge Cells

CURTIS has suggested that the initial phase of aggregation of a mixture of cells from *Haliclona occulata* and cells from *Halichondria panicea* is not species specific, and he has questioned the general validity of descriptions of species specific aggregation in marine sponges[1]. Although aggregation is not totally specific in many combinations, it is so in some cases, including the one noted above[2,3]. I have evidence to support the assertion that species specificity is complete in certain combinations of cells, and to question the applicability of the indirect test for specificity used by Curtis.

Wilson described species specific aggregation of dissociated sponge cells[4] when he observed that mixed cells from two species of different colours formed aggregates of one or the other colour. There is no reason to question this observation: the important question is, How do these cells arrive at this configuration? In many cases the initial phase of aggregation is not specific[2,3] and sorting occurs only after this phase, if at all.

I have examined combinations in which aggregation is completely species specific from the beginning. Of these only mixtures of cells from *Microciona prolifera* and *Haliclona occulata* have been studied extensively. This combination was chosen because the species are different colours and because virtually all cells of both species are well pigmented. I have generally used an ecological variant of *Haliclona occulata* which can be bright lavender when it grows near the low tide mark. *M. prolifera* is bright red, so the colour difference is very distinct and the species of most of the single cells in a suspension can be distinguished.

Microscopic observation, especially by time lapse photography (films available, see ref. 5), of the initial phases of aggregation of a mixture of *M. prolifera* and *Haliclona occulata* cells shows that single cells aggregate species specifically. While two cells of the same colour form the close association described by Wilson[4] as "coalescence", cells of different colours rarely form any kind of stable association. They often bump into each other, but there is no evidence of the establishment of a closer association. The films show clearly that the cells of *M. prolifera* and *Haliclona occulata* behave selectively from the beginning of aggregation. It is more difficult to be certain how many cells in the mixtures make "mistakes". In rare cases cells of different colours seem to adhere for an extensive interval. These examples in the time lapse films[5] where cells of one species emerge from an aggregate containing mostly cells of the opposite species. The significance of such "mistakes" is not clear, but they do not invalidate the general selectivity shown by more than 95 per cent of the cells. Curtis raised the possibility that the cells of the two species do not aggregate at the same time. Even casual observation of aggregation of mixtures of *M. prolifera* and *Haliclona occulata* eliminates this suggestion, and it is refuted by the time lapse films[5].

Species specific aggregation of mixtures of cells of *Haliclona occulata* and *Halichondria panicea* is not documented as extensively as for *M. prolifera* and *Haliclona occulata*, but the basic observations are the same using lavender specimens of *Haliclona occulata* and yellow-brown specimens of *Halichondria panicea*. If the lavender variant of *Haliclona occulata* were not available, it would be impossible to carry out direct observations, for the cells from *Haliclona occulata* and *Halichondria panicea* usually look very similar. The indirect assay used by Curtis to test specificity is based on the fact that aggregation is dependent on cell collision which is a second order reaction, the rate of which is affected by the concentration of the cells. A dilution of the reacting cells, which occurs when cells from two species which show specificity are used, should result in a lower rate of aggregation. Curtis, however, did not demonstrate that aggregation was following second order reaction kinetics in the conditions he used.

My data suggest that the rate of aggregation is dependent on the gradual resynthesis of cell surface macromolecules necessary for aggregation[6]. Treatment with ethylene diamine tetraacetic acid (EDTA) completely and irreversibly destroys the activity of these molecules. Because Curtis's test was conducted on cells treated with EDTA they must have had to replenish these macromolecules in order to reaggregate. My experiments with cells subjected to this treatment show that aggregation is much slower in such cells. Mechanically dissociated cells still have the macromolecules necessary for aggregation and they aggregate more rapidly than cells dissociated by removal of divalent cations. The macromolecules can, however, be isolated and returned to these cells, which then also aggregate much faster than cells which have to resynthesize it.

Consideration of the actual kinetics of aggregation becomes very complex if it involves several serial reactions including the conversion of a freshly dissociated cell, C, to a cell, C*, capable of adhering, and the collision of two cells to form an aggregate CC*. These two reactions can be diagrammed according to two schemes depending on the assumption made about capabilities of cells to adhere

$$(A)\ (1)\quad C \xrightarrow{k_1} C^* \qquad (B)\ (1)\quad C \xrightarrow{k_1} C^*$$

$$(2)\quad C + C^* \xrightarrow{k_2} CC^* \qquad (2)\quad 2C^* \xrightarrow{k_2} CC^*$$

In scheme *A*, the two cells in a collision can adhere even if only one has resynthesized its cell surface molecules; in scheme *B*, cells in a collision can adhere only if both cells have resynthesized their cell surface molecules. Although the kinetics predicted in these two cases are very complex even with simplifying assumptions, and are substantially different, the rate of aggregation in both is dependent principally on the rate of reaction 1 when k_1 is much smaller than k_2. Because reaction 1 is not dependent on the concentration of the cells, the test for specificity would not work in such conditions. Whenever the test is used, therefore, it must be used under conditions where k_2 is smaller than k_1 so that the rate of the reaction is completely concentration dependent.

Although the rate of aggregation need not be concentration dependent, it should be easy to set up the test for specificity in such a way that the rate of aggregation is dependent on reaction 2, which is the second order reaction dependent on cell concentration. Mechanically dissociated cells should aggregate in this way. Aggregation may, however, be more complex than expected, so it is necessary to demonstrate that the rate of aggregation is actually concentration dependent before the test can be accepted, even with cells whose adhesion is believed to be limited only by collisions.

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One-dimensional Pattern found in Blue-Green Algae

MANY filamentous blue-green algae, when grown in the absence of combined nitrogen, produce morphologically distinct cells (heterocysts) "often at more or less equal intervals" along the filaments[1]. In the presence of combined nitrogen, heterocysts occur only rarely in the algal filaments[2]. Fogg therefore proposed[2] that the formation of a heterocyst from a vegetative cell occurs when the concentration within the cell of a specific nitrogenous inhibitory substance, either ammonia or a simple derivative of ammonia, falls below a critical level. In accordance with this hypothesis there is evidence that the heterocyst is the site of nitrogen fixation in the algae[3], but this has been challenged[4]. The possibility that the concentration of a simple nitrogenous compound could determine spacing of heterocysts suggested a biochemical approach to the problem and provoked this study of the pattern in *Anabaena cylindrica*, the organism used for most earlier studies. A heterocyst pattern emerged in filaments within 12–16 h (that is, within about two-thirds of the doubling time of the organism) of their transfer from medium containing ammonia (+N medium) to medium lacking combined nitrogen (−N medium). It therefore seemed possible that the pattern was preformed in filaments grown in +N medium. Further study has confirmed this. I have demonstrated that a visible regular pattern of presumptive heterocysts is present in filaments grown in +N medium and that this pattern corresponds to that in filaments from −N medium.

A. cylindrica (Cambridge Culture Collection No. 1415/2) was grown axenically in plugged 250 ml. conical flasks containing 75 ml. of a defined salts medium[5] (−N medium) at 24° ± 1° C in an orbital shaker (200 r.p.m.) illuminated by 2 × 30 W warm-white lamps, and in an atmosphere of air containing 3 per cent CO_2. For growth in the presence of ammonia −N medium was supplemented with 0·02 per cent NH_4Cl (+N medium). The −N medium was inoculated with 3–4 day −N cultures, and the +N medium with 2–4 day +N cultures. The doubling time of the organism was 18–20 h.

Fig. 1*a* shows part of a typical filament from a 72 h −N culture. A close pattern of heterocysts and presumptive heterocysts ("proheterocysts"[7]) is apparent. Sixty to seventy-five per cent of non-vegetative cells in such filaments were recognizable as mature or maturing heterocysts[6]. A histogram of distances from each heterocyst or proheterocyst to the next (inter-heterocyst distance) in typical filaments from a young −N culture is shown in Fig. 2 (−N). The mean inter-heterocyst distance is six cells. As a measure of the regularity of the pattern let $R = 2\rho\bar{r}$ (by analogy with Claxton[8]), where ρ = density of heterocysts and proheterocysts and $\bar{r}$ = mean nearest neighbour distance. Thus with $\rho = \frac{1}{7}$, R has a value of 1·07 for a random distribution and attains a maximum value of 2 for a perfectly regular pattern. For young −N cultures $R = 1{\cdot}70$, indicating that the pattern has a strong tendency to regularity.

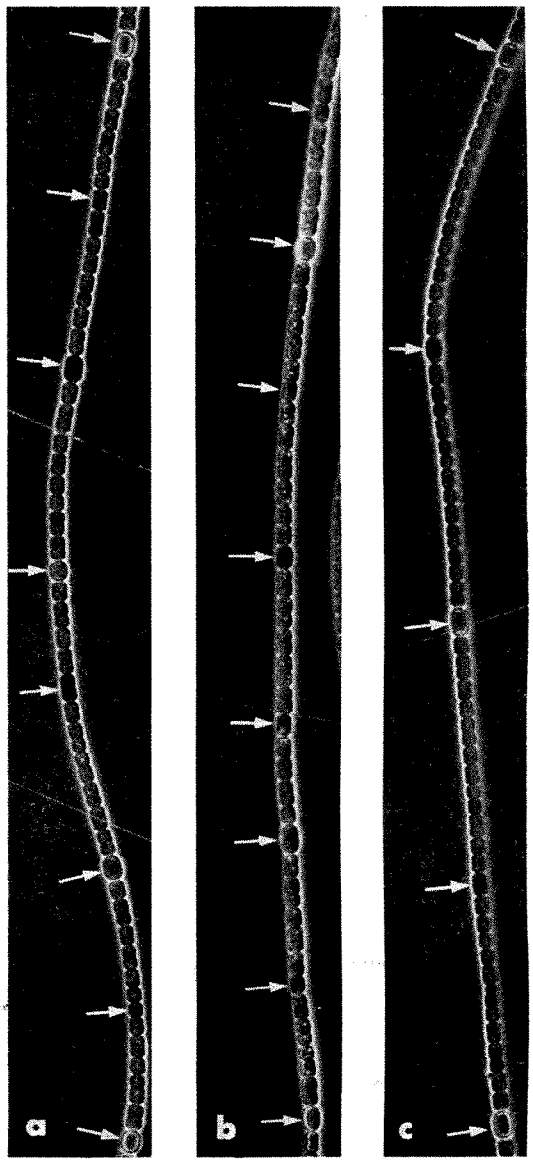


Fig. 1. Phase contrast light micrographs of filaments of *A. cylindrica*. (*a*) 72 h −N culture, (*b*) 36 h +N culture and (*c*) 8 day −N culture. Heterocysts and proheterocysts are indicated by arrows. ×460.

Proheterocysts are also present in filaments from 24–48 h +N cultures (Fig. 1*b* and ref. 9). The pattern spacing of all non-vegetative cells is similar to that found in −N cultures (Fig. 2, +N). $R = 1{\cdot}72$; mean inter-heterocyst distance = six cells. In such cultures, however, only 14 to 20 per cent of the differentiated cells are clearly heterocysts. Of the remainder, some, although distinctive (characterized by their grey appearance and relative loss of granularity[9]), could not be positively identified by direct observation as proheterocysts. It was necessary then to show that these cells were in fact presumptive heterocysts. This was accomplished by using time-lapse photography to observe the development of early proheterocysts in individual filaments which had been transferred from +N medium to thin layers of −N medium solidified by the addition of 1 per cent agar. Almost invariably these cells developed into heterocysts. Thus the pattern visible in early +N filaments is a partially expressed heterocyst pattern.

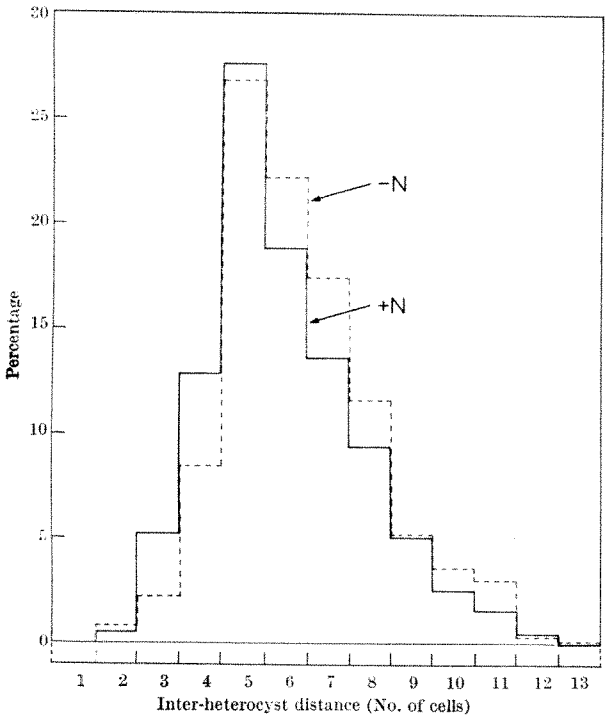


Fig. 2. Distribution of inter-heterocyst distances in filaments from early −N and +N cultures. Samples were taken from 72 h −N and from 36 h +N cultures and filaments were observed and inter-heterocyst distances (in numbers of cells) counted at × 400 magnification using phase contrast. A minimum of 200 inter-heterocyst distances (from a minimum of ten filaments) was counted in each case. The histogram is a plot of the number of occurrences (expressed as a percentage, ordinate) of each inter-heterocyst distance (abscissa). Inter-heterocyst distance means the number of vegetative cells between adjacent morphologically distinct cells; that is, heterocysts and all classes of proheterocysts were taken as equivalent. As growth is asynchronous in these conditions, cells in various stages of division were counted as one cell. These counts correlated in all cases with the actual distances in microns (determined by measurement following photography).

Although no systematic study of the heterocyst pattern in *A. cylindrica* has appeared in the literature, measurements of heterocyst frequency have been reported[2,10]. In the main, these reports indicate a larger mean inter-heterocyst distance than that described here. Some, but not all, of this discrepancy can be explained by my inclusion of all proheterocysts in the pattern, whereas the earlier reports dealt only with mature or maturing heterocysts. In addition I have found that the visible pattern widens appreciably with age of both +N and −N cultures. For example, in 4–5 day +N cultures and 7–10 day −N cultures the mean inter-heterocyst distance is about ten cells compared with six cells in young cultures. A typical 8 day −N filament is shown in Fig. 1c. At present, it is not clear whether this observation represents a true widening of the pattern, or merely reflects a slowed rate of heterocyst development relative to the rate of division of the vegetative cells. It is interesting that transfer of wide patterned −N filaments to fresh −N medium leads to the re-emergence of the close pattern within 12–18 h. Further observations on the growth and differentiation of individual filaments may help to resolve this question.

This study shows that a comparable pattern of heterocysts and proheterocysts exists in filaments grown in the absence and presence of ammonia. Fogg's hypothesis[2] therefore seems unlikely to be correct, for it contains implicitly the assumption that the production of a simple nitrogenous compound by heterocysts leads to the setting up of an inhibitory zone around them, thereby ensuring the preservation of the pattern in growing filaments. Proheterocysts, which constitute 80–90 per cent of the non-vegetative cells in +N cultures, seem unlikely to fulfil such a function. Their inability to reduce tetrazolium or silver salts suggests that they do not contain the high levels of reducing power found in heterocysts[3] and necessary for nitrogenase activity. This is corroborated by the finding that +N cultures possess no detectable nitrogenase activity when assayed by the acetylene reduction method[3]. Although it is therefore unlikely that proheterocysts are capable of fixing nitrogen, it is possible that they are involved in some other aspects of nitrogen metabolism. More knowledge of the biochemical changes occurring when a vegetative cell develops into a proheterocyst and of the role of the heterocyst may lead to insight into the mechanism underlying the transmission of positional information along the filaments. Currently we are seeking evidence of chemical and biochemical changes which can be correlated with altered patterns found in some mutants of *A. cylindrica*.

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Further Evidence for Reduced Role of Photorespiration in Low Compensation Point Species

MOST plant species cannot show a net photosynthetic uptake of CO_2 when the CO_2 concentration of the surrounding atmosphere falls below about 50 p.p.m., because at this point photosynthetic carbon fixation is reduced by lack of substrate to the rate at which CO_2 is being released from the plant by photorespiration. The concentration of CO_2 at which this occurs is called the CO_2 compensation point. A few species, including such important crop plants as *Zea mays*, have unusually low CO_2 compensation points, typically near zero. Such plants may not photorespire, or alternatively may be capable of refixing all the photorespired CO_2 by an unusually efficient photosynthetic mechanism.

The discovery in *Zea* of enzymes normally associated with photorespiration has been interpreted to mean that its low compensation point results from efficient refixation of photorespired CO_2 (ref. 1). This appears to be at variance with the observation of Goldsworthy[2], later confirmed by Zelitch[3], that in conditions where photorespiration is inhibited, both high and low compensation point species have similar affinities for externally supplied CO_2. It was thought possible, however, that the two kinds of species may have differing potentialities for the fixation of photorespired CO_2, which would, of course, be generated internally. Because, in low compensation point species, most of the photosynthetic tissue is arranged in two concentric layers of cells surrounding the vascular bundles[4], this hypothesis was tested by examining the ability of such species to fix CO_2 supplied through the vascular bundles.

The upper portion of a leaf of *Zea mays* var. 'Golden Bantam', about 40 cm² in area, was excised under water

and its base placed in a vial containing 0·05 M $NaH^{14}CO_3$. The surface of the liquid was covered with a 0·7 cm thick layer of corn oil to prevent direct $^{14}CO_2$ exchange with the atmosphere. The leaf and vial were enclosed in a chamber 550 ml. in volume at 25° C and illuminated at 600 foot candles from a tungsten source. The chamber was flushed at 500 ml. min^{-1} with 350 p.p.m. CO_2 in nitrogen for 2 h. The $^{14}CO_2$ released by the leaf was determined by bubbling the effluent gas through 1·0 M ethanolamine and counting aliquots. At the end of the period the leaf was blotted lightly to remove adhering isotope and homogenized in 0·1 M ethanolamine. Aliquots of the resulting homogenate were counted. The ^{14}C released by the leaf as CO_2 was expressed as a percentage of the total ^{14}C taken up. The whole experiment was then repeated using a leaf of similar size and shape from the high compensation point plant, *Phragmites communis*.

Table 1. CO_2 RELEASE IN LOW OXYGEN

Material	Leaf area (cm^2)	Bicarbonate taken up (μmoles)	Percentage of bicarbonate released as CO_2
Zea	43	26·5	7
Zea	40	26·0	6
Zea	46	30·7	9
Phragmites	44	18·0	6
Phragmites	52	14·3	6
Phragmites	43	22·1	11

The results of a number of experiments can be seen in Table 1. In low oxygen conditions both species released about 6 per cent of the radioactive carbon incorporated into the atmosphere as CO_2. The radioactivity remaining in both types of leaves seemed to be almost entirely in the form of photosynthate, for no significant loss of radioactivity occurred when the alkaline leaf extracts were acidified and boiled. We may therefore conclude that, when photorespiration is inhibited, the rate of fixation of CO_2 originating within the vascular bundles is of the same order in *Zea* and *Phragmites*.

When, however, these experiments were repeated using air containing 350 p.p.m. CO_2 in place of the nitrogen, the amount of $^{14}CO_2$ released from *Phragmites* increased several-fold, whereas that released from *Zea* was virtually unaffected. These results (Table 2) suggest that it is photorespiration which, by permitting the oxidation of photosynthate, causes *Phragmites* to become inefficient in fixing internally derived CO_2.

Table 2. CO_2 RELEASE IN 21 PER CENT OXYGEN

Material	Leaf area (cm^2)	Bicarbonate taken up (μmoles)	Percentage of bicarbonate released as CO_2
Zea	32	9·2	4
Zea	50	15·3	6
Zea	50	19·9	8
Phragmites	31	5·5	28
Phragmites	38	11·5	31
Phragmites	38	8·7	20

Because *Phragmites* can fix internally derived CO_2 as efficiently as *Zea* when its photorespiration is inhibited, the low compensation point of *Zea* may be explained by a lack of photorespiration, either in the whole leaf, or in at least the outer of the two layers of photosynthetic tissue surrounding the vascular bundle. The hypothesis that it is only the outer layer, the so-called mesophyll, which does not photorespire is extremely attractive, for the enzymes of photorespiration appear to be concentrated in the inner of the two layers, the so-called bundle sheath[1]. Further, it seems that the main Calvin cycle activity of the leaf is also located in the bundle sheath[4–6] and it is believed that the substrate for photorespiration is derived from the Calvin cycle[7].

We might speculate that low compensation point species photorespire as much as high compensation point types, but because their photorespiring tissue is surrounded by a non-photorespiring photosynthetic layer, they are able to refix all the photorespired CO_2, without loss, before it can reach the external atmosphere. This seems unlikely because no significant increase in net photosynthesis at low light intensity occurs in low compensation point species when photorespiration is inhibited by lack of oxygen[8,9]. Light energy is required by photorespiration in order to carry out the conversion of serine to carbohydrate[10,11], and also for the refixation of the photorespired CO_2. This energy could not then be available for the fixation of fresh CO_2. Thus we would expect inhibition of photorespiration in light limiting conditions to result in an increase in the rate of both gross and net photosynthesis, the increase in the latter being equivalent at least to the normal rate of photorespiration. The fact that no significant increase occurs[8,9] suggests that photorespiration plays only a very minor part in low compensation point species. This is confirmed by Zelitch's[12] observation that, in conditions where its oxidation is inhibited, the rate of synthesis of glycollate in *Zea* is only a small fraction of that found in the high compensation point species tested. Glycollate seems to be the primary substrate for photorespiration, and it does not normally accumulate in photorespiring tissues[12], so that its rate of synthesis should be the factor which normally limits the rate of photorespiration. The low rate of glycollate synthesis in *Zea* suggests, therefore, that this organism has but a low rate of photorespiration.

To some extent, low photorespiration in low compensation point species could be explained by postulating that if only a proportion of the leaf cells, for example those in the bundle sheath, photorespire, then the photorespiration of the whole tissue would be reduced on a *pro rata* basis. It is doubtful, however, if this would be sufficient to explain the almost total lack of effect of low oxygen on net photosynthesis. We believe the most likely explanation is an additional effect attributable to the C-4 carboxylation pathway. This pathway would be expected to generate CO_2 from malic acid within the bundle sheath chloroplasts[4,5]. Should this result in a higher concentration of CO_2 within these chloroplasts than would be normal in a true "Calvin plant", then it could result in the partial inhibition of glycollate synthesis. This has been observed in many other tissues[13–16].

Our results indicate that the carboxylation reactions of *Zea* and *Phragmites* are about equally efficient at fixing internally derived carbon dioxide. The differences in compensation points are explained as a lack of photorespiration in the mesophyll layer, and the possible partial inhibition of photorespiration in the bundle sheath of low compensation point species.

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Book Reviews

ENGINEER AND SCIENTIST

Osborne Reynolds and Engineering Science Today

Edited by D. M. McDowell and J. D. Jackson. (Papers presented at the Osborne Reynolds Centenary Symposium, University of Manchester, September 1968.) Pp. xiii + 263. (Manchester University: Manchester, September 1970.) 72*s*.

OSBORNE REYNOLDS was appointed first professor of engineering at Owens College, Manchester, in 1868 at the age of twenty-seven, and this book consists of the papers presented at a symposium held at the Victoria University of Manchester in 1968 to commemorate the centenary of that historic appointment. It is prefaced by a paper in which Jack Allen, professor of engineering at the University of Aberdeen, most ably summarizes Reynolds's life and work. This is followed by nine papers, each written by an expert, dealing with the different fields of research in which Reynolds so fruitfully engaged during his lifetime at the college and indicating the important developments which have stemmed from them.

The influence of science and scientific method on British engineering practice in the nineteenth century is a fascinating subject for debate. To say which came first, the science or the engineering, is very like the problem of the chicken and the egg. In my opinion, the great pioneer engineers of the late eighteenth and early nineteenth century relied far more on intuition based on practical experience than on exact science. The very success of these empirical methods led the British engineer to affect a certain contempt for the scientist as an unpractical theorist, an attitude which contributed not a little to the loss of British supremacy in engineering. That Reynolds was fully alive to the danger of this attitude he made clear in his introductory address at Owens College in 1868 when he said: "There is plenty for the British engineer to do, and for the British workman too, but they must both look out. They have enjoyed monopoly for a long time, and now their neighbours are wishing to taste their good things. . . . We have much of our capital locked up in plant which has become antiquated; and many of the systems in vogue in our workshops have been handed down to us by our fathers, whilst our friends over the water have the advantage of a fresh start. To supply their want of skilled engineers and workmen, they have founded schools to give technical instruction, combined with a scientific education and . . . they have created a class of men which does not exist in this country, eminently qualified to hold their own at a time when science is every day taking deeper hold on all our manufactures."

It is arguable that this warning, so clearly sounded by Reynolds more than 100 years ago, was not fully heeded in Britain until after the Second World War. The sequence of events seems to have been that the British engineer, having created the hardware by guess and by God, became self-satisfied with his handiwork and neglected until too late to subject it to scientific study and scrutiny with a view to determining the fundamental mechanical laws governing its structure and operation.

Of this fundamental and long overdue applied research Reynolds was a pioneer. He was also a bridging figure because he was primarily a practical engiener in spite of his great scientific and mathematical attainments. With him, the theory never preceded practical experiment; it was always the other way round. He was a master at devising ingenious practical experiments, and it was upon the results so derived that his great contributions to engineering theory were based. His study of the lubrication of bearings fully justifies the claim that he was the founder of modern tribology, while his work on estuarial flow with scale models had world wide influence and was the seed from which the present Hydraulics Research Station at Wallingford sprang sixty years later.

Like all men of genius, Reynolds had his curious blind spots. As early as 1875–76 he experimented with a small axial flow multi-stage steam turbine with a rotor diameter of six inches running at 12,000 r.p.m. In this he anticipated Sir Charles Parsons, but he apparently lost interest in the steam turbine because he judged that it could never compete with a reciprocating steam engine owing to the extravagant steam consumption of his model. This was evidently caused by leakage of steam arising from the relatively large clearance between the blades and the casing, and it is strange that it should never have occurred to a mind so acute that this source of leakage would greatly diminish relative to an increase in the size of the turbine. But for this lapse he might have anticipated Parsons in the invention of the steam turbine. Professor Allen conjectures that Reynolds may have been influenced subconsciously by the great affection he felt for the steam engine. Whether this is true or not, such near misses on the part of men of genius are one of the fascinations of engineering history.

Considering his stature as an engineer-scientist Reynolds is surprisingly little known. Perhaps his greatest contribution to knowledge was made in the field of fluid mechanics, yet even here I wonder how many of those familiar with the term "Reynolds number" know anything of the man behind the term. It is to be hoped that this book will help to restore Reynolds to his true position as a great pioneer and original thinker.

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INSIDE A COMPANY

A History of the Marconi Company

By W. J. Baker. Pp. 414 + 23 plates. (Methuen: London, September 1970.) 100*s*.

THE study of the relationships between science, technology and economic growth that have attracted the attention of both scholars and politicians since the end of the Second World War has been continually frustrated by lack of appropriate data on industrial innovation. This shortage has been particularly acute in the area of contemporary industrial history. Histories abound of the textile and steel industries of the industrial revolution, but up to date accounts of modern technological developments like petrochemicals, electronics and aviation seem to have been largely ignored by the current generation of historians.

W. J. Baker's new history of the Marconi Company is a much appreciated addition to this neglected area of research. Although the author is an "insider" in that he has been an employee of the Marconi Company for many years, he is careful to point out that the book is not a product of company sponsorship and "the 'downs' are recorded as well as the 'ups' ".

The first section of the book is a detailed account of the early history of the company and traces events from Marconi's early years through the trial and tribulations of the embryonic company, up to the end of the First World War.

In the second section, the author discusses the Marconi Company's involvement in the development of the international broadcasting network, the birth of the BBC and the launching of the world's first television service.

The impact of the rapid growth of the electronic industry on the company, both organizationally and technically, forms the major part of the discussion in the third section.

Throughout the text the author has taken pains to relate the developments in technology to the overall philosophy of the firm which Marconi started and gives much detailed information on the individuals responsible for the successive transformations which the company underwent. In his summary the author concludes that the company has flourished during the seventy year period largely because of "top quality in engineering craftsmanship and materials; a thorough training of youth for the future and a generous feedback of profits into research and development".

It is to be hoped that this work will stimulate other historians to undertake the essential task of compiling contemporary industrial histories which alone can provide the basis of more analytical work on the relationship between technology and industry. MICHAEL GIBBONS

PRAGMATIC ECONOMIST

Papers on Planning and Economic Management

By Ely Devons. Edited by Alec Cairncross. Pp. vii + 278. (Manchester University: Manchester, September 1970.) 60*s*.

ELY DEVONS was not one of the leading theorists of his generation. Reared in the traditional political economy mould of the University of Manchester he bore the stamp of that institution throughout his career. Ever sceptical of high-flown theories and acutely aware of the dangers that arise from divorcing fact and theory, Devons was, by his very nature, a pragmatic man ready to see issues at their face value and anxious to analyse problems simply but positively. He was probably too wide-ranging in his thoughts and writings ever to be a leading theorist, and in any case he was happy to let the facts speak for themselves rather than to devise hypotheses or theories about what might happen outside reality. He was also too busy teaching and looking after successive intakes of students to have much time left for intensive research.

To his students (and I number one of them) Devons had an acute intellect which could cut through any problem incisively and which could also, much to the chagrin of those concerned, quickly detect the weakness of his opponents' arguments. He was sparing of words but what he had to say was expressed with great clarity. In general Devons was not content to specialize exclusively on one small area of study, though he will be best remembered for his books on planning and economic statistics. He preferred, however, to operate on a wider plane often at the frontiers where different disciplines met. Much of the time he spent debating general issues relating to economic management, planning, administration, and, later, university affairs, and the essays in this volume (introduced with a memoir by Professor Cairncross) reflect his interests in these matters. Many readers may regard them as rather lightweight, and it must be admitted that one or two of them, especially the reviews of books, might have been left out. Nevertheless, they are nearly all worth reading again, for they show Devons's sharp grasp of essentials, his ability to analyse problems clearly, his deliberate attempt to question accepted notions and, above all, the self-critical expression of his own profession. In this last respect Devons (perhaps with tongue in cheek occasionally) was at his best, and economics and economists often came in for some hard knocks. For example, he noted the pitiful way underdeveloped countries have been bombarded with successive theories of development receiving, as they do, "ever-changing diagnosis and advice from the peripatetic group of economists tripping from one to the other giving them the benefit of the latest advances in the so-called theories of economic growth and economic development. In all this there seems to be a recklessness in putting forward newly formulated but untried theories as guides to policy".

The general reader in particular will find these essays to his liking. They cover issues of topical interest, they are elegantly written and free from professional jargon and they are spiced with sarcastic wit.

DEREK H. ALDCROFT

PLEISTOCENE TOPICS

Ice Ages

Their Nature and Effects. By Ian Cornwall. Pp. 180. (John Baker: London; Humanities: New York, July 1970.) 63*s*.

THIS book, somewhat expensive for its length and style, takes a general view of the Pleistocene ice ages. It is written for students of the natural sciences and of archaeology and for the general reader—certainly a wide field of readership. It is really an introduction to the subject and deals in an elementary fashion with the many facets of studies relating to ice ages and their changing environments—geological, geographical, biological and archaeological.

Such an introduction has a very useful part to play, especially now that environmental studies of past and present are becoming, quite rightly, more and more popular. How far does this book satisfy the requirements of an introductory text? It is certainly written in a readable style, the figures are clear and most of the plates provide helpful illustrations. But the reader is entitled to expect correct information and an up to date approach to the subject, and criticism can be made of this book on both these scores. A single example of the former will suffice: many of the distributions of plants given in the section on Pleistocene floras are incorrect.

A more important criticism is that the approach to parts of the subject is antiquated. In the section on Pleistocene fauna, we are told only of mammals, in spite of the great interest of recent work on molluscs and beetles, both of which groups are very revealing in interpreting past environments. Moreover, the account of the mammal fauna is clearly based on Zeuner's 1945 lists, and much has happened to our knowledge of Pleistocene faunas and chronology since then. Another criticism concerns the absence of any clear definitions of terms used in defining climatic periods. In the absence of such clear definitions, the author perpetuates ideas current in Pleistocene chronology some thirty years ago. Thus the idea that Penck and Brückner's classification of the Alpine sequence can be used as a stratigraphic basis for the rest of Europe is not questioned or considered. As a result, the alpine terms and the term "great interglacial" are liberally used in reference to areas beyond the Alps; it has yet to be proved that a "great" interglacial exists in north-west Europe. These difficulties partly stem from lack of definitions. Nevertheless, to the beginner in the subject it should be made quite clear that correlation of major ice advances and interglacials in the Alps, northern Europe and Britain is a subject of great difficulty, and that there is still no clearly accepted scheme of cold and temperate periods which brings these three areas together chronologically.

Another difficulty for the reader stems from the acceptance of Milankovitch's radiation curve (described as a method of absolute dating) as a basis for stratigraphic correlation. What is really required is a discussion of the stratigraphical evidence for the hypothesis that the course of the curve is related to geological events.

Apart from these considerations, the book has a reasonable coverage of Pleistocene topics. But it is unfortunate that the chronological and stratigraphical framework lacks a modern perspective, and a good opportunity for the presentation of the position in Pleistocene studies today has been lost.

R. G. WEST

WELSH GEOMORPHOLOGY

The Glaciations of Wales and Adjoining Regions

Edited by Colin A. Lewis. (Geographies for Advanced Study.) Pp. 378. (Longman: London, September 1970.) 40*s*.

THE elucidation of the part that glaciers have played in shaping the landscape requires the mind of a detective. The glacial geomorphologist must be prepared to work hard and long in his analysis of the forms and deposits left behind by the glaciers, but above all he must be able to appreciate the significance of the vital clues. That these clues are very varied in character is amply shown in this book. It is divided into twelve chapters, ten of which are devoted to the separate regions of Wales and the area around it. The introduction, by the editor, sets the scene by ranging widely over Pleistocene and Holocene chronology.

Each region is described by a geomorphologist who has a very intimate knowledge of it. Naturally their approaches to their areas differ according to the nature of the evidence, and they are by no means unanimous in their views. At times the accounts are so detailed that a reader unfamiliar with the area tends to lose the thread of the argument. J. B. Whittow discusses north-west Wales and C. Embleton describes north-east Wales. J. Worsley links the glaciation of Wales to that of the Midlands in his chapter on the Cheshire–Shropshire lowlands, which is a key area. The Lower Severn is discussed by N. Stephens, and E. Watson stresses the importance of periglacial activity in his account of the Cardigan Bay area. The Upper Wye and Usk and the Hereford basin are described by C. A. Lewis and B. H. Luckman respectively. D. Q. Bowen and B. S. John complete the area by their descriptions of south and south-west Wales. The adjacent area of the West Country and Southern Ireland is considered in the last regional chapter by N. Stephens.

F. M. Synge sums up the evidence in the final chapter. His main conclusion is that final agreement in the subject is far from achieved. In fact the diversity of views on the glaciation of Wales is the outstanding impression gained from the book. Every type of evidence is explored by the different authors. The book thus provides a useful account of possible methods of studying glacial chronology and land forms, as well as providing the latest views concerning the activity of the glaciers and the processes associated with them in Wales. The book is well illustrated by maps, sections and photographs. Many chronological correlation tables and long reference lists are included. The price of the book, however, seems rather high in view of the relatively small area covered and the obvious lack of definitive conclusions. It is of considerable value, however, as a record of the present state of knowledge in that it points out where more work is needed before there is sufficient evidence to write a consistent and more complete account.

C. A. M. KING

RURAL PASTIMES

Land and Leisure in England and Wales

By J. Allen Patmore. (Problems in Modern Geography.) Pp. 332+26. (David and Charles: Newton Abbot, September 1970.) 84*s*.

THIS book is a comprehensive study of the problems posed in Britain by the increasing demand on the country's limited resources for outdoor pastimes and pursuits. Mr J. A. Patmore details and quantifies the various ways in which the British spend their spare time in the open. There is an abundance of graphs, charts, maps and statistics which have been culled widely.

The favourite outdoor pursuits are swimming and angling. One illustration of the strength of the latter sport is that the London Anglers' Association recently paid £37,000 for fishing rights on an 8,200 yard length of the Wiltshire Avon. It is surprising to find that wild-fowlers may number as many as 500,000; it is surprising also that it is estimated that 50,000 people weekly attend meetings of foxhounds. Mr Patmore gives plentifully the sources of his information.

There are the horror figures too that are seen more often—21¼ million motor cars by the year 2000, and rural car parking at a density which will cover vast acreages. Indeed, quite understandably, the author gives a great deal of space to the way in which people use the car and to its impact on the countryside. The questionnaires, reports, and investigations on the behaviour of the motorist in the countryside are now numerous, and, generally speaking, these reach the conclusion that most visitors to the countryside are simply looking for an open space, preferably near water, where they can park the car and enjoy the view and play.

Though the shadow of the motor car hangs over the book, Patmore is not discouraged or pessimistic about the future. There are solutions, some which he rightly says may offend the precepts of rigid rural conservationists. In this category must surely come the suggestions that at Malham, between the village and the tarn, there should be widened roads with overlooks (viewpoints) and access to the famous Cove and gorge on made footpaths (tarmac or concrete?) provided with explanatory displays. Another suggestion is that there should be permitted residential development around country parks, where the enhanced site values would help to pay for the recreational provision within. Patmore is undoubtedly influenced in his solutions by what he has seen in America. The idea of the "heritage highway", a route which links places in the life of national figures or strings together a story of industrial archaeology, seems to me to be a sort of motorized nature trail in which the use of personal initiative is at a low ebb.

A greater degree of planned provision for leisure will have to be made; more thought given to the segregation of conservation areas from recreational areas, a policy for the greater spreading of holidays, and in particular some measures for taking the pressure away from the pronounced Sunday afternoon peak. Patmore deals with all these and many other problems in a book which is interesting, useful and provocative.

GEOFFREY BERRY

STRESSES AND STRAINS

Yield Point Phenomena in Metals and Alloys

By E. O. Hall. Pp. viii+296. (Macmillan: London, July 1970.) 76*s*.

A YIELD point, in the sense in which Professor Hall uses the term, is a sharp discontinuity on a tensile stress–strain plot at which the elastic (or nearly elastic) stage is abruptly replaced by plastic deformation. The phenomenon owes its practical importance to the fact that mild steel and light alloy sheets subject to such behaviour cannot be shaped by deep-drawing or pressing without displaying unacceptable local distortions: these are due to the fact (intrinsic to yielding) that once plastic deformation begins at one point, it tends to continue there rather than spread elsewhere. Thus the superplastic alloys now being studied so intensively are at the opposite pole to alloys showing sharp yield points.

The study of yielding came to life in 1949, when Cottrell and Bilby published their detailed theory of the locking of dislocations in mild steel by "atmospheres" of carbon atoms. This theory, which in its chief features has stood the test of time, opened the way to a quantitative understanding of many mechanical features of this most

important of engineering alloys, and it was soon supplemented by the equally fruitful discovery by Petch and Hall (the author of the book under review) of the relationship between yield stress and grain size. The study of yielding built up to a peak in the early sixties, since when the impetus has gradually departed, leaving, however, a very substantial body of knowledge and insight. It is this material which Hall has very ably marshalled in his book.

The text begins by analysing the features common to all forms of yielding, including its propagation in the form of Lüders bands and also "false yield-points". He goes on to a very thorough disquisition on yielding, strain-ageing, quench-ageing and blue-brittleness (dynamic strain-ageing by an old-fashioned name) in iron alloys. This chapter is the core of the book. It is surprising to find that, in the author's view, the theoretical details of the strain-ageing process and the associated yield behaviour are still not entirely clear, but his conscientious literature survey certainly confirms his conclusion. This is one of the few fields of modern metallurgy where the electron microscope has failed to contribute anything of value. Later chapters deal with a range of other metals and also with the special features of hydrogen as solute. Short sections also deal with ordered alloys, whiskers, ionic crystals and semiconducting materials.

All in all, this is a very professional, thorough and notably critical survey of its field, and, indeed, its comprehensive coverage of the literature alone would guarantee it a place on the shelves of metallurgical libraries.

R. W. CAHN

CONSTRUCTION KITS

Orbit Molecular Building Set

(Designed and Manufactured by RJM Exports Ltd. Distributed by Wiley: Chichester (universities and technical colleges); Macmillan: London (schools), July 1970.) 210*s* (demonstration kit); 56*s* (student kit). (Sample kit free on request.)

Elements of Protein Synthesis

An Instructional Model. By Thomas Peter Bennett. $3. A Guide to the Instructional Model. Pp. 40. $0.75. (W. H. Freeman: San Francisco and Folkestone, 1969.)

IN an age when model building is all the craze for molecular biologists, it is important that students should be able to indulge in this art if they are to appreciate its usefulness and limitations. The *Orbit Molecular Building Set* is one of the most promising models on the market, with the advantage that it is easy to use and the student set is cheap enough for the student pocket. The atoms are precast spheres with arms at the correct bond angles and they are joined together by short lengths of plastic tubes rather like drinking straws. By cutting the straws provided into suitable lengths, any convenient scale can be chosen, from about 2 cm per angstrom upward.

The kit is best for constructing rather small assemblies such as one base pair of a DNA double helix or a short oligopeptide. It is more difficult to build complicated tertiary structures; it is not easy, for example, to construct a DNA double helix of five–ten base pairs, without knowing in some detail just what positions the atoms take up about bonds of free rotation. One difficulty is that the atoms do not rotate very freely. A space filling model would perhaps give a better idea of the real configuration of molecules, but in lieu of the ready availability of such a kit, the Orbit kit can be recommended to students who will undoubtedly enjoy piecing macromolecules together.

The *Elements of Protein Synthesis* model consists of a two-dimensional cardboard cut-out kit, with parts to represent the ribosome, mRNA, and aminoacyl-tRNA. In short, it is a sort of do-it-yourself kit for producin a moving cartoon from the types of illustration favoure in molecular biology articles in the *Scientific Americar* To suppose that this will enhance a student's understandin of how the components of protein synthesis interact i optimistic. I advise students to save their money an buy a well illustrated text.

BENJAMIN LEWIN

ONE SIDED MICROCIRCULATION

Microcirculation, Perfusion and Transplantation of Organs

Edited by T. I. Malinin, B. S. Linn, A. B. Callahan an W. D. Warren. (Proceedings of a Conference held i Miami, Florida, October 30, 1969.) Pp. xvi + 423. (Aca demic: New York and London, June 1970.) $12.50.

THIS volume is a collection of communications delivere at a conference sponsored by US Naval Research an the University of Miami. The chief aim of the conferenc was to bring together the views of clinicians and physiolo gists on the complexities of organ transplants, rejection storage and perfusion and their relation to the micro circulation.

There are three principal sections to this volume—th microcirculation under normal conditions, the patholog and pathophysiology of the microcirculation, and th perfusion of organs. In the first section, the papers ar of a general nature and no effort is made to relate ther to transplantation problems. And yet this was wha the conference was all about. The speakers in all thre sections should have met before the conference an worked out together some aspects of the theory an practice of organ transplantation because without discussion at the end of each section the communication seem isolated.

A review of the ultrastructure of freeze-cleared capil laries is interesting but of limited interest if no effort i made to relate detectable damage to functional impair ment. After all, electron microscopy has given us ver little insight into glomerular protein leakage in mos forms of the nephrotic syndrome. Considering tha arteriographic and blood flow studies, indicating th principal features of rejection of organ transplants, wer published in Europe more than a decade ago, it is sur prising that this aspect of the microcirculation was no mentioned.

Only American researchers attended this conferenc and it is exactly under such circumstances that a splendi isolationism develops. One or two references are mad to European work which is all the more absurd becaus much of the early work on the microcirculatory problen of transplanted organs appeared first in European journals Of course, most authors made it clear that they woul not be presenting an extensive list of references, and s instead of presenting confirmatory evidence of pre viously published data one is left with what could seen to be an original statement requiring confirmatio itself.

What is so refreshing about the attitude taken by som American authors is that there is always somebody ther to debunk it. F. Gollan, in his introductory remarks t "*In vivo* Perfusion of Hearts", felt constrained to stat "I do not suggest a complete or even comprehensiv bibliography in every paper in traditional physiologica fields, but I do suggest, however, that results which wer obtained by other investigators, no matter how long ag and in what country, should be examined and relie upon before subsequent experiments are proposed in th same field of investigation. The tendency in many recen publications to cite primarily only contemporary author appears to lead to much unnecessary work and reduplica tion of effort".

W. J. DEMPSTER

Correspondence

Palace and Pollution

SIR,—I was disappointed at the lack of sympathy displayed in your article entitled "Palace and Pollution" (*Nature*, **228**, 398; 1970), for the various causes being argued at "The Countryside in 1970" symposium.

It is surely hardly realistic to expect such a meeting to resolve conflicts of amenity and economic interests; though you are right to point out that practical measures must take account of the wishes of people to exploit the environment for short term sectional advantages.

In view of the prestige which *Nature* enjoys, I am sorry that your contributor felt obliged to suggest that the Duke of Edinburgh was probably misguided to publicize the view that more people lead to a meaner life for all, and I am depressed by your calm acceptance that a population rise to 70 millions in Britain is inevitable. This will be the case only if nothing is done to avoid it. The Duke of Edinburgh suggested that population growth would be much slower and might be arrested if unwanted children were never born. This is a matter of the will to encourage people to take contraceptive measures and to make the means freely available, a matter about which the Duke surely displayed greater wisdom than your contributor.

Yours faithfully,

A. C. MASON

12 Collingham Green,
Little Sutton, Wirral,
Cheshire L66 4 NX.

SIR,—In your comment on the "Countryside in 1970" conference (*Nature*, **228**, 398; 1970) you rightly draw attention to the glossing over of the relationship between the spread of affluence and environmental destruction. However, you appear to consider that this must be accepted and do not mention the other alternative to the unacceptable maintenance of inequality: the levelling down of material consumption. This may seem more desirable than the alternative you describe: the growth of population beyond 70 million, since you say that it would be unjust (to whom ?) to keep it down, and the reduction of large parts of the countryside of south-east England to "isolated pockets of greenery". Do you really believe that we should pursue our immediate ends in this way with no thought for the future ?

Yours faithfully,

JOHN DAVOLL

Chairman of the Conservation Society,
10 Broadlands Avenue,
Shepperton.
Middlesex.

Nuclear Tests and Earthquakes

SIR,—I agree with Newstead and Pile[1], that the French test on May 24, 1970, was unlikely to have "triggered" the Peruvian earthquake. It was this coincidence, however, that focused press attention on the possible connexion between bomb tests and earthquakes. I do not agree that atmospheric tests are necessarily poorly coupled to the ground, especially when conducted over water[2], nor are time delays unreasonable. Delays of many days in the build-up of seismic activity often occur near test sites and in one instance a zone of 40 km from ground zero was active following a test[3]. Whatever the mechanism might be, such delays do occur.

The frequent incidence of fairly large earthquakes in Peru is simply a measure of the seismic instability of the region. Bomb tests adjacent to such regions must remain suspect until proved harmless. The possibility of a connexion between the tests and the Peruvian earthquake cannot be dismissed by means of statistics applying to other areas.

Statistical arguments by Emiliani *et al.*[4], similar to those of Newstead and Pile, have shown a correlation between underground tests and distant earthquakes. Later work[5] has shown no such correlation. In all cases, data from bombs of all sizes and earthquakes of all sizes were used. I am unaware of any detailed analysis of large bomb tests. It is here that correlations might be expected to show, if they exist.

Healy and Marshall[5] do give a table of eleven tests with a yield of over 200 kilotons. They give the number of earthquakes recorded in 24 hour periods before and after the tests. There were five in the periods before and sixteen afterwards.

Twelve of the post bomb earthquakes were after the Stones and Bilby tests. These were the second and third tests, and the authors say that this abnormal activity "may have been contemporaneous with natural aftershocks". The measurements were made in a circle of 860 km radius around the Nevada test site. Whether or not there were effects further afield is not known. The comparatively large effects after early tests, if indeed they were not natural, lend some support to the original proposal of Emiliani *et al.*[4]. It is possible that some seismic stresses were relieved by the early tests so that later ones occurred in a more stable environment.

Further information about the Bilby test is contained in a paper by Archambeau and Sammis[6]. In Figs. 6 and 7, they show a best fit between their theory and the measured values of particle velocity. Fig. 6 is for the Rayleigh wave of period 20 s and Fig. 7 is for the Love wave of the same period. In both cases there are anomalously high values towards the east coast of the United States. These can be attributed to an anomalously high main field or to a separate disturbance. In the case of the Love wave there is also an anomaly to the north-east, on the Canadian border. It would be interesting to see similar data for other tests.

Archambeau and Sammis also show the contribution of seismic energy to the total energy released in an underground test. The question to answer is whether this release of seismic energy is restricted to the immediate neighbourhood of the source or not.

Yours faithfully,

DAVID S. ROBERTSON.

"Maroonika",
Milan Terrace,
Stirling, SA 5152.

[1] Newstead, G., and Pile, M., *Nature*, **227**, 317 (1970).
[2] Harkrider, D. G., *Reviews of Geophysics and Space Physics*, **8**, 501 (1970).
[3] Hamilton, R. M., McKeown, F. A., and Healy, J. H., *Science*, **166**, 601 (1969).
[4] Emiliani, C., Harrison, C. G. A., and Swanson, M., *Science*, **165**, 1255 (1969).
[5] Healy, J. H., and Marshall, P. A., *Science*, **169**, 176 (1970).
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Obituaries

F. W. R. Brambell

FRANCIS WILLIAM ROGERS BRAMBELL, the zoologist famous for his work on animal reproduction, died on June 6, 1970, at the age of sixty-nine.

He was born at Sandycove, County Dublin, where as a young child he developed the interest in natural history that led him to Trinity College, Dublin, in 1918, with an entrance prize in Natural Science. There he was awarded a foundation scholarship and graduated with senior moderatorship and the gold medal for Natural Science in 1922. With a postgraduate research prize he obtained his PhD under Professor J. Bronte Gatenby.

By this time his main interest was in animal reproduction, and when in 1924 he was awarded a science research scholarship of the Royal Commission for the Exhibition of 1851, he decided to work at University College London, under Professor J. P. Hill and Professor A. S. Parkes. This arrangement was eminently successful, and led to the publication of a large group of papers on changes in the ovary caused by X-irradiation, and of the chapter on "Tissue Culture" in the 1928 edition of Bolles-Lee's vade-mecum. In 1926 he was awarded a fellowship of the International Education Board (Rockefeller Foundation), which he held at University College London until 1927 when he was offered a lectureship at King's College. He gained his DSc (London) in 1927, and in 1930, when only twenty-nine, he was appointed Lloyd Roberts Professor of Zoology at the University College of North Wales, Bangor. Here he remained until his retirement in 1968, working on the oestrus cycle and the reproduction of mammals (the common shrew, the bank vole, the lesser shrew and the wild rabbit) and on a local Enteropneust and its relatives. The first paper on what became his main study—prenatal mortality in the rabbit and the transfer of antibody from mother to young—appeared in 1942. In 1953, the Agricultural Research Council set up the unit of embryology at Bangor and appointed Brambell honorary director.

Of the many scientific and public offices that he held, he was a fellow of the Royal Society from 1947 and served on the council from 1954 to 1956. He was one of the society's royal medallists in 1964. He achieved public recognition chiefly from his chairmanship of what has always been known as the Brambell Committee, which undertook a considerable investigation of the methods used in maintaining farm animals in intensive conditions. The committee's report is a model of its kind of the consideration of the humane as well as the economic side of intensive farming. Soon after its publication, Professor Brambell was awarded the CBE.

He spent most of his retirement writing a book, *The Transmission of Passive Immunity from Mother to Young*, and seemed good for a long period of happy work. But early in 1970 he developed symptoms of a malignant disease, and in spite of extensive operations he died a few days after his book was published. His wife Margaret and his two children Anne and Michael survive him.

Dr M. J. Dijkman

DR MARINUS J. DIJKMAN, an internationally known authority on tropical agriculture and professor of tropical botany at the University of Miami, died on October 6 at the age of sixty-three.

He obtained his PhD as a plant physiologist at the University of Utrecht and began his career in 1935 as secretary to the West Java Experimental Station. Four years later he became director of the Agricultural Extension Service for South and West Sumatra. In the war he was a prisoner of the Japanese for four years and he was credited with helping to keep the mortality rate down by using his knowledge of jungle plants and insects as food and medicine. After the war, he worked as a consultant to the Netherlands Indies government for the mechanical cultivation of rice crops.

In 1947 he joined the staff of the University of Miami to work with his father in law, Dr Ochse, who was one of the world's outstanding experts on tropical economic plants. During this time Dr Dijkman initiated coffee studies in El Salvador and organized an agricultural training programme for the Pan American School of Agriculture in Zamorano, Honduras. He was made professor of tropical botany in 1953. Besides his interest in the purely practical field of improving agricultural production, he was also a pioneer in senescence and cancer research. Towards the end of his career he spent much of his time studying the effects of light on cell senescence. He was the author or co-author of a number of books including *Tropical and Subtropical Agriculture*, which has recently been reissued.

Dr Dijkman leaves his wife, Nelly, and their son Dr Dick Jaap Dijkman.

Announcements

University News

Professor J. E. Dowling, Johns Hopkins University School of Medicine, has been appointed professor of biology at **Harvard University.**

Mr P. Holgate has been appointed to the chair of statistics at Birkbeck College, **University of London.** The title of professor of psycholinguistics has been conferred on **Dr Frieda Goldman-Eisler** in respect of her post at University College. **Professor L. R. B. Elton,** University of Surrey, has been appointed visiting professor in the Department of Physics, University College. **Professor E. Shils,** University of Chicago, has been appointed visiting professor in the Department of Anthropology, and **Sir Frederick Warner,** of Cremer and Warner, consulting chemical engineers, has been appointed visiting professor in the Department of Environmental Studies.

Miscellaneous

Dr Gerald J. Wasserburg, California Institute of Technology, has received the Arthur L. Day medal of the **Geological Society of America.**

Entries are invited for the **1971 science writing award of the American Institute of Physics and the US Steel Foundation.** The $1,500 prize will be awarded for a article or book published in 1970 and intended for general readership. Entries should be submitted before January 31, 1971. Further information can be obtained from Public Relations Division, American Institute of Physics 335 East 45th Street, New York, NY 10017, USA.

The recipients of Soviet State prizes for science in 197 were announced, as is customary, on November 7, the anniversary of the October Revolution: **M. S. Ioffe** sector head at the Kurchatov Institute of Atomic Energy

B. B. Kadomtsev, R. I. Sobolev, E. E. Yushmanov, Yu. T. Baiborodov of the same institute, and **V. G. Tel'kovskii,** head of department in the Moscow Institute of Engineering Physics, for research into the instability of high temperature plasma in a magnetic field and the creation of the "magnetic well" method of stabilizing it; **Dr V. P. Silin,** laboratory sector head at the Lebedev Physics Institute, for his work on the theory of the electron Fermi-fluid of metals; **Dr E. V. Shpol'skii,** head of department in the "V. I. Lenin" Moscow Pedagogic Institute, for his discovery, investigation and implementation of fine-structure electron-oscillatory spectroscopy of complex organic molecules (Shpol'skii effect); **Dr S. V. Tyablikov,** for his monograph *Methods of the Quantum Theory of Magnetism*, published in 1965; **Academician A. G. Kolesnikov,** director of the Institute of Marine Hydrophysics of the Academy of Sciences of the Ukrainian SSR; **A. S. Sarkisyan, G. P. Ponomarenko** and **S. G. Boguslavskii** of the same institute; **G. N. Grigor'ev,** chief marine inspector of the Department of Marine Expeditions of the Academy of Sciences of the Soviet Union; **Dr S. S. Voit, Dr V. G. Kort, K. A. Chekotilo, Yu. A. Ivanov** and **V. G. Neiman** of the "P. P. Shirshov" Institute of Oceanology at the Academy of Sciences of the Soviet Union; and **N. K. Khanaichenko,** deputy director of the Institute of Biology of the Southern Seas of the Academy of Sciences of the Ukrainian SSR, for experimental and theoretical research on the Lomonosov currrent and other currents in the tropical Atlantic; **E. N. Mishustin,** head of department at the Institute of Microbiology of the Academy of Sciences of the Soviet Union, for research into soil microbiology and study of the biological fixation of nitrogen; **A. V. Artsikhovskii,** head of department at Moscow State University; **Professor V. L. Yalin** of the same university; **Dr B. A. Kolchin, A. F. Medvedev, P. I. Zasurtsev** of the Institute of Archaeology of the Academy of Sciences of the Soviet Union, and **V. I. Borkovskii,** sector head in the Institute of the Russian Language of the Academy of Sciences of the Soviet Union, for their work on the Novgorod archaeological expedition; **Dr M. F. Ternovskii** and **A. I. Teremt'eva** of the All-Union Tobacco Research Institute, for work on the hybridization of plants to produce disease-resistant strains; **Dr L. A. Lunts,** of the All-Union Scientific Research Institute of Soviet Jurisprudence, for his three-volume work, *Course of International Personal Law*, published in 1959–66, and **Academician N. N. Nekrasov,** chairman of the Council for the Study of the Forces of Production in the State Plan of the Soviet Union, and **Academician N. P. Fedorenko,** director of the Central Economic-Mathematical Institute of the Academy of Sciences of the Soviet Union, for research on the scientific problems of the development of the chemical industry of the Soviet Union.

ERRATUM. In the article "Three-dimensional Fourier Synthesis of Human Deoxyhaemoglobin at 3·5 Å Resolution" by Hilary Muirhead and J. Greer (*Nature*, **228**, 516; 1970), Fig. 2 is upside down. The part labelled *a* should be labelled *b* and vice versa.

ERRATUM. In the article "Three Dimensional Fourier Synthesis of Horse Deoxyhaemoglobin at 2·8 Å Resolution" by W. Bolton and M. F. Perutz (*Nature*, **228**, 551; 1970), the data on the left hand side of Table 1 refer to oxy and the right hand side to deoxyhaemoglobin: the printed headings should therefore be reversed.

ERRATUM. In the article "Nomenclature of Immunoglobulins" by D. S. Rowe (*Nature*, **228**, 509; 1970), column 2 of Table 1 should read

12
2
1·2
0·03
0·00025

British Diary

Monday, November 16

Developments in the Science of Wood Preservation (10.30 a.m. symposium) Society of Chemical Industry, Pesticides Group, at 14 Belgrave Square, London SW1.

Inflation and Eruption of Kilauea Volcano, Hawaii, 1965–68 (5.30 p.m.) Dr R. S. Fiske, University of London, in the Chemistry Lecture Theatre, King's College, Strand, London WC2.

Space Technology and the Future (6.30 p.m.) Mr G. K. C. Pardoe, Institution of Electrical Engineers, at the University, Liverpool.

The Origins of Money (5.30 p.m.) Mr P. Grierson, University of London, at Senate House, London WC1.

The University's View of Industry (6 p.m.) Professor J. W. Linnett, FRS, Royal Society of Arts, at John Adam Street, London WC2.

Tuesday, November 17

Cardiac Metabolism (5.30 p.m.) Professor R. B. Fisher, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Eighth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Electronics—Its Future in Navigation (6.30 p.m.) Institution of Electrical Engineers, in the Department of Electrical Engineering, The University, Liverpool (Silvanus P. Thompson Lecture).

Nature's Messengers (3 p.m.) Professor R. King, Royal Institution, at 21 Albemarle Street, London W1. (Lecture for preparatory school pupils from Schools in London and the Home Counties. To be repeated on November 18 and 19.)

Protein from Oil (6.15 p.m.) Mr G. H. Evans, Society of Chemical Industry, Food Group and Heavy Organic Chemicals Group, at 14 Belgrave Square, London SW1.

Submarine Ignimbrites? (5.30 p.m.) Dr R. S. Fiske, University of London, in the Chemistry Lecture Theatre, King's College, Strand, London WC2.

The Automatic Landing of Aircraft (6.30 p.m.) Mr S. A. W. Jolliffe, Institution of Electrical Engineers, jointly with IERE, at Leicester Polytechnic.

The Physics of Polymerized Material (5.30 p.m.) Professor S. F. Edwards, University of London, in Mechanical Engineering Lecture Theatre A, Imperial College, London SW7.

The Use of Organometallic Compounds in Synthetic Organic Chemistry (10 a.m.–6 p.m. symposium) Society of Chemical Industry, Fine Chemicals Group, at the Royal Society, 6 Carlton House Terrace, London SW1.

This Engineering Age (5.30 p.m.) Sir Henry Jones, Institution of Civil Engineers, at Great George Street, London SW1 (Graham Clark Lecture).

Wednesday, November 18

Biochemical Aspects of Insulin Secretion and Action (2 p.m.) Dr C. N. Hales, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

Chemical Magnification—Induction, Amplification and Catalysis in Analysis (6.30 p.m.) Mr A. Townshend, Society for Analytical Chemistry, jointly with the Carlett Park Chemical and Physical Society, at West Cheshire Central College of Further Education, Carlett Park, Wirral, Cheshire.

Electronics in Cars (6.30 p.m.) Mr L. G. Cripps, Institution of Electrical Engineers, at NE Essex Technical College, Colchester.

Fire Protection in Industrial Buildings (6 p.m.) Mr M. J. Smith, Royal Society of Arts, at John Adam Street, London WC2 (Alfred Bossom Lecture).

Investigations of the Liver, Spleen and Pancreas Using Radionuclides (10 a.m.) British Institute of Radiology, Nuclear Medicine Committee, in the Edward Lewis Theatre, Middlesex Hospital Medical School, Cleveland Street, London W1.

The Application of Ultrasonic Holography in Non-Destructive Testing (6 p.m.) Mr E. E. Aldridge, Institution of Electronic and Radio Engineers, Instrumentation and Control Group, at 8–9 Bedford Square, London WC1.

The Origin of the Metric System: Instruction sur les Mesures Déduites de la Grandeur de la Terre, by the Commission Temporaire des Poids et Mesures, Paris, Year 2 (1793–1794) (1 p.m.) Dr W. A. Smeaton, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

The Simultaneous Determination of All Zeros of a Polynomial (5.30 p.m.) Professor P. Henrici (Zürich), University of London, at Imperial College, London SW7.

The Tower Subway—The First Tube Tunnel in the World (6 p.m.) Mr C. E. Lee, Institution of Mechanical Engineers, jointly with the Newcomen Society, at 1 Birdcage Walk, London SW1.

Thursday, November 19

Analytical Applications of Lasers (6.30 p.m. discussion meeting) Society for Analytical Chemistry, Midlands Section, in the Chemistry Department, The University, Birmingham 15.

Automatic Methods and Assessment of Environmental Pollution (3 p.m. discussion) Society for Analytical Chemistry, Automatic Methods Group, at the Wellcome Building, Euston Road, London NW1.

Chemical Aspects of the Sodium Pump (5.30 p.m.) Professor R. Whittam, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Ninth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.).

Communications Satellites (5 p.m.) Professor B. Cheng, University of London, in the New Chemistry Theatre, University College London Gower Street, London WC1.

Electronics Education in Eastern Europe (6.30 p.m.) Professor G. D. Sims, Institution of Electrical Engineers, jointly with IERE, at Bournemouth College of Technology.

High Fidelity Loudspeakers and their Evaluation (6 p.m.) Dr A. R. Bailey, Institution of Electronic and Radio Engineers, Communications Group, at 8–9 Bedford Square, London WC1.

How to Achieve the Torque Curve You Want (6 p.m. discussion) Institution of Mechanical Engineers, Combustion Engines Group, at 1 Birdcage Walk, London SW1.

Laboratory Meeting (7.30 p.m.) Royal Society of Tropical Medicine and Hygiene, at the Liverpool School of Tropical Medicine, Pembroke Place, Liverpool 3. Chairman: Professor B. G. Maegraith, CMG, FRCP.

Mineralized Fluids in Ancient and Modern Sediments (2.30 p.m.) Institution of Mining and Metallurgy, at the Geological Society, Burlington House, Piccadilly, London W1.

Ocean Currents and Their Dynamics (two-day discussion meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

Progress in Organic Chemistry (2 p.m. symposium) Chemical Society, in the Scientific Societies Lecture Theatre, 23 Savile Row, London W1.

Some Investigation into Building Problems (5.30 p.m.) Mr R. T. Kelly, Society of Chemical Industry, Road and Building Materials Group, at 14 Belgrave Square, London SW1.

Stereo Transmission and Reception (6.30 p.m.) Mr J. H. Brooks, Institution of Electrical Engineers, London Graduate and Student Section, at Hatfield Polytechnic.

Systematic Application of Palynology (5 p.m.) Dr Wm. T. Stearn, Dr W. G. Chaloner and Mr A. Clive Jermy, Linnean Society of London, at Burlington House, Piccadilly, London W1.

The Frontiers of Farming (8.15 p.m.) Professor D. K. Britton, University of London, at Swanley Hall, Withersdane, Wye College, Ashford, Kent (Inaugural Lecture).

Friday, November 20

Dye-Sensitized Photo-Oxidation of Nucleotides (1 p.m.) Miss Gillian Mautner, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Gloss and Semi-Gloss Latex Paints (6.30 p.m.) Mr G. Willison, Oil and Colour Chemists' Association, at the Chamber of Commerce House, 75 Harbourne Road, Birmingham 15.

Monoamine Oxidase Inhibitors (10 a.m. colloquium) Biochemical Society, Pharmacological Biochemistry Group, at the Research Institute, May and Baker Ltd., Dagenham, Essex.

Muscular Contraction (9 p.m.) Dr H. E. Huxley, FRS, Royal Institution, at 21 Albemarle Street, London W1.

Storm Effects in the Ionosphere and Magnetosphere (2 p.m. discussion meeting) Royal Astronomical Society jointly with the Institute of Physics and the Physical Society, at the Scientific Societies Lecture Theatre, 23 Savile Row, London W1. Chairman: Professor V. C. A. Ferraro.

Monday, November 23

Building Linear Filters from Logic Circuits (7.30 p.m.) Mr M. H. Ackroyd, Institution of Electrical Engineers, at the University of Leicester

Engineers in General Management Posts—the Army's Experience (5.30 p.m.) Major General R. L. Clutterbuck, Institution of Civil Engineers, at Great George Street, London SW1.

Fire Precautions in Locomotives and Rolling Stock (5.30 p.m.) Mr J. M. Jarvis, Institution of Mechanical Engineers, Railway Division, at 1 Birdcage Walk, London SW1.

Founders of Radio-Analytical Chemistry (6 p.m.) Professor R. Spence, Society for Analytical Chemistry, Radiochemical Methods Group, at the Polytechnic of the South Bank, Borough Road, London SE1.

Microwave Ultrasonic Devices (6 p.m.) Mr R. F. Humphreys, Institution of Electronic and Radio Engineers, Aerospace, Maritime and Military Systems Group, at 8–9 Bedford Square, London WC1.

Some Aspects of the Educational System in the USSR (6.15 p.m.) Mr B. Nitikin (USSR Embassy), Institution of Electrical Engineers, in the Renold Building, UMIST, Manchester.

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Reports and Publications

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Great Britain and Ireland

West of Scotland Agricultural College. Current Experimental Programme No. 13, May 1970. Pp. 23. Experimental Record No. 15: The Effect of Heavy Dressings of Fertilizer Nitrogen on a Timothy/Meadow Fescue Mixture Under Three Cutting Systems. By I. V. Hunt. Pp. 35. (Auchincruive, By Ayr: West of Scotland Agricultural College, Grassland Husbandry Department, 1970.) [210

Department of Education and Science, Ministry of Technology. Statistics of Science and Technology, 1970. Pp. vii+127. (London: HMSO, 1970.) 25*s* net. [510

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 259, No. 831 (1 October 1970): *Ficus* Subgen. *Ficus*. Two Rare and Primitive Pachycaul Species. *Ficus* Subgen. *Pharmacosycea* with reference to the Species of New Caledonia. By E. J. H. Corner. Pp. 353–433. (London: The Royal Society, 1970.) 42*s*; $2.10. [510

Concorde. Pp. 64. (London: British Aircraft Corporation; Paris: Aerospatiale France, 1970.) [610

Imperial College of Science and Technology. Calendar 1970/1971. Pp. xi+569. (London: Imperial College of Science and Technology, 1970.) [710

Building Research Station Digest, No. 121: Stainless Steel as a Building Material. Pp. 4. (London: HMSO, 1970.) 9*d* (4*p*). [710

Other Countries

Cellular Immunology, Vol. 1, No. 1 (May 1970). Editor-in-chief: H. Sherwood Lawrence. Pp. 1–146. Vol. 1, 1960 (6 issues). $30. (New York and London: Academic Press, 1970. Subscription orders to 111 Fifth Avenue, New York.) [289

US Department of the Interior: Geological Survey. Water-Supply Paper 1899-G: Measurement of Mixing Characteristics of the Missouri River Between Sioux City, Iowa, and Plattsmouth, Nebraska. By N. Yotsukura, Hugo B. Fischer and William W. Sayre. Pp. iv+29. (Washington, DC: Government Printing Office, 1970.) $0.25. [289

Canvassing for Peace: a Manual for Volunteers. By Robert P. Abelson and Philip G. Zimbardo. Pp. vii+75. (Ann Arbor, Michigan: Society for the Psychological Study of Social Issues, P.O. Box 1248, 1970.) $1. [299

Proc. of the North American Paleontological Convention, Field Museum of Natural History, Chicago, September 5–7, 1969. Part A: Teaching Paleontology. Pp. 5. Part B: Computers in Paleontology. Pp. 55–154. Part C: The Genus—a basic Concept Paleontology. Pp. 155–320. Part D: Paleoclimatology. Pp. 321–408. (Lawrence, Kansas: Allen Press Inc., 1970.) $13.50 the four parts. [299

Metropolitan Life. Statistical Bulletin, July 1970: Diabetes at Midlife. Longevity at 65—International Trends. Trend in Work Injuries. Profile of Our Children. Pp. 12. (New York: Metropolitan Life, 1970.) [299

Smithsonian Contributions to Zoology, No. 54: Cyclopoid Copepods of the Genus *Pseudanthessius* Associated with Crinoids in Madagascar. By Arthur G. Humes and Ju-Shey Ho. Pp. 20. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) $0.35. [299

Le Bi-Repertoire Nucleaire: Techniques Nouvelles, Techniques de Pointe. Edition 1970 (Seconde Edition). (Paris: CID, Centre d'Informations et de Diffusion, 28 rue de Trévise, 1970.) [299

Agricultural Research Service, US Department of Agriculture, in cooperation with South Carolina Agricultural Experiment Station. Production Research Report No. 114: Pathogens and Chemicals Tested Against Caterpillars on Cabbage. By C. S. Creighton, T. L. McFadden and J. V. Bell. Pp. 20. (Washington, DC: Government Printing Office, 1970.) $0.15. [309

Mental Health Book Review Index: an Annual Bibliography of Books and Book Reviews in the Behavioral Sciences, Vol. 15. Compiled by the Editorial Committee and Contributing Librarians. (Whole No. 20.) Pp. xxxviii+88. (New York: Council on Research in Bibliography, Inc., c/o Research Center for Mental Health, New York University, 1970. Orders to Mental Health Book Review Index, c/o Paul Klapper Library, Queens College of the City University of New York, Flushing, New York.) $10. [309

World Health Organization. Technical Report Series, No. 451: Wholesomeness of Irradiated Food with Special Reference to Wheat, Potatoes and Onions—Report of a Joint FAO/WHO Expert Committee. Pp. 44. (Geneva: WHO; London: HMSO, 1970.) 3 Sw. francs; 6*s*; $1. [210

Rebublic of Cyprus: Ministry of Commerce and Industry. Annual Report of the Geological Survey Department for the year 1969. By Y. H. Stavrinou. Pp. 43. (Nicosia: Geological Survey Department, Ministry of Commerce and Industry, 1970.) [210

Arbeitsgemeinschaft Deutsche Kunststoff-Industrie AKI. Jahresbericht 1969. Pp. 16. (Frankfurt-am-Main: Arbeitsgemeinschaft Deutsche Kunststoff-Industrie AKI, 1970.) [210

World Health Organization. Technical Report Series No. 542: Requirements of Ascorbic Acid, Vitamin D, Vitamin B_{12}, Folate, and Iron—Report of a Joint FAO/WHO Expert Group. Pp. 75. (Geneva: WHO; London: HMSO, 1970.) 4 Sw. francs; 8*s*; $1.25. [510

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NOVEMBER 21, 1970

Will the Coelacanth Survive?

THE British Association has been in trouble of one kind and another for several years, so that the committee of review which has now been set up (see page 702) is by no means a frivolity. For a long time it has been clear that some radical reshaping of the association's function would be necessary. Moreover, it has become clear that the devices by means of which the association has in the past decade sought to extend its influence in public life by the encouragement of young scientists and the provision of public lectures, valuable though these have been, are not sufficient to provide a pattern for continued existence. So taking stock of the present and charting a course for the future is a necessary and welcome step.

What are the questions to be asked? If the committee of review is sensible, it will start with the question of what the British Association is for. Sad though it may be to see honourable institutions disappear, there is no excuse for keeping them alive simply because they exist already. And it must be said that the annual meeting which for decades has been the chief of the British Association's claims on public attention has become a muddled affair. Everybody agrees that there is no longer a need for a general forum for the presentation of new concepts in science —other institutions have now replaced the association in that way. To be sure, the annual meeting still gives pleasure to those who attend, but the number of people willing to pay for that (and able to afford a week away from home at the same time) is understandably dwindling. And the association's interest in young people and public lecturing could at a pinch be done as well by other organizations.

So what is there left to do? It is a great surprise that in the past several years the association seems almost wilfully to have neglected its own origins and the reasons which made its existence seem logical for the first fifty years or so after its foundation in 1831. Then, the association was valued not merely as a means of communication among scientists but also as a forum for the discussion of issues which are important to science as a whole and as a means of advancing causes which might help the development of science. What reason is there to believe that these functions are now less necessary than in the nineteenth century? If anything, there is now a more urgent need than ever for some means of exploring in public issues of importance to professional science and in the relationship between professional science and the wider community. The place of science teaching in British universities and the organization of the undergraduate curriculum as a whole are important public issues calling for an independent examination. Moreover, a body like the British Association could function well as a public arbiter of the quarrel between the nuclear physicists and the rest over the question whether to participate in the scheme to build a new proton accelerator at Geneva. On these and a host of other matters, the British Association, if it had the will, could play a valuable and as yet unfilled role in British public life.

Will it have the will? And will it have the courage to make the other necessary changes of organization which are necessary if such a task is to be undertaken? The first thing to be said is that the constitution of the association, hallowed by 140 years of tradition, is now quite unsuitable for the performance of any task. The interlocking series of committees which pass as its machinery of government are a means of sharing status among large numbers of people while ensuring that nobody need feel responsible. There is nowhere for Mr Harry Truman's buck to stop. It is no wonder that in the past six months, the association has been plagued with a rash of conflicts between the committees supposedly responsible for policy and the staff saddled with the thankless task of executing it.

In circumstances like these, it is also no surprise that hardworking professional scientists tend on the whole to spend their energies elsewhere than in the British Association—the association must count the continued support of many devoted professional scientists not as a mark of active respect but rather as a sign of the affection in which it continues to be held in spite of recent failings. And is it any wonder that keeping body and soul together and balancing the budget has turned out to be a daunting process?

Against this background, it must seriously be doubted whether the committee of review which is now established will be sufficiently radical in temper and sufficiently persuasive in the presentation of its eventual report to bring about the necessary reforms. The trouble is that the committee which has chosen to take responsibility for charting the future course of events is also for practical purposes that responsible for day to day management of the association's affairs. Although there is some new blood on the committee, it will evidently require an unwonted degree of courage for such a committee to acknowledge that the association's strategy for the past decade has been pusillanimous. By the same test, it will be hard for the committee to acknowledge some of its own recent folly, particularly in its dealings with staff. And finally, it is inevitable that when the committee eventually makes public its recommendations, those which argue for the continuation of present practices will seem unconvincing because they will also appear insufficiently detached. In short, it is a mistake to have chosen such an introspective method of inquiry.

Universities Once More Under Pressure

THESE are sombre times for those who would form a coherent view of the development of British universities in the next few years. To begin with, the change of government has spread a little alarm and a good deal of despondency, particularly among those who cannot easily reconcile a generous policy towards the universities with the government's apparent belief that reductions of public expenditure are innate virtues. The news that the government has also asked the Open University to study what would be involved in taking on to its books not the stream of self-improving adults for which the university was originally intended but, rather, students straight out of school has added to the sense of alarm, if only because it suggests that the government is planning to meet the demand for more higher education, commonly acknowledged to be a reality, by setting up methods of higher education which are alternatives to conventional universities.

In circumstances like these, the universities should be careful not to panic. To cry WICKED TORIES! would probably help to set the blood flowing again in the veins of many nostalgic academics but there is much more point and potentially much more profit in a radical re-examination of the present system of higher education. First, there is at least a case to be made for thinking that young people in the United Kingdom might find some benefit in a university that would allow them to obtain degrees while working at some regular job. After all, Birkbeck College in the University of London has an honourable reputation. The University of South Africa, which functions as a correspondence college and which is much despised by academics, is nevertheless a good deal better than nothing. It is true, of course, that the Open University would be better able to defend itself against an attempt at converting it into a substitute for more university places of an orthodox kind if it had had time to demonstrate that it could operate successfully with more mature students.

No amount of dickering with the Open University will, however, solve the problems with which orthodox universities are concerned. The overriding problem is how to cater for much larger numbers of students while still providing a curriculum that is at once educative and a preparation for more exacting things to come. The system proposed by Professor Brian Pippard and his colleagues (about which an article will be published next week) does point to one possible solution. Briefly, there are potential advantages in a system in which, in Britain, the usual undergraduate course lasts two years and serves as a foundation for vocational studies or more advanced scholarship. There is particular benefit to be expected from an attempt to make a liberal course for undergraduates based on science, if only because it is by now plain that the specialist curricula inherited from the time when the university system was much smaller are unsuited to the present needs of a technical society in which large numbers of people must take on work which is technical in its connexions but neither research nor development in the old sense. The universities should therefore regard the exploration of innovation of the kind now known as Pippardism with all the vigour at their command, for in the long run these are typical of the ways in which they must hope to be able to provide higher education for more people in a manner better suited for modern needs.

More Games with Numbers

DR EUGENE GARFIELD'S Science Citation Index has become not merely a bibliographical tool for a disputed phenomenon in its own right. Some weeks ago (*Nature*, **227**, 669; 1970) Dr Garfield himself argued how his compilation could be used as a basis for historical research of various kinds. The Science Citation Index is in fact a list of all the pieces of scientific literature referred to in scientific articles published in some 2,000 scientific journals with, of course, suitable references to the articles in which the items were first cited. Dr Garfield himself suggested that his compilation can be used as a means of tracing the development of new ideas through several decades of contemporary history, and this is evidently a fruitful field for research. He did however go further by suggesting that the numbers of occasions on which particular articles were cited in the literature could in some sense be taken as a measure of their importance and that—this is where the trouble begins—the productivity of individuals might be measured by the numbers of occasions on which their names are cited in the literature in any year. This is the point which has been attacked by several correspondents in the past few weeks, and which is now once more defended (see page 789) by Mr A. E. Cawkell from Dr Garfield's Institute for Scientific Information.

Where does the truth lie? What value is to be attached to the number indicating how often a particular article or the author of a series of articles is referred to in the literature? And is there any foundation in Dr Garfield's suggestion that a discriminating use of numbers like these might help to identify creative people early in their careers, to award grants and fellowships without "wasteful infighting and manoeuvring" and to provide outsiders with a sense of "where the action is"? Much depends on knowing why authors cite particular papers when writing an account of their own work. This is a subject on which much remains to be said. The chances are, however, that some kinds of articles will be more frequently cited than others because of the character and not necessarily

the importance of their contribution to the development of science. In theoretical physics, for example, authors frequently refer to accounts in the mathematical literature of the development of a new technique simply as a perfectly proper means of shortening their own account. The same is true in many other fields in which a reference to a convenient description of a standard technique may save a lot of space and explanation. Elsewhere, however, it is well known that radically innovative papers are often neglected for several years until they have somehow become absorbed in the mainstream of development—Einstein's classic paper on the stimulated emission of radiation and Onsager's calculation of order–disorder phenomena in crystal lattices are two examples of crucial papers that would hardly have shown up on Dr Garfield's index when their importance was most in need of recognition. In short, the snag in the operation of this numbers racket is the danger that, with the best will in the world on everybody's part, the numbers will merely accentuate a conventional description of "where the action is".

Signs of Reason

THE leader of the House of Commons, Mr William Whitelaw, has shown reason. His proposals for the select committees of the House of Commons (see *Nature*, **228**, 401; 1970) which were debated last week are much more sensible than his warnings last July about avoiding "developments which will lead to a position like that reached in the United States Congress where committees are all-important". Mr Whitelaw was then concerned to preserve the chamber of the House of Commons as the place where "true debate must take place", in the best traditions of Pitt and Disraeli, but his friends and other colleagues have luckily brought him back into the twentieth century.

What Mr Whitelaw now suggests is that the select committee system should not be allowed to mushroom to such an extent that important decisions are taken outside the chamber of the House of Commons but, on the other hand, he sensibly says that there is considerable value in maintaining a few specialist select committees to look at the workings of the executive departments. This—Mr Whitelaw may be comforted—is a system with a good pedigree. In 1918, Lord Haldane said that any increase in the efficiency of the departments of state "should have as its correlative an increase in the power of the legislature as a check on the acts and proposals of the executive". This need is probably most obvious with the Select Committee on Science and Technology, which has done much to bring to the attention of members of Parliament and the public the issues underlying decisions in the field of nuclear energy, for example, and it has also been valuable in stimulating debate outside Westminster and in the press. The reappointment of the Science and Technology committee will be welcomed; there is an obvious need for it to embark without delay on a study of the present chaos in which Britain's space research finds itself.

But is Mr Whitelaw in any case justified in holding on to his belief in the ability of Parliament to engage in productive debate? All too often the agenda is filled with matters which keep it occupied until the early hours of the morning, but even on important issues the result of a debate is a foregone conclusion. Apart from the operation of the party system, the Members of Parliament are incapable of keeping themselves fully informed of many of the issues which underlie government policy, so that debates all too often turn out to be a series of rather bald statements. An answer could be to extend the select committee system even further, by setting up *ad hoc* bodies to look into important issues as they arise. A select committee to examine the consequences of Britain's entry into the Common Market, for example, would not go amiss. But for the future, Mr Whitelaw should take careful note of the point made repeatedly in last week's debate that if a select committee is to function properly, it must have a permanent and competent secretariat and pool of knowledgeable advisers.

Emperors without Clothes

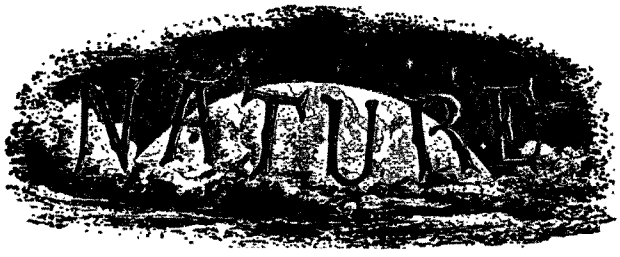
THE trouble at Rolls-Royce is a smack in the eye for British policy towards industrial development. For two decades now, the manufacture of aircraft engines has been regarded as an activity as important to the survival of the United Kingdom as the preservation of the pound sterling and (more difficult) of the white cliffs of Dover. Over the years, Rolls-Royce has paid more in taxes than it has earned by government contracts or subventions, and its export record has been splendid. Why, then, has it suddenly fallen flat on its face?

First of all, it must be acknowledged that the company has been exceedingly complacent in its belief that it would be possible always to earn a living for itself by applying a variety of high engineering skills to the manufacture of a single product, particularly when the market for that product is likely to contract and when the company could not hope to establish itself as a prime contractor in the United States as well as Britain. In terms of industrial strategy it would have been wise, more than a decade ago, to have plumped for a measure of diversity, partly as an insurance against the kind of setback which has now occurred and partly as a way of making fuller use of the skills available within the organization. This, one would have thought, is one of the simple

facts of life that an indulgent government could have spoken of to its protégé.

But would not greater diversity have blunted the flair with which Rolls-Royce was able to exploit its skill in the manufacture of aircraft engines? This is what the company would have said. Outsiders and even some of those within the company may now agree that the flair for exploiting high technology in a narrow market, which has kept Rolls-Royce going for twenty years, may in the end have been the company's downfall. How much better might it not have been to have devoted some of this skill to the manufacture of electric typewriters, sewing machines or other objects which could have been sold for a profit?

100 Years Ago



DR. MICHAEL FOSTER (the newly-appointed Prælector of Physiology at Trinity College, Cambridge) commenced on the 14th inst. his course of lectures in a part of the new museums, which has been temporarily fitted up as a Physiological Laboratory. He gave a lucid and able exposition of the three great factors of life—contractility, as evinced chiefly in muscles; irritability, as evinced chiefly in the nervous system; and secretion. Dilating upon the much-vexed question, how far these are attributable to physical agencies, or are to be referred to another agency called "Life," he compared the latter view to a fortress closely besieged by an able band of investigators who are ever narrowing its area, and pressing the physical forces closer and closer upon it. But it has not yet capitulated. No one has a right to say that it will or will not capitulate; and till it has done so we are perfectly justified in regarding it as an entity, as a something to be taken into account in the investigation and the attempts at the explanation of living processes. He should still, therefore, use the term without committing himself to either view. He gave definitions of Physiology and Morphology. He spoke of the enormous importance of vivisection to the advance of Physiology. By it Vesalius might be said to have laid the foundations of Physiology; by it Harvey had been enabled to obtain the proofs of his great discovery. Without it all that had been written on Physiology would have gone for very little, and we should still have been in the Aristotelean mists darkened by the theories of the Schoolmen. He wished, however, to state that in the teaching of Physiology it would be necessary for him to resort to it much. He stated the plan he intended to pursue in carrying out the intentions of those who had placed him in that honourable position. Lectures he did not regard as a very fructifying mode of sowing seed. He thought it far better that men should work and see for themselves. With the munificent aid of Trinity College, he hoped, ere long, to make the physiological laboratory in Cambridge one of the best working laboratories in the country. He intended to have practical classes in addition to the lectures; and students who were competent would have opportunities for private work. It would be a labour of love to him to render practical aid to those who needed it, and to promote the study of physiology by every means in his power.—

From Nature, **3**, 73, *November* 24, 1870.

OLD WORLD

SATELLITES

UK-5 to Study Cosmic X-rays

THE Science Research Council has announced that next satellite in the series being launched for Britain NASA will be designed to study cosmic X-ray sour Called UK-5—a successful launch in 1973 will re in its being renamed Ariel 5—it will be the sec satellite to be wholly manufactured in Britain. UI which should be launched in 1971, is now being built the British Aircraft Corporation, and both the sa lites will carry an experiment for a United St research group in order to obtain a relatively low launch.

UK-5 will be a squat cylinder 34 inches high 37·75 inches in diameter, its sides being almost cove with solar cells to provide electrical power. attitude of the spacecraft will be controlled by its computer in accordance with instructions from ground, so that most of the X-ray detectors can pointed at individual sources while the one-side moun experiment will scan the whole sky as the sate rotates, searching for new objects.

There will be a total of six experiments carried UK-5, two of which are the responsibility of University of Leicester group which has beer successful with other X-ray detectors. One will meas the polarization of X-rays, while the other is overall sky survey mentioned above. The Mullard Sp Sciences Laboratory is providing a proportic counter to study individual sources in the 2–30 band, while higher energy sources (up to 2 MeV) be studied by a detector designed at Imperial Coll London, to investigate known X-ray sources to, search for X-ray emission from, pulsars. Anot pointing detector, which incorporates a photomultip for detecting visible light as well as several detect intended to carry out a sky survey in the range 0·2 30 keV, is also the responsibility of the Mullard labo tory and the "guest" American experiment to Goddard Space Flight Center will search for transi effects against the X-ray background radiation.

On this occasion, the spirit of Anglo-American operation will be maintained after the launch of UK when ground based observations will be carried out the Mount Wilson and Palomar Observatories correlate the X-ray observations with simultane optical measurements. One group at the Hale Obser tories are already compiling a library of plates obtai with their 48 inch Schmidt telescope, and these will made available by the time UK-5 is in operation. Ot groups, possibly including one in Chile, will also t part in the observational programme being plann but the Hale survey will be particularly useful beca it covers the galactic plane out to a latitude of ±9°, a most X-ray sources lie in the plane of the galaxy.

This highly sophisticated satellite has been announ at a time when interest in X-ray astronomy is at height (see p. 715) and will provide just the type detailed information which is required before any gr progress in understanding high energy sources can achieved.

LUNA-17

Automatic Exploration

from our Soviet Correspondent

THE landing of Luna-17 on the surface of the Moon, and the safe deposition and operation of the Lunokhod-1 "mooncar", following hard on the heels of the Luna-16 project, show that Soviet space research is soldiering on with the first, reconnaisance, stage of lunar exploration. This stage, according to Academician B. N. Petrov (*Nature*, **228**, 6; 1970) will depend only on automatic spacecraft.

From the pictures released so far, Lunokhod-1 with its eight wheels seems to be capable of proceeding at more than one speed. There are also reports that it is "proceeding at its first velocity". The weight of the vehicle has not so far been released, but its equipment, in addition to the radio and television links needed for its control from Earth, includes a laser-reflector, developed on a basis of Soviet–French co-operation, and a number of as yet unspecified experiments.

Because of the small amount of Moon-rock (about 100 g) recovered by Luna-16, it had been suggested that the mission of Luna-17 would be merely a repetition of Luna-16. The rapid progress from the stage of simple retrieval of specimens to the operation on the lunar surface of an explorer craft indicates an acceleration in the tempo of the Soviet space programme. But the highly publicized setting up of Soviet flags and pennants, together with a portrait of Lenin, suggests that this successful attempt at a new "first" in space hardware has been undertaken for prestige as much as for scientific purposes.

INDEPENDENT UNIVERSITY

Hazy Plans command Support

by our Education Correspondent

A MEETING of supporters of the Independent University last week failed to remove most of the question marks still hanging over the proposed venture. What emerged from the meeting was a woolly idea of the possible academic policy of the institution, an even hazier picture of how it may be financed and the knowledge that the planning board is not sure whether to plump for a site very close to London or one in the country. In other words, the plans do not seem to have matured much since the idea was first discussed in January 1969 (see *Nature*, **221**, 119; 1969). One thing the meeting did demonstrate, however, is that the Independent University now commands a growing band of influential supporters—the list of patrons, for example, looks like an extract from *Who's Who*.

United in the belief that academic freedom, and in particular the freedom to innovate, are being eroded in the traditional state-supported universities, protagonists of the Independent University are attempting to set up a university free from financial ties to the exchequer. Under the chairmanship of Sir Sydney Cain, former director of the London School of Economics, a planning board has been looking at ways in which such an establishment could be financed and what sort of teaching it should go in for. The venture is expected to be financed by foundations, industry and contract research, and its students will pay their own fees.

Describing the possible academic policy of the Independent University, Professor Max Beloff, from the University of Oxford and a member of the planning board, said that the institution would stand or fall on its ability to attract good staff. The emphasis, he said, will be on intensive teaching, "which will bring out intensive learning", and the curricula will be something of a compromise between the specialist and non-specialist schools of thought—an undergraduate's first two years would be spent in general studies, while the third year would be devoted to a specialist course.

One of the chief criticisms often levelled at the proposals is that the university will not be able to support a good science faculty on a limited budget, but according to Professor G. H. Rawlcliffe of the University of Bristol, and also a member of the planning board, this attitude is a "delusion". The university, he said, will concentrate on those branches of science which do not require heavy capital expenditure, and science will be taught in a new way. It is possible, Professor Rawlcliffe maintained, to teach "science for the citizen" rather than "science for the specialist", and to teach concepts very cheaply.

But this hazy argument did not entirely appeal to Professor Philips Griffiths, of the University of Warwick, who doubted the ability of the university to attract good teaching staff without spending much money on research equipment. Other speakers also raised the question of the provision of computing facilities and there was some discussion of the viability of a two-year generalist course. But the trend of the discussion was in favour of breaking down the barriers between subjects.

Discussion of the finance of the Independent University was non-specific—little mention was made of how much the institution will need, or how much has been promised. A system of student support by loans was also talked about in theoretical terms, with Dr Margaret Woodhall of the University of London Institute of Education providing the basis for discussion with a review of the loans system in Scandinavia. It was broadly accepted that a loans system is a good idea, but again, how it will be administered at the Independent University was not discussed in detail.

The final session was devoted to a discussion of the progress made so far by the planning board. Sir Sydney Cain said that the "project is increasingly in line with current thinking on university problems" and the board has been much heartened by the expressions of goodwill it has received. The chief task during the past few months has been to secure a site. Several are under consideration and one at Croydon has been investigated very thoroughly, in spite of "rather considerable" planning problems. Once a site has been secured, Sir Sydney said, the Independent University will petition for a charter and it hopes to take about 400 students in its first year of operation—possibly 1973—and eventually to build up to a student population of 3,000.

Because nobody at the meeting really challenged the basic idea of the Independent University, much of the discussion tended to revolve around particular points. The upshot was that the ultimate viability of the institution was barely questioned and some of the chief criticisms that can be levelled were not brought up. The problem in attracting good staff,

for example, was mentioned only in passing, and the problem of building up a good science faculty with limited funds was not dealt with very convincingly. But the biggest assumption, although backed up by favourable results from a questionnaire answered by 117 sixth formers, was that bright students will be interested in the university in spite of the fact that they will have the chance to go to a traditional university with state support.

MATHEMATICS TEACHERS

The Vicious Circle

THE continuing crisis over the shortage of mathematics teachers has been brought back into the public eye by the *Second Report on the Shortage of Teachers of Mathematics*, published by the Institute of Mathematics and its Applications. The report emphasizes that the official definition of a "qualified" teacher, which includes any graduate who has completed a course of teacher training, allows those with arts degrees and no formal training in mathematics to teach the subject and to receive full graduate allowance in their salaries. There is thus no financial incentive for teachers to teach the subject in which they have been trained and, with a surplus of arts graduates, this works against the best interests of pupils taking all science courses. Although salary scales in which there are built-in subject differentials would probably prove impractical, the institute suggests much more flexibility could be provided by giving outstanding teachers of any subject the opportunity for rapid promotion and other incentives with which other professions recognize unusual ability.

The Institute of Mathematics and its Applications suggests that as many as 50 per cent of pupils in maintained secondary schools have mathematics teachers who themselves lack even O-level qualifications in the subject. Although that is probably an exaggeration, the overall picture is now sufficiently clear—unless action is taken soon, the vicious circle whereby pupils are so put off mathematics by bad teaching that the proportion taking the subject (and hence the eventual proportion of mathematicians in succeeding generations of teachers) is falling drastically every year will become unbreakable. The Department of Education and Science had no comment to make on the detailed evidence put forward in the report. A DES spokesman was only prepared to say that the situation has been known for a long time, and the department has nothing to add.

CIVIL RIGHTS

Sakharov Again

by our Soviet Correspondent

ANDREI D. SAKHAROV, the Soviet nuclear physicist, is again at the centre of a controversy in the Soviet Union. Together with his fellow physicists Tverdokhlebov and Khalidze, he is proposing to set up a committee for the defence of human rights. Although such a committee, if registered with the state, would in theory be legal, it would be an embarrassment to the Soviet authorities to have a watchdog body to safeguard the rights of man, and in particular intellectual freedom, operating within the Soviet Union. To sanction the setting up of such a committee would be a tacit admission on the part of the Soviet authorities that it is needed.

Sakharov's proposed committee would lie within the bounds of Soviet law because the Soviet Union was a signatory to the Universal Declaration of Human Rights in 1948. But although the Soviet press reported the signing, the text of the declaration has never been published in the Union. The committee, its supporters maintain, is non-political and will not associate itself with any organization which is in any way anti-Soviet. In previous manifestos, Dr Sakharov has always denounced arbitrary and illegal behaviour towards individuals as being typical of the Stalin regime and he has urged that all such action should be stamped out as unwelcome inheritance from the past.

The formation of this committee will in many ways be a culmination of Dr Sakharov's previous efforts to establish a more liberal atmosphere in the Soviet Union. He published in 1968 an open letter, "Thoughts on Progress, Peaceful Coexistence and Intellectual Free-

BA under Scrutiny

THE British Association for the Advancement of Science has set up a committee to scrutinize and reinterpret the association's objectives and to review its future activities, finances and management. The setting up of such a committee was proposed at the British Association's annual meeting last September (see *Nature*, **227**, 1074; 1970), and its chairman will be the association's president, Sir Alexander Cairncross, master of St Peter's College, Oxford. Other members will be Sir Vivian Fuchs, the president elect and director of the British Antarctic Survey, Dame Kathleen Lonsdale, Sir Eric Mensforth, chairman of the Council of Engineering Institutions and general treasurer of the association, Dr H. M. Finniston, deputy chairman of the British Steel Corporation, Professor G. E. Fogg, professor of botany at Westfield College, London, Dr A. H. Hughes, managing director of Arthur Guinness Son & Co. Ltd, Lord Rennell of Rod, Sir David Martin, Dr J. D. Carthy, scientific director of the Fields Study Council, and Mr P. W. B. Semmens, products works technical manager of the ICI Agricultural Division.

The committee will be responsible for reviewing the economy of the association and it will seek new sources of finance and new means of co-operating with other scientific organizations with similar and overlapping interests. As for the association's task of considering the impact of science and technology on the quality of life of the community, the committee will enlist the further support of scientists and technologists and the goodwill of the press, radio and television. The committee will submit its findings to the council of the association in time for the annual meeting which is to be held at Swansea next September.

dom", which included an attack on the "crippling censorship" in the Soviet Union, and a demand for greater intellectual freedom. This was followed in March 1969 by an open letter to Brezhnev, Podgorni and Kosygin which suggested that intellectual repression can only act to the detriment of the state by hampering the whole process of economic growth and development. In particular, he pointed out that brainpower is wasted by the imprisonment or internment in mental hospitals of intellectuals for opposition "in the field of ideas and convictions" (see *Nature*, **226**, 210; 1970).

When Zhores Medvedev was arrested and taken to Kaluga mental hospital for just such "opposition", Dr Sakharov again wrote to Brezhnev. This letter was not only a reasoned plea for the release of the geneticist but virtually a manifesto of human rights as well.

COMPUTERS

Privacy and the Computer

THE British Computer Society has strongly criticized the approach of the Parliamentary Committee on Privacy to the problem of safeguarding the public against the computer. In a letter to the chairman of the committee, Mr A. Agapeyeff, president of the society, points out inadequacies in the committee's approach and suggests forms of legislation urgently required to prevent encroachment on privacy by the abuse of computerized personal data. He also says that the committee seems to be ignoring the dangers involved in using computers in public spheres.

Mr Agapeyeff points out that as computer systems become more complex, privacy is threatened not only from deliberate abuse of computer records of personal details but also from accidents in the programming and processing of the data. There are already too many victims of faulty bills, financial statements, automatic reminders and threats of legal action sent out by haywire computers and the situation is getting steadily worse. Moreover, Mr Agapeyeff suggests that public safety is at stake for the same reason. Computers are already deeply involved in monitoring, for example, nuclear power stations and military systems and before long they will also assist in air traffic control and in hospitals for monitoring intensive patient care. That there is no system for maintaining the standards of computing, says the society, represents a serious omission on the part of the computing industry.

Mr Agapeyeff proposes that there should be safeguards to protect the public from inexperienced computer operators and that computers should be checked by independent specialists before they are put into service. There should also be stricter systems for updating and "forgetting" incorrect data.

As far as intentional indiscriminate use of computerized personal data is concerned, Mr Agapeyeff suggests that the need for legislative control is obvious. Under economic pressure, the records have already become centralized and easily accessible and there are increasing incentives to market information to employers, customers and voters. The letter distinguishes between information that is inherently private such as medical and criminal records, and the semi- and potentially private information such as political affiliations and details of travel and companions. It goes on to suggest that in order to keep an eye on computers intending to process inherently private information, a system of licensing should be introduced and records should be kept of the type of data, its source, use and distribution. It should also be illegal for computer operators to reveal information to unauthorized third parties.

Whatever its fears, the society remains resolute in its belief that extensive computer applications are strongly in the national interest. The machine, says Mr Agapeyeff is morally neutral and it is up to the computer professionals to detect and alleviate the growing dangers which arise from faulty computing systems.

FOWL PEST

Epidemic Loses Momentum

THE fowl pest epidemic which has been afflicting British poultry for almost three months may have passed its peak, but it seems, nevertheless, to be trailing fresh hazards in its wake. Although it is not uncommon for poultry workers to contract a mild form of conjunctivitis—inflammation of the membranes around the eyes—following exposure to fowl pest virus (*Myxovirus multiforme*), a speaker at a recent meeting of the British Veterinary Association (BVA) warned that human response to the strain of virus causing the present epidemic is unusually severe. The symptoms include a fever resembling influenza, conjunctivitis, swollen glands and a variety of vague aches and pains. The BVA was swift to emphasize, however, that the virus can be contracted only by contact with live infected poultry, and that the disease in humans is not serious.

The present epidemic, hopefully, seems to be on the downswing. Outbreaks recorded up to last weekend totalled 1,690 and the number of fresh outbreaks is decreasing daily. Well over 4,000,000 birds have died since the epidemic began, mainly in East Anglia, but outbreaks have been reported in 38 counties in England with isolated cases in Scotland and Wales.

The epidemic seems largely to have been the result of complacency amongst poultry breeders. Over the past few years the incidence of Newcastle disease has been so slight that many breeders were lulled into a false sense of security and neglected to maintain full and regular vaccination and more than half the national poultry stock was not protected when the virus struck. As a result, the disease has ravaged disastrously through the unprotected stock, whereas vaccinated poultry has remained comparatively untouched.

Poultry vaccines used in this country are of the inactivated type, killed by treatment with formaldehyde or irradiation. In most other countries where poultry are reared intensively, live vaccine is used. This makes mass inoculation considerably simpler, even though the immunity conferred by this method has not always proved reliable. The Ministry of Agriculture has taken advantage of the present epidemic to test the live vaccine in the field. Trials are being carried out in specially chosen infected areas of East Anglia under close ministry supervision, but it is too early yet for the results to be assessed.

Poultry breeders are angry that the ministry did not use the live vaccine earlier in an effort to crush the epidemic in its initial stages; a more telling criticism is that it did not take a strong enough line with the poultrymen in the first place to ensure that a high standard of vaccination was maintained.

ATOMIC ENERGY

Construction Costs Soar

THE Central Electricity Generating Board has adopted a new strategy for the construction of its latest nuclear power station, Sizewell "B", by splitting up the contract so that the nuclear components will be tendered for separately from the generators and the civil engineering work. This practice of splitting up contracts has been common for conventional stations, but has never been applied to nuclear plant.

The CEGB has awarded the design contract for the 2,500 MW Sizewell B station to the Nuclear Power Group, one of the two British design and construction consortia, and it seems certain that the NPG will be given a firm contract next year for the construction of the nuclear islands, estimated to be worth about £140 million.

Sizewell B will be the largest nuclear power station in Britain when the last of its four reactors goes critical in 1977. The total cost is expected to be about £250 million which marks a considerable increase in capital cost per kilowatt since the Hinkley Point B station, also an advanced gas cooled reactor system, was started in 1967. Hinkley Point B cost about £76 a kilowatt whereas expenditure on Sizewell B is expected to reach about £100 a kilowatt. The CEGB points out, however, that the costs have still to be finalized.

UNIVERSITIES

Selection Procedures Condemned

A STINGING attack on the system of entry to British universities was delivered last week by the National Association of Careers Teachers. In a letter sent to the Committee of Vice-chancellors and to other bodies responsible for university admissions, the association points to many faults in the workings of the Universities Central Council on Admissions, and it is particularly scathing about the lack of liaison between universities and schools. There is rivalry between universities, faculties and departments, the association suggests, "and in consequence heads and careers teachers receive little help from these sources which may be used as a basis for reports on applicants from their schools".

The accusations are based on a recent survey of university applications carried out by the association. The letter points out, for example, that the apparent choice of five universities offered by the UCCA is a choice "on paper only". "It is patently obvious," says the association, "that for popular courses such as law, medicine and languages, those universities placed fourth and fifth rarely give serious consideration if any at all to the applicants concerned".

What is to be done? The association suggests that one of the more obvious drawbacks in the present selection system is that the universities select students before their A-level results are announced, and this results in undue complication. There is therefore a good case for reviewing the whole system of university selection, adjusting examination times so that the results are published by the end of July and moving the start of the first year of university courses to November. Alternatively, the association suggests, a student could be compelled to break off his studies for one year between A levels and university.

Parliament in Britain

Transfer of Research Establishments

RESPONSIBILITY for the Water Pollution Research Laboratory, the Hydraulic Research Station, the Forest Products Research Station and the Fire Research Station will be transferred from the Department of Trade and Industry to the Department for the Environment on January 1, 1971. Announcing this transfer, Mr David Howell, Parliamentary Secretary to the Civil Service Department, said that the functions of these laboratories fall chiefly within the ambit of the new Department for the Environment. By the same token, the Department for the Environment will take over the responsibility for administering grants to the Water Research Association and to the Construction Industry Research and Information Association. All these responsibilities were previously assumed by the Ministry of Technology. (Written answers, November 10.)

Research and Development Estimates

THE government intends to spend about £63 million on research and development in its own establishments in 1970–71. This figure includes expenditure on the Atomic Energy Authority, and it is about the same as the estimated total expenditure in 1968–69 and 1969–70. This announcement was made by Mr Nicholas Ridley, Under Secretary of State, Department of Trade and Industry, in reply to a question from Mr J. H. Osborn. (Written answers, November 9.)

British Steel Corporation

MR JOHN DAVIES, Secretary of State for Trade and Industry, hinted in a reply to Mr Gregor Mackenzie that the government is considering making changes in the structure of the British steel industry. He said that he is engaged in discussions with the British Steel Corporation to try to develop a structure for the industry which will assure it of a prosperous and satisfactory future. He also said that it would not be correct to assume that the management of the industry believes that it should be left exactly as it is. (Oral answers, November 9.)

National Health Service

SIR KEITH JOSEPH, Secretary of State for Social Services, gave details of how the government plans to spend the English share of the extra £110 million which will be made available to the social services over the next four years. A large share of the money will be spent on provision for the mentally handicapped and on improvement of hospital and local authority services for old people and the mentally ill. Each of these services will receive £40 million from the £110 million.

The Secretary of State also said that he hopes to make a statement soon about family planning, and about improvements in the accident and emergency services. A review of what should be done in these areas, he said, is under way. £3 million will also be set aside over the next four years for special units for the chronic sick, and an extra £2 million to improve facilities and advice for alcoholics.

The total resources available for the hospital, local authority and welfare services for 1971–72 will be about £5 million more than during the present financial year. This is an increase of 6 per cent, compared with an increase of 4·3 per cent during 1969–70. (Statement, November 11.)

Rolls-Royce Looks Happier

THE instability of the economic tightrope which Rolls-Royce has been obliged to walk almost since the start of development of the very successful Spey engines in the early 1960s has been highlighted during the past week. Rolls-Royce has announced a loss of £48 million during the first six months of this financial year, compared with a profit of £2·5 million last year, and the government has stepped in with massive additional launching aid of £42 million for the RB 211 turbofan engine. This aid will be supplemented by bank loans of £18 million and repaid by a levy on sales. Sighs of relief from both sides of the Atlantic that Britain's only aero engine manufacturer has been saved from financial disaster have shown how important Rolls-Royce is in the complex international aerospace web.

The firm's trouble is rooted in the ever escalating costs of the development of the "Dash 22" version of the RB 211 which is to be used in the Lockheed TriStar airbus. The engine contract was plucked from the hands of American competitors in 1968, but the penalty of landing such a prestigious order was a fixed quotation which required more than the average amount of guesswork. As it turned out, estimates for the total development cost have soared from £65 million in 1968 to £135 million at 1970 prices, and launching aid of £47 million granted by the government in 1968 is now seen to be entirely inadequate. A measure of the immediate seriousness of the situation is the provision by Rolls-Royce in its accounts of £45 million to offset the predicted loss on the 600 engines to be sold to Lockheed during the next five years.

The Rolls-Royce problem is essentially one of cash shortage, the source of which can be traced back to a change of accounting procedure in the early days of the Spey engine. At that time it became impossible to write off research and development totally against profits without depleting the latter to such an extent that the problems of raising further capital in the money market would become insuperable—about £90 million has been raised by loans and share issues in the last four years alone. The problem was solved by the expedient of charging only a proportion of research and development against profit and entering the remainder as a capital item in the balance sheet, to be written off as profits increased during production. The result was a healthy looking increase in profits from year to year but a hidden strain on cash resources which eventually forced the recent injection of £60 million.

The Industrial Reorganization Corporation (IRC) made a general loan of £10 million to Rolls-Royce earlier this year and several new directors were appointed, including Mr Ian Morrow, who now heads the new four man executive committee. It was Mr Morrow's probings and the financial monitoring by the IRC which revealed the company's critical financial situation and gave the government much of the factual information necessary for assessing the aid to be given. Ironically the IRC will be wound up at a time when its usefulness has been highlighted; it would have been the ideal body to watch over the now considerable public investment in Rolls-Royce, and to assess any future requirements for public money which might arise.

In some ways, Rolls-Royce has been shown to suffer from its own tradition of excellence. For example, some 2,000 new parts used to be produced each year as optional extras for existing designs of engine. The IRC investigations revealed too much self confidence and often a refusal to face the economic facts of life. The Rolls-Royce management was, for example, considering a draft contract with Lockheed which specifically promised carbon fibre reinforced fan blades and it was only at the insistence of Lockheed that a back-up programme of research into titanium blades was started. In the event the break-up of carbon fibre blades under adverse conditions, such as heavy rainstorms, made the titanium research vital, even though the extra weight of titanium blades in turn made other design changes necessary.

The Rolls-Royce situation will certainly have a bearing on the choice which the government has to make soon between the European airbus (the A300B) and the British Aircraft Corporation BAC 3-11, both of which are contenders for government support. If the government chose the BAC 3-11 it would saddle itself with two large items of expenditure: £75 million towards the development of the airframe and about £60 million for the development of the RB 211-61, an improved and lighter version of the "Dash 22". Even the offer of up to £50 million from the West German Government does not necessarily make the A300B project more attractive. General Electric, with one foot already in the door, is likely to win a considerable proportion of the engine orders for the United States, thus denying Rolls-Royce the long uninterrupted production run so badly needed and offered by the BAC 3-11 with its estimated sales of at least 280 aircraft (*Nature*, **228**, 496; 1970.)

The plain fact is that a single private firm does not have the boundless capital resources seemingly needed to support the research and development functions necessary in the aerospace industry, or the resilience to survive unaided the inevitable setbacks. It can be argued that Rolls-Royce channels too little of its effort (about 20 per cent) into bread-and-butter products such as marine engines, but although the non-aero side of the business is expanding well, it could never provide the degree of cushioning required. The continuing injection of government money and the appointment of watchdog directors are probably too close to nationalization to be contemplated by the government, although the aerospace industry in the United States is subsidized extensively and successfully in the guise of defence grants.

It has been suggested that the next logical step would be the formation of a European company with Rolls-Royce as its nucleus and comprising firms like the French SNECMA and Turbomeca and the West German group Motoren und Turbinen Union which are all intimately involved with Rolls-Royce in one or other of the joint ventures.

European Space Research in the Melting Pot

THE role to be played by Europe in space will probably be decided within the next two months. Following the abortive second session of the European Space Conference (ESC) which met in Brussels recently to consider NASA's proposals for a European share in the post-Apollo programme (see *Nature*, **228**, 596; 1970), the next planned step is the report now being prepared by a joint group from ELDO and ESRO on the feasibility of the shuttle proposals put forward by NASA. Moreover, the German delegation to the ESC, supported by the Belgian and French delegations, suggested that the deadline by which interested countries must decide which proposals they are prepared to support (if any) should be December 31.

Like the Concorde project, the question of cooperation in space research is subject to political as well as economic factors. West Germany is particularly enthusiastic about working with NASA on the next generation of spacecraft, while France and Belgium support the idea with some reservations. Britain has refused to make any commitment without a more well-defined proposal from NASA, which at present wants the ESC to commit itself to something like a 10 per cent share in the still undefined cost of a project. Italy, Holland, Sweden and Norway are, like Britain, interested in the proposal but refuse to commit themselves without more information. Denmark is not interested in participating in any post-Apollo programme, and is also withdrawing from ESRO.

On their own, the three firm supporters of participation in NASA's programme could supply only about 8 per cent of the total cost, and would therefore be in a very weak bargaining position when high level policy decisions are made. With the four more reluctant members of the ESC joining in, Europe might be able to provide a sufficient stake in the project for its voice to carry some weight at policy making levels. But even in the United States there is no consensus in favour of the shuttle and there is a possibility that if a majority of the European countries are against participating, the US Congress could be sufficiently influenced to cut back its space budget still further—as yet, no endorsement of any post-Apollo manned space flight programme has been given.

A major source of the present confusion is the differing interpretations placed on NASA's offer to launch satellites for European countries provided that they join the space shuttle programme. Some have interpreted this as implying that NASA will not provide launch facilities for those who do not help with the shuttle; the British view is that American launch facilities will still be available on a basis similar to the arrangements under which the Skynet and Ariel satellites have been launched for Britain by NASA. This is also the reason for Britain's split with Germany and France over the development of the Europa 3 launcher and which caused the British withdrawal from ELDO. On the basis of three failures to send into orbit a European satellite, and Britain's own unhappy record with Black Arrow, the argument that Europe should concentrate on satellite development and leave launch facilities to the Americans is very strong. There is no question that the British Ariel satellites have contributed greatly to scientific knowledge (see page 700), and there are now financial rewards such as the recent contract between the Hughes Aircraft Corporation and the British Aircraft Corporation.

Already the largest supplier of satellites and components in Europe, BAC now has an order book worth roughly $10 million, the recent contract with Hughes for components for the second generation of Intelsat 4 satellites accounting for one tenth of this total. BAC is also a partner in European aerospace consortia, and an interesting situation could arise if the countries in which their partners are based officially join the post-Apollo programme while Britain does not. In spite of the possibility of some political backlash affecting BAC, it would still have a good chance of playing a major role in the development of Europe's share of the programme. Commercially, a continued European space effort on Europa 3 and applications satellites plus a share in the American space effort is the most attractive prospect, but in the face of any extravagant speculation it is salutary to remember that the highly successful Skylark sounding rocket has been both BAC's greatest single money earner in the field of space research and has also provided a wealth of scientific data for relatively little cost.

The political situation is, as ever, extremely complex. Non-cooperation by Britain could be used in Europe as a barrier to the application to join the Common Market, while at the other extreme, complete European participation in the shuttle project might be viewed with alarm in the Pentagon by those who are impressed by the military applications of the new spacecraft.

The future for a cooperative European space effort therefore looks extremely gloomy. Even the proposed merging of ELDO and ESRO to form a European Space Organization dealing with both launchers and satellites seems to be merely an administrative shuffle. But there are two points yet to be decided which could even now result in a combined European partnership working at a high level (rather than merely as glorified sub-contractors) on the next stage of the US space programme. Mr Frederick Corfield, the Minister of Aviation Supply, has made it clear that Britain is quite prepared to examine any concrete proposals made by NASA, and he is at present simply refusing to sign a blank cheque with little say in what the money is spent on.

If any progress in this direction is made, the Swiss have offered to act as hosts to the next meeting of the ESC, and this could make for a more coherent conference than the recent shambles in Brussels, which arose partly because the chairman (traditionally provided by the host country) was an outspoken supporter of the acceptance in full of NASA's proposals, and did not provide the relative degree of impartiality appropriate for his position. The exact position here is far from clear because technically the last meeting of the ESC was adjourned unfinished, and traditionally it should therefore reconvene in the same country.

NEW WORLD

Gas and Germ Warfare Renounced but Lingers On

by our Washington Correspondent

A YEAR ago, on November 25, 1969, President Nixon renounced the first use of chemical or biological weapons by the United States. He said he would submit the Geneva Protocol of 1925 to the Senate for ratification, declared that the United States would confine its biological research to defensive measures and announced that preparations would be made to destroy existing stocks of bacteriological weapons. Lack of evident progress towards these goals in the last twelve months suggests that, like King Richard II, the President is finding the enactment of his dramatic renunciation harder than the recital of it.

The Geneva Protocol, which prohibits the first use in war of gases and bacteriological agents, was not submitted to the Senate until nine months after the President's speech. Further delay has been caused by the reluctance of Administration witnesses to appear before the Senate Foreign Relations Committee, which will now probably be unable to start its hearings on the Protocol until the new Congress meets next year.

Progress has been equally slow on the plans for destroying the present stockpiles of biological weapons. The consensus of two recent accounts*, widely regarded as accurate, is that the biological warfare arsenal at Pine Bluff, Arkansas, still includes stocks of the agents causing tularaemia, Q fever, anthrax and Venezuelan equine encephalitis. There are anti-crop agents causing diseases such as rice blast and wheat rust, and an arsenal of bullets and darts loaded with botulinum toxin. Unlike the deteriorating nerve gas rockets that gave the Army such a disposal problem two months ago, these agents are not, as far as is known, encased in concrete coffins along with explosives. Proteins being denaturable by heat, it would seem that a procedure not greatly more complicated than boiling would suffice to destroy the contents of Pine Bluff. But twelve months after the President's statement, the stockpiles are still there.

So too is Fort Detrick, the Army's chief biological research centre, which was to have been handed over for peaceful uses to the National Institutes of Health. The transfer, approved by government officials at one level, seems now to have been vetoed at another.

Dissension is also apparent within the Administration on the question of classified biological research. At a background briefing in February a "high White House official" seems to have stated that classified research in biological warfare would henceforth be unnecessary. This position has been frankly repudiated by the Army, which makes little effort to deny that it is still conducting classified research and expects to continue doing so. The military view is that certain of the defensive research sanctioned in the President's statement "raises requirements that some of our biological research would have to be classified", an Army spokesman said last week.

The general absence of forward movement in the direction pointed by President Nixon is ascribed by some to skilful delaying tactics by the Army, which is held to be determined not to drop its biological weapons until its hand is forced. In the most open aspect of these affairs, the ratification of the Geneva Protocol, the Administration appears to have given little leadership to those pursuing the new policies it initiated, and it could be that in the more covert ramifications of his decision as well, the President is meeting a certain amount of inertia. But whatever obstructions the Pentagon may have placed in the path of the renunciation of biological weapons, there are less devious reasons for at least some part of the delay.

Destruction of the stockpiles, for example, is not the Army's sole responsibility but must be agreed as to method with the Surgeon-General's Office in the Department of Health, Education and Welfare, with the Office of Science and Technology and with the Department of Agriculture. There have clearly been problems in getting the several agencies to agree on a visibly safe method for disposing of the stockpiles. The interagency coordination is still not yet finished but agreement is now very close, a member of the Office of Science and Technology said last week. "The plan has not yet been finalized but we know pretty much how it is to be done."

On the vexed issue of classified research the same official did not deny that some secret research would go on, but stressed that it would be kept to a minimum. "I'm sure if we do have any classified biological research it will be only if it is judged absolutely necessary to protect the national security," he said. This position seems the likely compromise to the present dispute now going on within the Administration between those who argue that defensive research on matters such as immunization procedures can be conducted openly and those who hold that secret research is necessary. Proponents of classified work see the need to develop offensive strains of microorganisms—research which it is generally agreed should be classified—in order both to develop protection against them and to analyse strains gathered abroad by intelligence agencies.

Another sign of the Army's reluctance to follow President Nixon's directive is said to be the $23·2 million requested for biological warfare research in the present financial year, which in fact exceeds the $21·9 million spent last year. Army spokesmen reply that the budget request for the present financial year—which Congress has already reduced to an authorized maximum of $21·1 million and may cut still further in the appropriation process—was made before the President's November 25 speech. The budget request for 1971–72 is in fact as low as $10 million, a more than 50 per cent reduction. Administration officials explain that this year's authorization for biological warfare research is nearly as large as last year's expenditure because of the heavy costs incurred in winding up installations and paying termination benefits to staff.

Financial rather than political reasons may also underlie in part the apparent failure of the National Institutes of Health to capture the nearby Fort Detrick. The NIH has proposed to convert the unique facilities

* Seymour M. Hersh, *Washington Post*, 20.x.70; Robert M. Smith, *New York Times*, 10.xi.70.

of the centre to research on slow and hot virus diseases. The proposals would have provided employment for 700 scientists and technicians (some of whom could have been supplied from the present staff of Fort Detrick) with annual running costs of $15 million. Several organizations put in bids to take Fort Detrick off the Army's hands; "Ours," said an NIH official last week, "was considered especially propitious by higher echelons (of the Administration), but they weren't high enough." Although no final decision is known to have been made, it seems that a memorandum has been sent from the Office of Management and Budget to the Secretary of Health, Education and Welfare to the effect that the NIH proposal for Fort Detrick's future is too expensive.

Does the Administration's tolerance of these various impediments and delays betoken a shift of position on biological warfare, or merely preoccupation with larger issues? Apart from the troubles facing the official interpretation of the Geneva Protocol—President Nixon seems not to have been properly briefed on the extent of the likely opposition—the factors behind the renunciation appear not to have changed. The decision was certainly helped along by accidents such as the 6,000 sheep gassed at Dugway in March 1968, but it rested substantially on an Administration review of current programmes in biological warfare research. The review, the first for fifteen years, is said to have appalled many officials by the direction that the research was taking. Papers were presented giving the President his options and he made his choice. Unfortunately, in one point of view, the issue of using tear gas and herbicides in war was considered in conjunction with the bacteriological and other forms of biological warfare. The military as well as legal grounds for using these two types of agent in Vietnam are not particularly strong and had they been judged on their own merits the decision might have been to drop them. Considered along with other biological agents there was a natural pressure towards compromise, the terms of which could have been that bacteriological agents would be dropped in return for reservations to the Geneva Protocol regarding incapacitating gases and herbicides.

Thus or otherwise, in the letter transmitting the Geneva Protocol to the Senate Secretary of State William P. Rogers gave it as his understanding that the Protocol does not cover incapacitating gases and herbicides. The Senate Foreign Relations Committee has not yet been able to hold hearings on the Protocol —the earliest date the Secretary of State and Joint Chiefs of Staff, the customary first witnesses, can offer is mid-December—and the Protocol will probably not be reported to the floor of the Senate until early next year. There it seems set for a bruising debate. Senator Nelson has already introduced a contrary understanding to the effect that the Protocol does prohibit the use of chemical herbicides in war. In as far as this is just a legal issue, the Senate is likely to be influenced by the clear-cut 80 to 3 vote by which the United Nations last December asserted that tear gas and herbicides are covered by the treaty. The debate seems certain to bring up the divisive issues of the Vietnam war, although there could be some kind of coalition between hawkish Senators concerned with the effect of the reservations on arms control and those with doveish positions on Vietnam and the use of herbicides there.

President Nixon has not publicly committed himself to an interpretation of the Geneva Protocol and it looks as if the Secretary of State's letter of transmittal represents the Administration's final word, beyond which the matter is to lie in the lap of the Senate. Opponents of chemical and biological warfare take comfort in the fact that the statement of the Administration's views in the form of an "understanding" is less entrenched a position than a declaration or formal reservation would have been. But a formal reservation could force other signatories of the treaty to agree with or dissent from the US position, whereas an "understanding" presents a sufficiently low profile to be overlooked.

CANADA

Science Council Reveals All

by our Physical Science Editor

A THOUGHTFUL open letter has been written to the Canadian physics community in the shape of the Canadian Association of Physicists by the Science Council of Canada, and is published in this month's edition of the association's journal *Physics in Canada*. What the letter boils down to is that there will have to be some heart-searching among scientists in Canada, and the greater the number of people who take part in the debate the better the chances of everybody being happy with the results.

Canada, of course, is well known to be among the most introspective of nations when it comes to the management of science, yet for all the talk Canadian science is only in average shape. The chief reason is that expenditure on science is not rising at anything like the rate that people were hoping only a year or two ago. The target date for the attainment of an R&D expenditure running at the comparatively modest level of 2 per cent of the GNP is receding like a mirage. Last year the Science Council was recommending that 1973 be the target; now it seems unlikely to be reached even by 1975.

What this means is that belts will have to remain tightened, especially in what are called the basic sciences. It is now fairly well recognized that the more serious problems afflicting Canadian science are in what has become a no-man's-land between university and industrial research. With these problems in mind, the Science Council has decided to lay its cards on the table, setting out its views in an open letter to the physicists rather than in a formal report. The reasonable hope is that this will better stimulate the physics community to express its reactions. The letter ranges over all aspects of science funding where physicists are involved, and provides some new information about the standing of the Science Council on some particular questions. In Canada as everywhere else the support that should be given to what is called big science is the most hotly debated, and the open letter raises some questions which up to now have frequently been swept under the carpet. "The existence of a single big science project will determine the interests of the participating scientists, often for a decade or longer. The problem is potentially much more significant in the case of a scientific discipline which has invested a large share of its total resources in big science. Are big science projects self-perpetuating? Do the participants have many real options for significantly changing the course of their discipline?" Later in the letter the Science Council asks whether investment in high energy physics would be a godsend or a millstone for

Canadian physics. Big science projects generate a momentum of their own.

Debate along these lines has already weighed heavily in the Science Council's decision that it would be better for Canada that money be spent on a 50 per cent share of the Carnegie Institution's project for a 200-inch optical telescope in Chile than in buying a share in the 200 GeV accelerator at Batavia. (Since that decision was taken, however, a question mark must have arisen over the Carnegie project following the change of government in Chile.)

The reasons for not supporting the Batavia project are set out for the first time in the letter. In the words of the Science Council, the high energy physics group in Canada is "small, competent, well-coordinated and, in contrast to the astronomers, is thoroughly cohesive with no sign of internal stresses and strains", but the decision went against them because this is a field of limited interest, the investment would probably not guarantee a proportional share of time on the machine, and there is even less money to be made in high energy physics than in astronomy. In passing, the council mentions that it is still unhappy with the way that astronomy is being run in Canada; in particular the council is disturbed that the desirable coordination of Canadian astronomy, which was to have taken place under the NRC, has not yet materialized.

In the course of its discussion of the worth of big science, the council also has some home truths to tell about the desirability of internationally shared facilities. "The international nature of a proposed venture is not," the council bluntly states, "a strong selling point." That is something to be taken account of in Europe, where pressure for more international projects is to be expected on political grounds. International ventures, the council says, are hard to assess, slow in gaining approval, are not usually productive unless there is a strong home base, and lead to minority partners being dependent on the good graces of the major partner and to uncertainties about the distribution of any profits that may accrue.

Much of the remainder of the letter is rightly taken up with a discussion of how to break down the barriers between what the letter calls the four solitudes in Canada—government, industry, university and the financial community. The best way to let in the fresh air the council suggests is to have some major national programmes that would cut across the boundaries. But there are things that can be done even before these rallying calls are sounded:

- Government departments and agencies should contract out more of their R&D to the universities and industry.
- The government should use its purchasing powers to encourage innovation and better design.
- Individual scientists in government, industry and the universities should try to become aware of each other's problems. There should be applied institutes attached to the universities, and industry could subcontract some of its research work to the universities.
- There should be more support for postdoctoral fellows to work in industry and for industrial scientists to spend sabbaticals in the universities.

What part does the Science Council of Canada itself want to play in all this? The letter suggests that the council should act as arbiter between competing claims on the funds. Only when a new area of science arises with nobody to speak for it should the council enter the fray as a participant. Usually normal competition between scientists for funds will automatically lead to the best use of resources, but it is possible for monopolies to develop. For this reason there needs to be assessment from outside. It is good that the Science Council considers that the scientific societies, such as the Canadian Association of Physicists, should play an active part in helping to decide where the money goes.

TECHNOLOGY

No Longer Glamour Industries

by our Cambridge Correspondent

In the last ten years a new species of industry has emerged. Its rise and fall are worth careful study by anyone at least marginally concerned with science and government.

Along Route 128, a frantic highway which gives Boston a wide berth, has grown up a large number of moderately sized businesses selling, in the main, computer software, electronic components and other instrumentation necessary to a transistorized military. The Boston environment, with Harvard, MIT and a small number of defence-oriented laboratories, was undoubtedly the reason this phenomenon flowered most extensively in Massachusetts. Indeed, many firms were founded by research scientists from these institutions who found their salary not commensurate with the value of the things they were discovering. A few have become household words. A Route 128 industry can immediately be spotted by its discreet facade among shrubs and trees. Everything carefully landscaped, no vulgar neon signs or anything which obtrudes on the passing driver's vision. The main asset of these industries has been their brain-power—they are rich in PhDs and computers and employ relatively few manual labourers. Nevertheless, between them they constitute a major ingredient of the military–industrial complex, solving space age problems and cornering a huge share of the Defense Department's research budget. Much civilian work is also done, but it is the sustenance of the military money which has really made things tick.

Suddenly Route 128 has found itself synonymous no longer with a swinging young industry but with chronic financial troubles and huge lay-offs. Layings-off are, of course, nothing unusual in the volatile aerospace industry, but this is something different in that it is whole research teams that are being broken up. The most recent figures suggest that something like 2,000 highly qualified engineers and scientists have recently become unemployed in Massachusetts. This excludes the preposterous mishandling a few months ago of the Cambridge NASA Electronics Center which involved the tearing down of a sizable part of Cambridge to produce hideous laboratories which were promptly declared redundant by NASA once staffing was well under way. All in all, it has been a very bad year for technology based industry.

Some glib political commentators of which Boston has a surfeit see Nixon taking revenge on the liberal Senators Kennedy and Brooke who have not been of much help to the President in the last two years. A much more reasonable explanation lies in the harsh economic fact of a tightly restrained defence budget in an inflationary period and the harsh technical fact of a lack of impetus in a lot of science today.

It was inevitable that with a relatively stationary defence budget a lot of suffering would befall the less monolithic parts of the military–industrial establishment and to this extent Route 128 industries have been very much the jam on the bread-and-butter. Government contracts simply have just not materialized, nor is there any good sign for the future. Ironically, many of the employees now being laid off would be counted amongst the liberals who believe defence spending should be held, ABM deployment deferred and so on. The first tightening of the belt had some salutary effects. Ten years of unbridled affluence had left many firms much too fat, and early reductions in staffing removed some of the portliness. One personnel officer not afflicted by these troubles noted that the first ten applicants for a computer programming post had all been laid off, all described themselves as programmers and yet had minimal skills in that subject, for which they appeared to have been paid extraordinarily well.

But now the dismissals are cutting right into the research teams and leaving many highly qualified scientists and engineers jobless. One of the less palatable but more plausible explanations for this rapidly ebbing tide is simply that some branches of science are coming up hard against the stops. There just does appear to be less to get excited about in aerospace and defence research. Systems are big, roles small and objectives for each group limited. A certain dynamic seems to be lacking and perhaps this is because of an awareness that there are no breakthroughs just around the corner. Scientists and engineers don't retain their identity long as small cogs in big wheels and this sort of environment may have stifled the ingenuity of many. Certainly a fairly common feature today is the unhappy scientist—even unhappy if earning $30,000 and not in danger of being laid off. It may well be that after ten years of intensive vocational training many PhDs find that the frontiers to which they have been remorsely led are not anything like as glittering and amenable to being pushed back as they had fondly imagined.

With too many men chasing too few jobs and insufficient intellectual challenge, colleges ought to have seen the writing on the wall. Colleges may have an important role to play in dissuading entrants from becoming highly proficient in fields where their expertise is no longer wanted. The wisdom, for instance, of colossal courses on solid state physics, atomic and nuclear physics and high energy physics as the staple diet of the complete physicist is increasingly being brought into question and the more articulate students are saying in increasing numbers that they resent the time spent in this work. There are even very vague rumblings amongst the activists against what they regard as the deceptive practice of professors teaching vocational courses without revealing the lack of job prospects. Exponential growth is clearly at an end.

DETERGENTS

Good News for Soap

from our New York Correspondent

A TOTAL ban on the sale of a whole class of detergents, the first in the United States, will go into effect in Suffolk County, Long Island, on March 1, 1971. The ban is directed against detergents containing alkyl-benzene sulphonates, alcohol sulphates, and all methylene blue active materials except those used in automatic dishwashers, shampoo and toothpaste.

The ban has not satisfied all ecologists, however, because it fails to discriminate against phosphates, contained in nearly all detergents, and held to blame for the eutrophication of lakes and rivers.

The alkyl-benzene sulphonates, or ABSs as they are known, have been banned in Suffolk County in essence because they are not degraded by micro-organisms in anaerobic conditions. Suffolk County has no sewer system and nine tenths of its sewage is treated in cesspools, which are anaerobic.

Although phosphates are the generally more pernicious ingredients in detergents, the ABSs have been causing a more obvious problem in Suffolk County for the past fifteen years, and the county Board of Health had warned that continued widespread use of detergents would seriously pollute the county's underground water supply. The problem is primarily an aesthetic one in that the ABSs cause foaming, discoloration, and bitterness when they are present in the drinking water.

Nearly two hundred brands of detergents will be affected by the new law. The Soap and Detergent Association, which estimates total detergent sales in the county at $6 million annually, claims that with only minimal sewage treatment, present detergents would be totally biodegradable and that much of the present problem in Suffolk is probably due to the continued presence of totally nonbiodegradable ABSs that were used in detergents up to 1957. The Association argues that the ban is attacking only the effect, not the cause, of the pollution problem. What is needed, it says, is sewage treatment. The Suffolk County Water Authority agrees that the county is twenty years behind the times in sewage treatment and that the new law is merely a stop-gap measure, but claims it is necessary nevertheless.

The first referendum to install sewers in the county was passed only last year. The $275 million project will cover the south-west section of the county, and will take ten to fifteen years to complete. In the meantime, the population is growing rapidly but developers and homeowners are still unwilling to install a vastly more expensive sewer system as long as cesspools are adequate for the job. And they probably will remain so for most of the county once detergents are no longer present to contaminate the public water supply.

Although Suffolk has taken a first step in its ban on detergent sales, this is unlikely to mean a permanent end to the use of detergents in the county. In spite of the Soap and Detergent Association's outcry against the ban, it seems likely that the detergent manufacturers will quickly overcome the problem by the simple expedient of eliminating the nonbiodegradable ingredients from their products. The ingredients are not essential to the basic cleaning function of the detergents and, since detergents are simply a mixture of ingredients, it would be a fairly simple task to merely leave out the questionable compounds, still solving Suffolk's immediate problem, but continuing the phosphate controversy.

It now appears, however, that New York City itself may take the next step next spring by banning the sale of phosphate-containing detergents. The Environmental Protection Administration is considering this possibility, a situation that the detergent manufacturers will find considerably harder to resolve.

NEWS AND VIEWS

Factor Saga Continues

FINDING factors which control the transcription of RNA continues apace, for on page 748 of this issue of *Nature*, Travers reports the discovery of another such protein, which in the Hellenophilic tradition of Harvard he duly names psi, succeeding the sigma and rho factors previously discovered there. Psi enables RNA polymerase to transcribe specifically the genes of *Escherichia coli* which code for ribosomal RNA. This is perhaps the most significant factor report yet, for it makes the whole jigsaw of hitherto unrelated observations on RNA synthesis fall into place to suggest parallel mechanisms for the central control of messenger and ribosomal RNA synthesis in bacteria.

A curious feature of the systems used to transcribe RNA *in vitro* is that no ribosomal RNA seems to be synthesized. But *in vivo*, although the cistrons coding for ribosomal RNA comprise only about 0·3 per cent of the genome, the synthesis of ribosomal RNA can account for up to forty per cent of total RNA synthesis. (This implies that the overall rate of synthesis per cistron must be much greater for ribosomal than for messenger RNA.)

The absence of ribosomal RNA synthesis in the *in vitro* systems, Travers argues, means that they must lack some factor(s) responsible for directing the polymerase to transcribe the rRNA cistrons *in vivo*. He reports that a crude ribosome-free extract of *E. coli* contains the expected activity, which stimulates the synthesis of rRNA *in vitro* at least a hundred-fold. Almost a third of the RNA synthesized in the presence of the psi extract is ribosomal (although there is also an equivalent increase in the synthesis of other, unidentified RNA species).

Synthesis of ribosomal RNA in *E. coli* cells may at first seem quite unrelated to the replication of RNA which takes place when the bacterial cell is infected with an RNA bacteriophage, but Travers shows that there is a close, if not intimate, relationship between these two processes. For some time there have been indications that they may be connected, because some RNA phages inhibit the synthesis of ribosomal RNA during their infectious cycle.

RNA phages are replicated by a special enzyme, RNA replicase, which is found only in the phage-infected cells. The replicase of phage Qβ is an interesting protein which, as Kamen and Kondo, Gallerani and Weissmann showed two weeks ago (*Nature*, **228**, 525 and 527; 1970), consists of four different polypeptides. Only one of these (molecular weight 65,000) is coded by the phage itself; the remaining three (molecular weights 75,000, 45,000 and 35,000) are coded by the host bacterium and must presumably associate with the phage-specified polypeptide after infection to create the replicase enzyme.

Travers was spared the tedium of isolating psi from the crude extract because of the finding that, when added to the *in vitro* transcribing system, the two smaller subunits of Qβ replicase exercise an activity functionally identical with that of the extract from *E. coli*. (Travers does not yet know whether either or both subunits comprise the active fraction.) This explains the relationship between ribosomal RNA synthesis and RNA phage replication, which presumably compete with one another for psi.

The advantage to the cell—in selective terms—of sharing psi between these two functions is obscure, at best, but promises to prove interesting. One possibility is that because phage Qβ contains only three or four genes, it cannot itself code for a sufficiently complex replicating enzyme, and has therefore had to resort to making use of bacterial polypeptides. If psi really is either or both of the Qβ replicase subunits, the mechanisms which it uses to participate both in the transcription of RNA from DNA and in the replication of RNA should prove a fruitful field for study.

What does psi do in uninfected bacterial cells? Although it controls the transcription of specific RNA species, it is not another sigma factor. Sigma was the first transcription factor found, when RNA polymerase was separated into its subunits; the core enzyme, consisting of four polypeptide chains, synthesizes the phosphodiester bonds of RNA, and the single polypeptide chain sigma restricts the core enzyme to initiating transcription at certain genes. Transcription of some bacteriophages during infection is controlled by production of successive phage-coded sigma-like factors which replace the host sigma and direct the host core enzyme to transcribe specific phage genes.

It is an open question whether the action of sigma is to bind to core enzyme and indirectly influence the promoters recognized, or whether it directly recognizes certain sequences in DNA. It is unlikely, however, that psi works by the latter mechanism; its apparent involvement with the Qβ replicase suggests that it probably recognizes proteins rather than nucleic acids.

There seems to be only one sigma factor in uninfected bacterial cells, and psi acts on the complete RNA polymerase, which consists of both core enzyme and sigma factor. This action is strongly reminiscent of

another recently found control factor. The lactose and other inducible operons of *E. coli* cannot be expressed completely unless cyclic AMP is present in the cell. The effect of the nucleotide is mediated by a protein factor (termed CAP), which seems to act on the initiation of transcription by RNA polymerase at the promoter of the operon. This factor too seems to act on the complete enzyme.

The similarity between psi and CAP also extends to their interaction with nucleotides. Although no one has suggested that psi interacts with the all pervasive cyclic AMP, Travers throws out a tantalizing hint that it may interact with ppGpp, a guanosine tetraphosphate. The implications of this interaction are exciting. In normal (stringent) *E. coli* cells, starvation for essential amino-acids blocks not only protein synthesis but also the synthesis of ribosomal and transfer RNA, although messenger RNA synthesis seems to continue. The effect is abolished in a (relaxed) mutant strain, in which all RNA synthesis continues irrespective of the state of protein synthesis. Comparison of normal *E. coli* with the mutant suggests that the effect is mediated by ppGpp, which accumulates in stringent cells starved for essential amino-acids.

Psi is a good candidate for the controlling protein which must mediate the effect of ppGpp on stable RNA synthesis. Just as CAP is a protein which when activated by cyclic AMP acts as a general control element which is required for the complete expression of many bacterial operons, psi may be a general control element which, unless inactivated by ppGpp, keeps RNA polymerase transcribing ribosomal RNA, and perhaps also transfer RNA genes. (The action of psi may also explain the greater overall rate of expression of ribosomal RNA genes.) This response to small molecules would allow bacteria independently to adjust the synthesis of different classes of RNA to any changes in the environment.

PROTEINS

Sequence Sequel

from our Molecular Biology Correspondent

FOLLOWING on Jörnvall's sequence of the 374 residues of liver alcohol dehydrogenase, about which I wrote a few weeks ago, comes another *tour de force* of protein chemistry. With five of his colleagues, E. L. Smith reports an all but complete sequence for bovine glutamate dehydrogenase (Smith *et al.*, *Proc. US Nat. Acad. Sci.*, **67**, 724; 1970). In its native state, at low concentration, this enzyme is a hexamer, which undergoes an indefinite concentration-dependent association to form long rods. The monomer has a considerably greater molecular weight than those of the other known NAD-dependent dehydrogenases: according to the new sequence there are 506 residues in the chain, and the corresponding weight of 56,000 agrees with physically measured values.

The sequence is regarded as tentative, because at two points overlap has not yet been achieved, and here and there a few residues have not been fully ordered. The first conclusion from this work is that, because the sequence is unique, the subunits are identical. Taken with the other known sequences for dehydrogenases of different quaternary structure, this seems to establish a clear pattern. There is some polarization of composition, with clusters of charged and hydrophobic residues at opposite ends of the chain. Since there is little perceptible homology of sequence between liver alcohol dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase, which are much closer together in size, it is not surprising that a comparison, using a computer search, of the glutamate dehydrogenase sequence with that of the latter also shows scant similarity. A strange exception occurs at one point in the chain, however: a run of twelve residues—94 to 105 in glutamate dehydrogenase, and 209–220 in glyceraldehyde 3-phosphate dehydrogenase—are identical in six positions, and have conservative substitutions, with single-base mutations in the rest. Moreover, both proteins contain enzymatically essential lysines, and Smith *et al.* have identified the one in their enzyme by way of its interaction with pyridoxal phosphate. This reagent can be used as a covalent label, for reduction of the labile Schiff base adduct leaves it irreversibly bound. The vital residue turns out to be lys-97, which is in the conserved peptide sequence. Glyceraldehyde 3-phosphate dehydrogenase also contains a lysine in the corresponding position of this segment, but the only lysine known to be functionally vital was identified by Harris and Perham as residue 183. It is not obvious what one is to make of this fugitive hint of common gene ancestry.

Smith *et al.* have in addition identified what seems to be an activity-controlling ligand binding site. It is known that modification of one tyrosine residue leads to loss of response towards the non-competitive inhibitor, GTP. The tyrosine, labelled with a 3-nitro group, has been identified as residue 412.

It is worth noting that subunit enzymes with identical chains now seem to be very common. An interesting example is the enzyme, thiolase, in which thiols operate as acyl acceptors in catalysis. The molecular weight is 170,000, breaking down to 42,000 in a denaturing medium. Gehring and Harris (*Europ. J. Biochem.*, **16**, 487; 1970) have ascertained that there is only one N-terminal tripeptide sequence, and that the number of tryptic peptides corresponds to the known content of lysine and arginine, if there are four chains. It is reasonable to infer that the subunits are identical. The protein is inactivated by reaction with iodoacetamide at the level of one molecule per chain. In their second article (*ibid.*, 492) Gehring and Harris report how they isolated peptides from the protein so modified, and showed that the substituent is an o-thiol, which is in a unique sequence at least to the extent of twenty-six residues. It is the same thiol groups which are acetylated when fragments are prepared from the enzyme-substrate complex, and are therefore marked as the catalytic residues.

VIRUSES

Doing without Cells

from our Cell Biology Correspondent

FOR the past several years the ribonucleoprotein particles which contain vaccinia virus DNA, and

which can be found in the cytoplasm of cells infected with this virus, have usually been refered to as "DNA factories". They have now been rechristened in the Latin by Dahl and Kates (*Virology*, **42**, 453; 1970), who apparently believe that virosomes is a better name because it does not "create a misleading impression concerning their composition and function". Be that as it may, Dahl and Kates have succeeded in isolating these complexes from infected HeLa cells. The virosomes are heterogeneous in size and density and the various classes of virosomes are related by some complex precursor product relationship which has yet to be fully resolved. What is more interesting is that the dense virosomes isolated three hours after infection contain nearly all the newly replicated viral DNA and an endogenous RNA polymerase activity (Dahl and Kates, *ibid.*, 463). *In vitro* the isolated virosomes can synthesize RNA which competes in hybridization experiments with both "early" and "late" viral RNAs made *in vivo*.

To convince themselves of the reality of "early" and "late" viral RNAs in infected cells, Dahl and Kates transcribed *in vitro* purified viral DNA with RNA polymerase from *E. coli*. They then analysed the sequences present in RNA extracted from infected cells at early and late times by competition hybridization experiments with the RNA made *in vitro* by the bacterial enzyme. The RNA isolated from cells six hours after infection competed with about 97 per cent of the RNA made *in vitro*. By contrast the RNA extracted from cells 1·5 hours after infection only competed with about 40 per cent of the *in vitro* RNA. Apart from proving that sequences are present in "late" RNA which are not present in "early" RNA, this experiment indicates that *E. coli* RNA polymerase transcribes one and the same strand of vaccinia DNA as is transcribed in cells. Green and Westphal have respectively shown that *E. coli* polymerase asymmetrically transcribes the correct strand of adenovirus 2 and SV40 DNAs. Presumably the bacterial enzyme readily recognizes the promoter sequences in these viral DNAs, which hints that such sequences may be universally similar.

In the same issue of *Virology* (page 508), Bukrinskaya, Bykovsky and Zhdanov report that a ribonucleoprotein complex isolated from Sendai virus particles associates with ribosomes from Ehrlich ascites or chick embryo cells; and, in a suitable cell-free system, it stimulates a five-fold increase in the incorporation of leucine and phenylalanine. This stimulation is inhibited by puromycin, the omission of ATP or the S-100 fraction, and the extent of the incorporation depends on the amount of ribonucleoprotein complex added. Bukrinskaya *et al.* suggest, therefore, that they are detecting protein synthesis directed by Sendai RNA. They further claim that because the incubation mixture after thirty minutes contains an RNA polymerase activity, which is insensitive to actinomycin D, one of the proteins being made is viral RNA polymerase. A full report of these experiments will be awaited with interest.

Jockusch, Bull and Kaesberg (*ibid.*, 401) report that an *E. coli* cell-free system programmed with bacteriophage Qβ RNA makes three of the four proteins which are found in extracts of infected *E. coli*. This result, therefore, leaves open the question of whether the Qβ genome contains four rather than three cistrons, as does the characterization of Qβ mutants reported by Horiuchi and Matsuhashi (*ibid.*, 49). They find that the twenty-two amber mutants which they have tested fit into only three complementation groups.

BACTERIOPHAGE

Heads and Tails

from our Cell Biology Correspondent

THE assembly *in vitro* of such macromolecular complexes as bacteriophage R17, tobacco mosaic virus and ribosomes of *E. coli* from purified preparations of nucleic acids and proteins leaves little doubt about the importance of self assembly mechanisms in the genesis of viruses and cell organelles. But such observations can belie the amount of genetic information which may be required for the synthesis of comparatively simple macromolecular structures, for work with the DNA bacteriophages, such as the T phages and lambda phage, indicates that the number of genes implicated in the morphogenesis of these phage particles far exceeds the number of genes which specify molecules actually found in the particles.

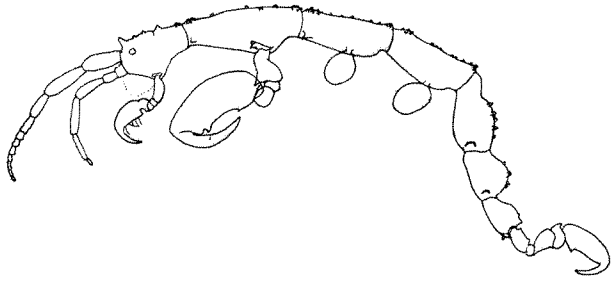
Bacteriophage lambda, for example, is by any standards a simple structure; each particle consists of a head, the phage DNA and associated internal proteins enclosed in a roughly spherical protein coat, and a tail, an aggregate of protein. It is perhaps surprising therefore that genetic analyses of the lambda chromosome have implicated at least eighteen genes in the production of heads and tails, especially since Siminovitch's group (*Virology*, **42**, 375, 390; 1970) have extended earlier work and proved that only three species of proteins account for at least 95 per cent of the total protein in each phage particle.

Buchwald, Steed-Glaister and Siminovitch, using two amber mutants, have isolated pure, empty heads which sediment at 114S and have a molecular weight of 18×10^6 and tails which sediment at 43S and have a molecular weight of $6{\cdot}1 \times 10^6$. These two components account for about 75 per cent of the protein in the phages. Buchwald, Murialdo and Siminovitch have analysed on SDS polyacrylamide gels the proteins of dissociated heads, tails and whole phage. The head protein has a molecular weight of 37,500, the tail protein has a molecular weight of 32,500; and a protein present in phage, but not in heads or tails, has a molecular weight of 11,000. These three proteins account for 57, 19 and 19 per cent respectively of the total phage protein. Minor protein components no doubt account for the other 4–5 per cent—three to six minor proteins can be detected by electrophoresing dissociated whole phage.

Genetic analyses reported by others and now confirmed by Buchwald *et al.* show that the head protein is coded by gene *E* while gene *V* specifies the tail protein. The gene which specifies the third major protein, that with a molecular weight of 11,000, has yet to be identified and the same is true of the genes which specify the minor protein components. If there are as many as six minor proteins specified by the phage, the roles of nine genes will be accounted for; but what do the other nine genes implicated in the morphogenesis of the phage do? Some at least may well specify proteins required for the assembly of the phage but not components of the particles. If that is the case it will be extremely difficult to define precisely the function of these putative proteins, but their existence should caution against over-emphasis of the role of molecular self assembly.

INVERTEBRATES

North Pacific Catch



This crustacean, *Caprella pustulata*, is one of six new species described, together with a new genus, *Pseudoliropus*, in an account of the Caprellidae of the American North Pacific (Laubitz, D. R., *Publications in Biological Oceanography* No. 1, National Museums of Canada; 1970). The animal shown here is a male *C. pustulata*, and it is 7·9 mm long.

RIBOSOMES

Phosphorylated for Storage

from our Nucleic Acids Correspondent

A STORAGE function has been suggested for ribosomes which are phosphorylated and apparently inactive. D. Khabat has made this suggestion after extensive investigations of the occurrence and properties of phosphorylated ribosomal proteins from rabbit reticulocytes (*Biochemistry*, **9**, 4160; 1970).

Ribosomes from this source have the particular advantage that the phosphorylation of the proteins can be studied by incubating cell suspensions with ^{32}P-orthophosphate, in the absence of any *de novo* synthesis of ribosomal RNA. Any other residual nucleic acid synthesis within the cells can be blocked by the addition of actinomycin and the bulk of the label is incorporated into the ribosomal protein fraction. Acid hydrolysis of the ribosomes yields phosphoserine and phosphothreonine.

Khabat found that about 40 per cent of the phosphorylation was associated with as many as ten loosely bound proteins, associated with polysomes, single ribosomes and ribosomal subunits. These can be removed by thoroughly washing the ribosomes. A further component, which may be GTP but is certainly not a protein, is specifically associated with the polysome fraction, and can be released by dissociating this fraction. He detected at least three *bona fide* phosphorylated ribosomal proteins among the remaining material, by electrophoresis through polyacrylamide gels containing sodium dodecyl sulphate.

One of these proteins, present in the larger subunit, is only found phosphorylated in single ribosomes, although the corresponding non-phosphorylated component is present in the other ribosomal fractions. The molecular weight of this protein is estimated to be about 21,000. A further phosphoprotein, with a molecular weight of about 70,000, is specifically associated with the smaller subunit, but is not found in single ribosomes or polysomes (or in the supernatant fraction) and might well be identifiable with some type of factor, particularly because it can be removed by washing the subunits with buffers of high ionic strength. A further phosphorylated protein, of molecular weight 33,000, is also present in the smaller subunit, and is present in all the ribosomal fractions.

Although ideas about the role(s) of such phosphorylation are very unclear, Khabat makes a salient observation about the kinetics of phosphorylation of the protein from the large ribosomal subunit. It seems that the turnover of the phosphate is comparatively slow, and therefore such phosphorylated single ribosomes do not participate in the usual subunit–polysome cycle of protein synthesis. He therefore suggests that these are inactive ribosomes, and that they may have a storage function. It seems unlikely that reticulocyte ribosomes are unique in this respect; Khabat also reports the occurrence of phosphoproteins in the ribosomes of various chick embryo tissues, promising further information later.

BACTERIA

Group with Obscure Function

from our Nucleic Acids Correspondent

THE little single stranded DNA bacteriophage φX174 continues to occupy a place in the hearts and minds of many molecular biologists, and is particularly associated with the untiring efforts of Sinsheimer and his colleagues, who have used it as a tool to examine many of the problems of DNA replication. Recently, Razin, Sedat and Sinsheimer have illuminated the abstruse but important problem of the methylation of φX174 DNA (*J. Mol. Biol.*, **53**, 251; 1970).

Although it is widely believed that the introduction of methyl groups into DNA *in vivo* must have some major significance, direct support for this article of faith is regrettably sparse. An exception is the likely relationship between methylation and host-controlled modification or restriction. (DNA from *Escherichia coli* strains K12 or B is "modified" in different ways, and if introduced into the other cell type is thereby recognized as "foreign" and degraded–restricted.) Razin *et al.*, however, studied the methylation occurring during growth of the phage in a C strain of *E. coli*. This host does not display modification or restriction, ruling out in this case any link between methylation and these phenomena. They used ^{14}C-methionine to label specifically the methyl groups introduced, with the additional precaution of the strain being a purine auxotroph, so that growth in the presence of adenosine depressed background labelling of the purine rings. Hydrolysis of the phage DNA with trifluoroacetic acid yielded principally radioactive 5-methylcytosine, although a far from negligible amount of thymine was also inexplicably labelled.

They determined the amount of 5-methylcytosine present in the DNA, and conclusively showed by four different methods that there is likely to be one methyl group in each DNA molecule. This suggests that here, as with methylation of RNAs, a specific site within the DNA is subjected to methylation. Additional support for this assumption was afforded by the finding that after depurination of the DNA with diphenylamine and formic acid, fractionation of the pyrimidine isopliths by chromatography on DEAE-cellulose yields a single radioactive dinucleotide. This was identified as either m^{5}C-T or T-m^{5}C, suggesting that the methylation occurs in one or other of the sequences pu-m^{5}C-T-pu or pu-T-m^{5}C-pu. The magical function of this solitary methyl group sadly remains unresolved.

In the same issue of the *Journal of Molecular Biology* (**53**, 205; 1970), an equally esoteric and important

study is described, concerning the way in which colicins act on bacterial cells. These substances are proteins (isolated from so-called colicinogenic strains of *E. coli*) which have powerful antibiotic activity against sensitive strains. They are singular in being adsorbed into specific sites on the membranes of sensitive cells, but not penetrating into the cells. Such sites are only present in sensitive strains. Senior, Kwasniak and Holland have studied the colicin E3 (molecular weight 60,000) which inhibits protein synthesis. In cells exposed to E3, protein biosynthesis is arrested after about five minutes, and is paralleled by the appearance of polyribosomes which dissociate into ribosomal subunits *in vitro* in 1 mM Mg^{2+}. 30S ribosomal subunits isolated from cells treated with E3 are correspondingly deficient in protein synthesis *in vitro*, and Senior *et al.* suggest that the lesion causing both the inhibition of *in vivo* protein synthesis and the curious instability of the polysomes is a modification of the 30S subunits. They show that this can occur whether or not the 30S subunits are actively engaged in protein synthesis. Further study of the mechanism whereby attachment of the colicin to the surface of a cell can cause such a modification of the ribosomal subunits will certainly be of great importance in explaining the means of communication between the cell membrane and the interior.

X-RAY ASTRONOMY

Radiation and Discrete Sources

by our Cosmology Correspondent

THE first of the new informal specialist discussion groups arranged by the Royal Astronomical Society to precede the regular monthly meetings was held on November 13. Topics for discussion were the isotropic diffuse background radiation, pulsar radiation and discrete X-ray sources. The meeting was an undoubted success, confirming the belief that small meetings of experts in one field are more profitable than larger meetings covering diverse topics each of which is understood only by a minority of the audience.

Dr M. Longair (University of Cambridge) gave his own interpretation of the shape of the spectrum of high energy background photons. This follows a power law with index roughly 1·5 for energies below 50 keV and 2·2 for energies above 50 keV but below 1 MeV. The few available data at very low and very high energies deviate from this simple behaviour, with an apparent excess of photons at each end of the scale. But the principal point of discussion concerned the reality of the "knee" at 50 keV. Some theoreticians have argued that this appears only because of observational difficulties, which arise because this change of slope occurs just in the energy range where the sort of detector used to study the radiation changes from proportional counters to scintillation counters.

It was generally agreed that this break is indeed real, and that the problem now confronting observers is to determine the exact range in energies over which the change in slope occurs. At present, the data are sufficiently inconclusive that almost any theory of the origin of this background radiation can be adjusted to allow for the change, but most will fall by the wayside if observations can be improved by a factor of 10 or more.

The isotropy of this radiation was also a point of discussion. Because an energy density of only 10^{-4} eV cm^{-3} is required to produce the radiation, many models can also be constructed to explain this. It is now clear that the radiation really is isotropic to a very large degree, and that if it is produced by many discrete sources of equal intensity then more than 10^4 sources per steradian are required. For this reason, models in which the radiation is produced uniformly throughout intergalactic space (for example, by bremsstrahlung radiation from a uniform hot gas) seem preferable to those which require the radiation to originate in discrete regions and become uniformly spread out during its passage to Earth. It is significant that the isotropy needed for a discrete source model is greater than the isotropy of the distribution of galaxies in space.

Discussion of high energy emission from pulsars was severely limited because only the Crab pulsar is known to emit optical and X-ray pulses. But a very significant experiment carried out by the Bristol group earlier this year has provided meaa sure of the X-ray pulses from this source (NP 0532) above 600 keV. Both the main pulse and precursor seen optically and at radio frequencies were detected, but at these high energies the precursor becomes three times more powerful than the main pulse, which is itself stronger than at lower frequencies. The fraction of the total emission energy which is in the pulse is therefore very large, in contradiction to the earlier belief that only a small part of a pulsar's emission occurs in the pulses. The most profitable way to find other high energy pulsars is therefore to look at young supernova remnants in other galaxies.

Dr M. Rees (University of Cambridge) suggested that the energy content of the pulses during the first months of a pulsar's life might be enough to make them visible as far away as the Virgo Cluster, covering a volume of space in which an average of one supernova a year occurs. Unfortunately, for the first few months of its life a pulsar would be obscured by the cloud of gas expanding away from it as a result of the explosion in which it originated, so that it is not certain that the pulsar will still be strong enough to be seen when the obscuration clears. But high energy astronomers will certainly be looking at hopeful cases in future.

Most of the discussion of discrete X-ray sources centred on the archetypes of the two types of source best known, Sco X-1 and the Crab. Very little of this covered any new ground, although it does seem that there is a consensus in favour of accepting the marginal evidence for greatly broadened lines of highly ionized iron in the spectrum of Sco X-1. The next new developments in this part of the field will probably result from the studies just commencing of the new class of bright low energy discrete sources (with emission only below ~2 keV) like the Cygnus loop. These should provide spectra in which many lines will stand out, and give the theoreticians another chance to revise their estimates of the relative abundances of the elements in our galaxy.

EARTH'S HEAT

Equality over Land and Sea

from our Geomagnetism Correspondent

THE equality of the average heat flows through continents and oceans is one of the major mysteries in geophysics. If, as has commonly been assumed, the principal contribution to the Earth's heat flux is from

the decay of radioactive materials, especially those in the crust, the heat flow at sea should be less than that on land. The fact that there is no such inequality suggests that the chemical composition of the mantle is different beneath continents and oceans; and this immediately creates problems for the new global tectonics, implicit in which is large scale lateral movement of upper mantle material. Several possible explanations have been put forward to account for the unexpected equality; but so far none has carried sufficient conviction to be widely accepted.

The idea that the oceanic and continental equalities might be fortuitous is apparently anathema to many geophysicists. Yet as a result of a new analysis of world wide heat flow data, this is basically what Sclater and Franchetau (*Geophys. J.*, **20**, 509; 1970) suggest. Their analysis is unique in that for the first time they have determined oceanic averages over defined geological age provinces rather than over latitude-longitude "squares" unrelated to geology. Thus the oceans were divided into strips of gradually increasing age as determined from seafloor spreading studies, and mean heat flows calculated as a function of age.

It emerges that in the North Pacific and South Atlantic, at least, average heat flow decreases with the age of the province from 2·82 μcalories cm^{-2} s^{-1} for provinces less than 10 million years old to 1·15 μcalories cm^{-2} s^{-1} through provinces older than middle Cretaceous. This result parallels that previously demonstrated for continents where heat flow decreases with the age of the orogenic province to an apparently constant value of about 1·00 μcalories cm^{-2} s^{-1} for Precambrian shields. In the oceans, the data are compatible with a model in which hot mantle material rises at ridge crests and loses heat by conduction as the new crust moves away laterally. But because in seafloor spreading a significant amount of heat must be lost from the oceanic crust by the creation of oceanic lithosphere—a process not paralleled on land to the same degree—there is no need to assume identical radioactive compositions beneath the surface of oceanic and continental crusts, and thus no need to assume differences in mantle composition. The sources of heat below continents and oceans must be different—a conclusion which is supported by the fact that the time scales for the thermal decay of heat flow beneath continents and oceans differ by an order of magnitude. These radical differences inevitably lead to the conclusion that the basic flow equality is purely coincidental.

So, according to Sclater and Franchetau, the quality is irrelevant. The important questions are rather: (i) why the heat flow decreases with the age of the rock; and (ii) why, in spite of differences in time scale, the heat flow in the older parts of the oceans is about equal to that for Precambrian shields. To solve these problems, Sclater and Franchetau consider two particular geophysical and geochemical models of oceanic and continental crust and upper mantle. They show that each model can explain the near equality of heat flow through Precambrian shields and old ocean basins when plates of oceanic and continental lithosphere move, and that each can account for the heat flow-age variations. The two models are similar in that the thickness of the oceanic lithosphere lies between 75 and 100 km and that the continental plate is about twice as thick, but they differ in the temperature at the base of the oceanic lithosphere. In each case, however, the composition of the mantle is the same under oceans and continents. Sclater and Franchetau have thus shattered a few illusions concerning the Earth's heat flow, and have convincingly demonstrated that unreasonably speculative hypotheses are not required.

HIGH ENERGY PHYSICS

The Intermediary Boson

One of the most formidable obstacles to detecting the intermediary or W boson, the particle believed to mediate the weak nuclear interaction in much the same way that a photon mediates the electromagnetic interaction, is the uncertainty about the exact way that the W boson decays. Experiments have had to be designed to allow for a great variety of possible decays into light particles (called leptons) and strongly interacting hadrons. This is why the results so far of this search for the boson have been quite inconclusive. D. Cline, A. K. Mann and C. Rubbia (*Phys. Rev. Lett.*, **25**, 1309; 1970) have now, however, proposed an experiment which avoids this difficulty and which may even make it possible to carry out a thorough search for the W boson without having to know the relative amounts of hadronic and leptonic decay.

It is perhaps appropriate that Cline *et al.* should have published their proposals so soon after the first successful trials on the intersecting storage rings at CERN (see *Nature*, **228**, 597; 1970), for the search for the intermediary boson is one of the most obvious experiments planned for the new machine. When energies of interaction may be as much as 1,500 GeV, the search for the W boson is more likely to bear fruit than the search for the quark.

Cline *et al.* claim that the hadronic decay modes of the W boson from high energy neutrinos provide a unique and unambiguous experimental signal of the production and decay of the W, even in the absence of information about the number, configuration and momenta of the decay products. The essential point is that the release of large amounts of energy to the hadrons in hypothetical W events is accompanied by a small transfer of momentum to the associated muon. If the boson exists it will therefore manifest itself by a tight clustering of W events in energy which will stand out against a smooth background.

Cline *et al.* point out that the experimental resolution necessary to observe this cluster is easily attainable in either a heavy liquid bubble chamber or some form of ionization calorimeter. The largest uncertainty will probably accrue from the effect of multiple scattering of the muon, they say, but this should not reduce appreciably the prominence of the cluster.

Many suggestions for detecting the W boson depend on detecting the μ-mesons in its decay. The principal decay mode of the W is believed to be into a neutrino and an electron or a μ-meson, but a persistent problem in its detection is a lack of knowledge about the branching ratio. This has produced nebulous conclusions to experiments appearing in the form of an upper limit of the product of the cross-section for W production by a particular leptonic or hadronic decay mode. Cline *et al.* recall that the present lower limit on the mass of the W is sufficient to allow multi-pion decay modes of the W, and that their experiment provides an unambiguous way of recognizing the W through its hadronic decay modes.

Future for Integrated Cognitive Systems

by
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Before the mid-1970s integrated cognitive systems are expected to attain performance levels comparable in some respects with a 2 or 3 year old human infant. Many people think that within the next 100 years the level of adult humans will be reached. At present limitations lie in inadequate understanding of mathematical-logical and programming principles rather than in hardware speeds or storage capacities. This article is based on a lecture delivered at the CERN computing and data processing school held at Varenna from August 31 to September 12.

WORK is in progress in several laboratories[1-6] directed towards the construction of an integrated cognitive system (ICS): I avoid the phrase "intelligent robot" because of its science fiction connotation of humanoid appearances and other attributes. The research is concerned with intellectual attributes, involving sensorimotor and reflex capabilities only to the extent that these form a necessary substratum for the acquisition or display by computing systems of purely intellectual skills.

At this early stage the "intellectual" skills which research aspires to emulate may seem to the onlooker so primitive as scarcely to deserve the name. Let him, however, reflect on the struggles of small children with the simplest tasks of deduction, generalization and description, and their dogged attempts to construct and refine world-models adequate for their growing needs, representing a succession through which every developed human intellect has passed. Even these first exploits of the infant mind are beyond the abilities of any computing system yet devised. Computers equipped with optical input and manipulative devices are available in at least two laboratories, but understanding of machine perception and cognition has not advanced so far that they could be programmed to compete with human infants, for example on tasks such as the following, which is taken from Stanford-Binet IQ tests[7]. The task involves obeying simple commands, and is designed for $2\frac{1}{2}$ year old infants. With a brick, a button, a dog, a box and a pair of scissors laid in order on a table, the child is told (*a*) "give me the dog"; (*b*) "put the button in the box", and (*c*) "put the scissors beside the brick". A machine passing tests of this sort would be disqualified if it had merely been pre-programmed *ad hoc* for each individual test. An artificial intelligence worth the name must show some degree of generality.

Problems of abstracting from the world of crude sensations and of planning and physically doing things in space and time are dominant in intellectual activity at this early age—possibly they also form an indispensable springboard for the flights of abstract thinking attained later. Emulation by machine therefore demands, as a matter of technical necessity, the design of non-standard computer peripherals to serve as "eyes", "hands", and the like. The alternative course would be to simulate entire real world problem situations inside the machine —a desperate measure of unimaginable scale and cost. R. L. Gregory (private communication) has truly remarked that the cheapest store of information about the real world is the real world, and this indeed is the rationale of the recent emphasis by artificial intelligence projects on "hand-eye" and "robot" devices.

How long is it likely to be before a machine can be developed approximating to adult human standards of intellectual performance? In a recent poll[8], thirty-five out of forty-two people engaged in this sort of research gave estimates between 10 and 100 years. There is also fair agreement that the chief obstacles are not hardware limitations. The speed of light imposes theoretical bounds on rates of information transfer, so that it was once reasonable to wonder whether these limits, in conjunction with physical limits to micro-miniaturization of switching and conducting elements, might give the biological system an irreducible advantage. But recent estimates[9,10], which are summarized in Tables 1 and 2, indicate that this is not so, and that the balance of advantage in terms of sheer information-handling power may eventually lie with the computer rather than the brain. It seems a reasonable guess that the bottleneck will never again lie in hardware speeds and storage capacities, as opposed to purely logical and programming problems.

Granted that an ICS can be developed, is now the right time to mount the attempt? Is it possible that effort should instead be put into some abstract field of philosophy, linguistics or pure mathematics? Perhaps only by postponing rash attempts to construct actual systems can a sufficiently deep understanding be gained to enable artificial intelligence problems to be tackled in the right way.

Theoretical studies are certainly central. But it is not clear that they would be aided by abstention from experimental work. Indeed the lessons of history point in the opposite direction, as with the relationship between classical thermodynamics and the development of the steam engine. Typically engineering artefacts come first, and provide the theoreticians not only with the needed spur to rationalize what is being done, but also with test gear on which to check their formulations. There are elements of a similar relationship between the robot building now in progress in various laboratories and the recent spate of activity by theoreticians in the same laboratories in two areas in particular: visual scene analysis by machine[1,3,11], and construction and formal description of abstract models of the world for use as the basis of plan-formation[12-14].

Yet the principle of "unripe time", distilled by F. M. Cornford[15] more than half a century ago from the changeless stream of Cambridge academic life, has provided

the epitaph of more than one premature technology. The aeroplane industry cannot now redeem Daedalus nor can the computer industry recover the money spent by the British Admiralty more than 100 years ago in support of Charles Babbage and his calculating machine. Although Babbage was one of Britain's great innovative geniuses, in terms of tangible return on investment, support of his work was wasted money. It is now appreciated that of the factors needed to make the stored-program digital computer a technological reality only one was missing—the means to construct fast switching elements. The greater part of a century had to elapse before the vacuum tube arrived on the scene.

Table 1. A COMPARISON OF INFORMATION-HANDLING POWERS OF BRAIN AND COMPUTER

	Brain	Computer
Speed	1,000 bits traverses 1 neurone in 1 s	1,000 bits transferred in or out of core memory in 1 μs
Store	$10^{12}-10^{15}$ bits	10^{12} bits, retrieval 50 ms

This table is based in part on data from ref. 9. The upper comparison, which appears to give the computer the advantage in speed, is compensated by the brain operating in a highly parallel fashion, as opposed to the sequential processing characteristic of the computer.

Table 2. THE SUPPLY POSITION OF MECHANICAL ENERGY AND MECHANICAL INFORMATION PROCESSING CAPACITY

(*a*) Ratio of energy available from mechanical sources and from human muscle power

Year	1500	1700	1800	1900	1945	1965
ER	10^{-4}	10^{-3}	10^{-1}	1	5	10

Total human muscle power potentially available is assumed to be of the order of $0{\cdot}25{\cdot}10^{9}P$ Wh/annum (P=world population). For 1965 it has been assumed that mechanical energy supply was of the order of 4×10^{16} kcalories or $0{\cdot}75\times10^{16}$ Wh at 15–20 per cent conversion efficiency.

(*b*) Ratio of mechanical and human information processing capacity

Year	1955	1965	1970	1975
CR	5×10^{-4}	10^{-2}	2	50?

This comparison is based on those tasks where the human channel capacity of *c.* 20 bits/s is a major rate determining factor, as may be the case in many routine clerical operations and computations.

As in (*a*), (*b*) attempts to compare facilities available world wide. Both assessments, and in particular (*b*), are obviously very tentative only.

It can reasonably be said that time was unripe for digital computing as an industrial technology. But it is by no means obvious that it was unripe for Babbage's research and development effort, if only it had been conceived in terms of a more severely delimited objective: the construction of a working model. Such a device would not have been aimed at the then unattainable goal of economic viability; but its successful demonstration might, just conceivably, have greatly accelerated matters when the time was finally ripe. Vacuum tube technology was first exploited for high speed digital computing in Britain during the Second World War[16]. But it was left to Eckert and Mauchly[16] several years later to rediscover and implement the conceptions of stored program and conditional jumps, which had already been present in Babbage's analytical engine[17]. Only then could the new technology claim to have drawn level with Babbage's design ideas of 100 years earlier.

How is ripeness of time to be diagnosed for projects to build intelligent machinery? The only absolutely safe method is to wait until someone does it, just as the only error-free pregnancy test is to wait for the birth of a demonstrable infant. We would prefer, I think, to detect what is still in the womb, and accept a risk of being wrong. Table 3 shows four possible criteria of ripeness which might be applied, from the outside as it were, to budding technological enterprises.

The four criteria are listed in decreasing order of superficiality. Criterion 1 is of a kind which can be applied by a policy-maker "off the cuff", without delving into technical considerations. It says "This is not a bee in an isolated bonnet. Laboratories all over the world are in the race. Can we afford not to join?". The fact that it is so widely persuasive stems chiefly from the fact that it is superficial, and hence cheap and easy to apply: also it acts on the "keeping up with the Joneses" reflex. For what it is worth it is favourable to the proposal to construct an integrated cognitive system, which is being studied actively in America, Britain and Japan. But we must remember that the highest recorded score for this particular criterion could probably be claimed for the alchemists.

Table 3. CHECK LIST OF SIGNS AND SYMPTOMS FOR THE EARLY STAGES OF FIVE TECHNOLOGICAL PROJECTS

	A	*B*	*C*	*D*	*E*
(1) Multiplicity of effort (i.e. how many laboratories?)	√	√	X	√	√
(2) Availability or feasibility of all essential instrumentation	X	√	X	√	√
(3) Demonstration of a working model	X	√	X	√	(?)
(4) Theoretical proof of overall feasibility	X	X	X	X	X
Fate: S succeeded F failed	F	S	F	S	?

(*A*) Transmutation of elements in the time of alchemy; (*B*) steam engines in the time of Watt; (*C*) stored-program digital computing in the time of Babbage; (*D*) heavier-than-air flight in 1900; (*E*) intelligent machinery in 1970. The symbol "(?)" is used to mean "possibly imminent" in distinction from "?" which means "undecided".

Jumping to the other end of the range, criterion 4 is in general of little assistance because of the "pregnancy test" argument. Overall theoretical analysis is usually only achieved on the morrow of success. An interesting counter-example is Lovell's project to build a giant radio telescope at Jodrell Bank[18]. Here criterion 4 was satisfied in advance. But of course there was never any problem concerning unripeness of time in the feasibility sense. All that was in question was the balance of expected benefits against expected costs. This issue was finally settled when the first Russian sputnik was successfully tracked by an instrument designed for other purposes, and the world found itself dependent on Jodrell Bank for accurate data on satellite orbits. This decisively ended the era of uncertainties of funding for the project. One may wonder whether some element of international "keeping up with the Joneses" was at work here, to the great good fortune of astronomical science.

An even more interesting case is radio broadcasting. Feasibility was shown theoretically by Maxwell in 1865, and verified experimentally by Hertz about 20 years later. Shortly afterwards Popov and Marconi independently achieved the first "working models". An equally clear example is the modern (junction) transistor, the basic action of which was predicted by W. Shockley in 1949. A further application of criterion 4 arises if theoretical infeasibility is demonstrated, as in the case of the perpetual motion machine. Anti-gravity is another example, in spite of rumours of continued multiplicity of effort behind the security curtain. But it is well to look on such negative proofs with caution. The possibility of broadcasting radio waves across the Atlantic was convincingly excluded by theoretical analysis. This did not deter Marconi from the attempt, even though he was as unaware of the existence of the Heaviside layer as everyone else.

Instrumentation

To summarize the uses of the four criteria, No. 1 is so weak that it is better left alone, while No. 4 is so strong that it is usually not available in real cases of doubt. We are therefore thrown back on criteria 2 and 3. It will be interesting to relate these, if possible, to the present scene in machine intelligence research. First, I shall consider availability of instrumentation. Four categories are involved: (1) computing hardware; (2) programming systems; (3) utility packages (such as deduction routines, parsing routines, learning routines, search routines and so on), and (4) "robot" input–output devices.

These can be interpreted as stages in a development programme. First, get your computer. Then develop a software and programming language base adequate for the needs of machine intelligence research. Only then is it feasible to build a library of useful packages and to construct special peripherals such as "hand-eye" attachments. The next step, at least in aspiration, is the construction of a working model of an intelligent machine. Most workers who partake of this aspiration would, I think, agree that categories 1–4 are beginning to be in reasonable shape to provide the tools for the job.

Working Model

A working model is almost a necessary condition of confidence in the feasibility of any proposed technological innovation. It is by no means a sufficient condition. New and possibly prohibitive difficulties may be brought into being by the scaling-up process. We would do right to be impressed by a power-driven model aeroplane. But suppose that an inventor proposed to develop a man-sized jumping machine able to clear the top of St Paul's Cathedral. A flea-sized model jumping a similar multiple of its own height would scarcely be convincing. So if the objective which I shall discuss were to be attained within the next few years, this would by no means imply that an intelligent machine was round the corner. But it might indicate that significant success at the scaled-up level was perhaps only a decade or two away.

What is meant by a working model of an intelligent machine? The best approach is to map out roughly the principal constituents of such a machine's "mental world", and then say that a working model is constructed on something like the same overall plan, differing only in the relative poverty of the individual subsystems which are linked together. Also it must be "working" in the sense of displaying the various constituents and their collective operation in interaction with real world problems—for example problems of the type illustrated by the Stanford-Binet tests for infants which I cited earlier. An ICS able to operate at this level would bear the same relation to the intelligent machines of the future as a powered toy aeroplane to passenger-carrying airliners. But even such a primitive ICS has two rather interesting features; first, its achievement lies, in the opinion of some workers, only a few years in the future; and second, such an ICS could almost certainly be made the basis of an industrial development programme to produce before the end of the 1970s a range of commercially useful devices.

Applications

At Edinburgh we recently commissioned the consultant firm Scicon Ltd to do a study addressed to the question: "Assuming solution of the technical problems, what industrial applications can be envisaged for the late 1970s"? The possibilities included in their report were, first, anchored devices for luggage-handling at airports, crane-controlled assembly, and automatic control and inspection of machine tool output; and second, free roving devices for exploratory vehicles for the space programme, ocean bed exploration, laying pipe-lines in deserts, and tree-felling in remote forests.

Activities such as these in inhospitable or inherently unstructured environments are difficult and expensive when conducted by conventional means, so that any prospect of delegating them to cognitive machines will have attractions. At Edinburgh we are assisting in a feasibility study of automatic parcel-handling for the Post Office Telecommunications Headquarters, and a Japanese group (see ref. 5) are interested in applications of ICS work to assembly line operations. The robot project at Stanford Research Institute envisages exploratory vehicles of various kinds as a major industrial payoff.

Programs and Plans

Leaving industrial implications, I shall now consider the quintessential activity of an integrated cognitive system, in the sense that locomotion is the quintessence of a motor car. This, it can be argued, is planning, for it is by the relative absence of this activity that we recognize that existing automatic systems of prediction and control, however sophisticated and "clever", are not true examples of intelligent behaviour. Further, when we speak of machines able to form plans and to reason about the adequacy of a plan to a given task we can be quite precise about what we mean, by pointing out that a plan of action can be usefully treated as formally equivalent to a computer program ("plan of computation"). Forming a plan is then seen as having the same logical status as writing a program, and validating a plan as utilizing the same mathematico-logical apparatus which programming theorists have developed in recent years for proving things about programs. This insight, elaborated recently by C. C. Green[12], is of profound importance for the future development of artificial intelligence, and may well be destined to occupy a place as central as, say, the equivalence of the corpuscular and wave models in the theory of optics.

An integrated cognitive system, then, can be conceived as a plan constructor and plan implementer. At any moment it is either in interactive mode or in planning mode. "Planning mode" is interpreted in a broad sense to include all processes of inference involved in the formation of new plans, including purely internal reorganizations or extensions of stored descriptions.

In interactive mode it is executing a plan. No reasoning in the sense used here occurs in this mode, until an interrupt generated by an input device causes reversion to planning mode. Input (for example "eye") and output (for example "hand") devices interact directly with an external world in such a way that the next sense-datum received is dependent on the past input–output sequence. The precise form of this dependency is governed by the laws of nature. It is part of the business of an ICS to form an approximate picture of these by processes of abstraction.

What else is in memory, apart from plans, and to what top-level control are the contents of the memory subject?

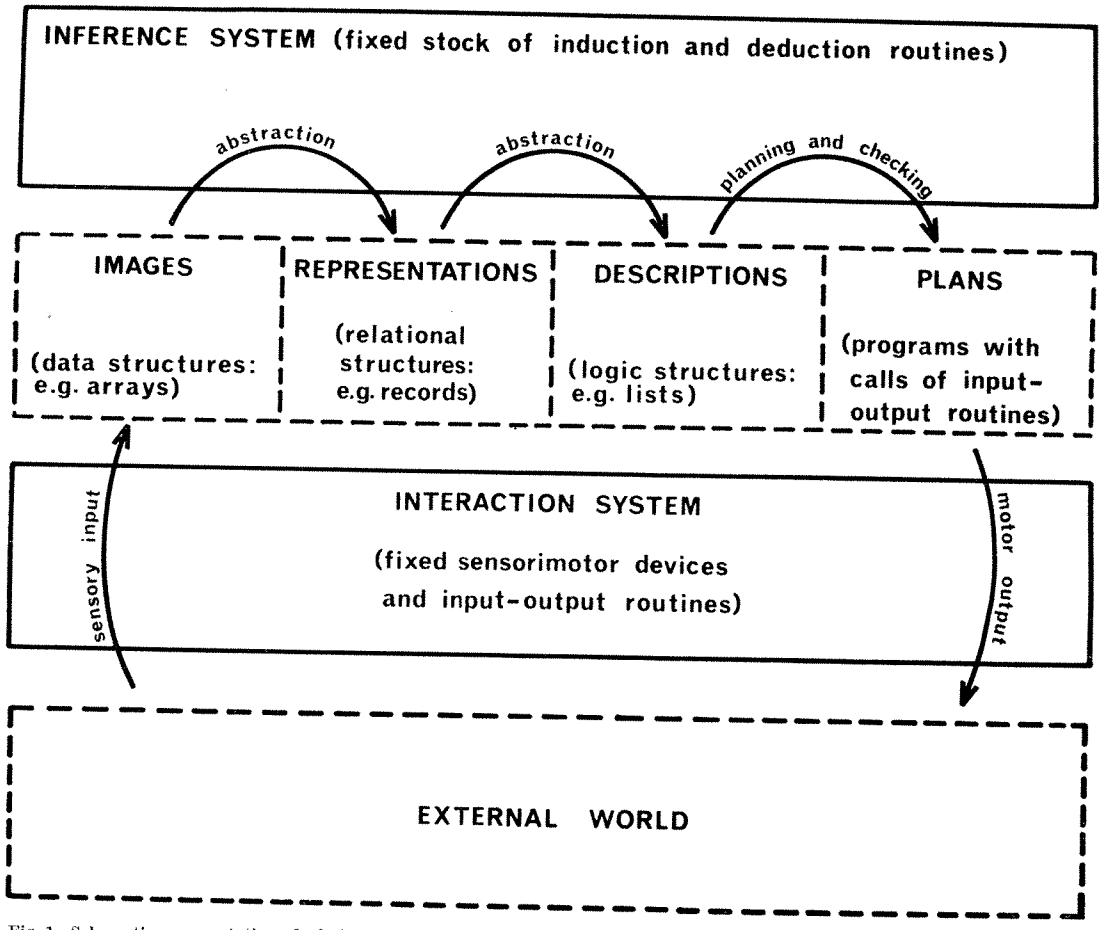


Fig. 1. Schematic representation of relations between an integrated cognitive system and its external and internal worlds. The fixed systems are shown with solid lines, while those which are subject to change in the interaction process are drawn with broken lines.

Four major categories are envisaged (Fig. 1): "plans" (in the form of programs); "images" (in the form of data structures); "representations" (for example in the form of relational structures), and "descriptions" (in the form of logic sentences).

At the top level a common inference system operates on: (*a*) plans, not only to construct them, but to verify in advance that they will work, using in the process: (*b*) images, which are direct point-to-point projections of objects in the external world (for example a map is an image of a particular geographical area); (*c*) representations, which model objects in the external world by abstracted features, and (*d*) descriptions, usually called "theories", which make general statements about objects in the external world and their relations to each other in space and time.

It is worth commenting on the extraordinary faithfulness with which the brain can store images: the extreme case is "eidetic imagery" in which a visual pattern can be stored for periods in considerable detail. It is possible, and has been argued by Richard Gregory[19], that this type of direct modelling is biologically more primitive, and hence has had time to evolve to a higher pitch, than the storage and sequential processing of symbolic information, as in natural language and in formal reasoning.

Automatic Plan-formation

The idea of getting a computer to write its own programs has appeared and disappeared several times in the past two decades. Early attempts, inspired by the example of biological evolution, were based on generating program symbols randomly, conserving the more successful sequences[20,21]. Such an approach is now considered naive, and nature tends to be thought a poor model for cost-conscious designers. Present ideas centre round the systematic construction of a program, either as a side product of mechanically proving from given axioms that the task which the program is to accomplish is theoretically capable of accomplishment, or alternatively as an end-product of a process of heuristic search. Keeping in mind the formal analogy between programs and plans, it may help fix ideas to consider an example from an unpublished study by Popplestone in which elements of both approaches are used.

The theorem-proving approach, developed by C. C. Green, uses the apparatus of formal logic to form plans of action. There are difficulties in the approach. One is the "frame problem": it is necessary to say not only what things are changed by an action, but also what remains unchanged. Not only are the frame axioms tedious to write, but they also tend to lead the theorem-proving process astray. This raises the second principal difficulty, the Achilles heel of present day mechanized proof procedures, that they very easily stray into unprofitable inference paths through lack of any adequate formulations of the notion of relevance.

An alternative approach to plan-construction, suggested by the work of Floyd[22], is to start at the goal and work backwards to the present situation. The goal is repre-

sented by a sentence, which is conceived as having been deduced from the conjunction of a preceding situation (represented by a sentence) and an antecedent action. A backwards search tree can be grown until a state-description is produced which is a logical consequence of what is known about the initial pre-planning situation. This process, which is currently being developed by R. J. Popplestone, is illustrated in Fig. 2 for a housekeeping task in a world furnished with a cupboard, a table and a chair. Initially the cupboard contains exclusively forks and the robot's hand is at the chair. A plan is required to create a situation in which at least one fork is at the table. The example is exhibited here for its didactic value rather than for its originality of approach. Novel features do, however, exist (*a*) in the way in which general statements, rather than detailed specifications, are handled in the search, and (*b*) in Popplestone's method for guiding the search heuristically. For this he uses a notion of approximation to the desired condition of being logically implied by the initial situation. The degree of approximation is estimated from the number of interpretations of the description of the initial situation which are inconsistent with the current one. The smaller this number the more "promising" the given situation as a point of departure for extending the backwards search.

"Hand-eye" Problems

The provision of a suitable formal basis for reasoning about even such simple systems is by no means trivial, and the properties which must be possessed by a satis-

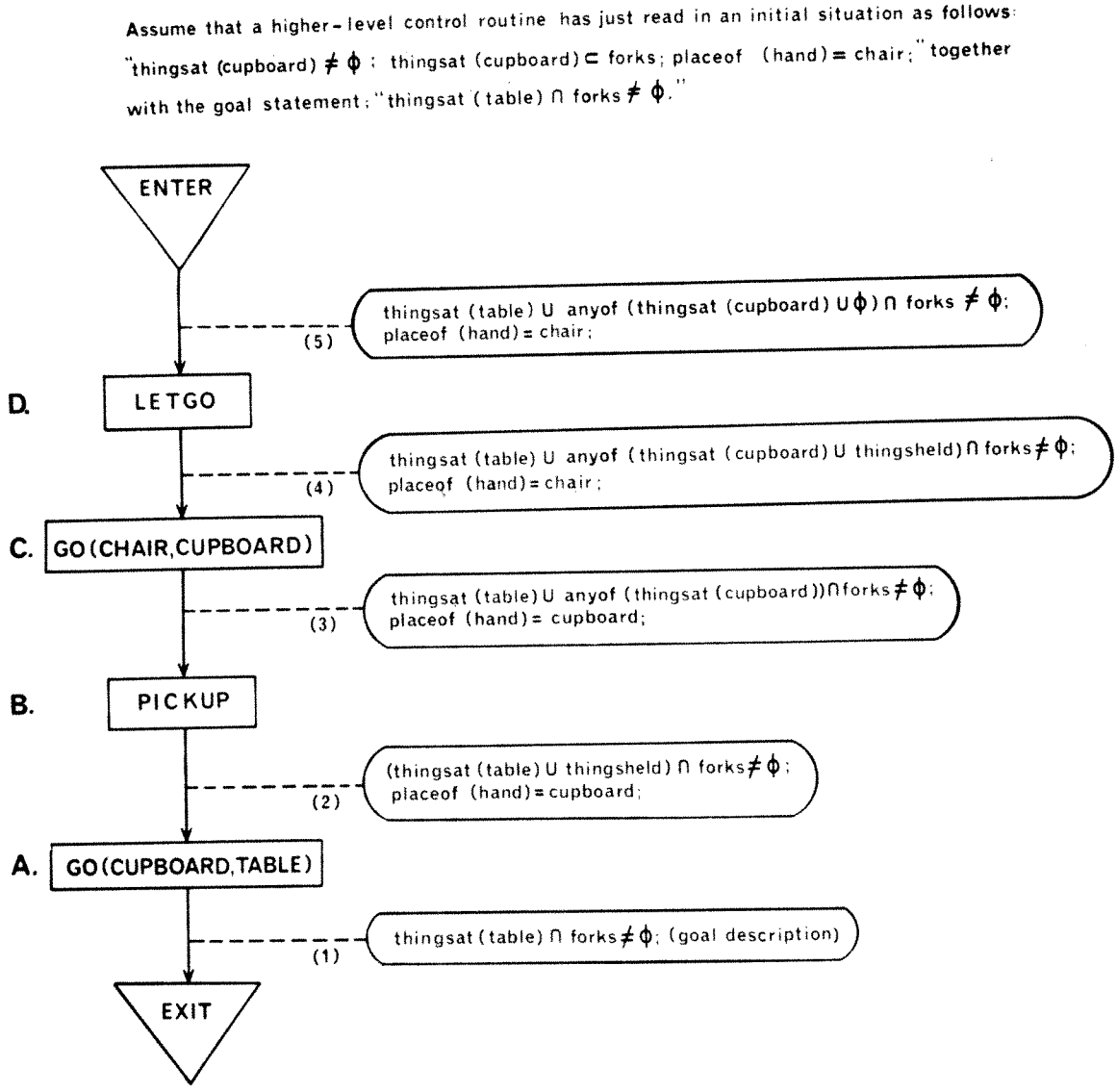


Fig. 2. Successful action-chain constructed by backwards search from the goal situation, using logical inference to associate descriptions with successively earlier situations. The process terminates when a description is produced which is a direct logical consequence of the initial state-description, in this case giving the sequence D, C, B, A as the answer. A plain-language transcription of comments 1–5 might read as follows: (1) there is at least one fork at the table; (2) the things at the table together with the things held in the hand include at least one fork, and the hand is at the cupboard; (3) the things at the table together with a random selection from the things in the cupboard include at least one fork, and the hand is at the cupboard; (4) the things at the table together with a random selection from the things distributed between the cupboard and the hand include at least one fork, and the hand is at the chair; (5) the things at the table together with a random selection from the things in the cupboard include at least one fork, and the hand is at the chair. This last statement can be obtained as a logical deduction from the initial state-description given in the text, so the process is terminated. The method by which description (2) is obtained from description (1) and action *A*, (3) is obtained from (2) and *B*, and so on is due to Floyd[22].

factory calculus of situations and actions have been re-examined by McCarthy and Hayes[13] and by Burstall[14]. In several laboratories, including our own in Edinburgh, experiments are in progress with various "hand-eye" and "robot" attachments to computers in order to provide instrumentation and a software base adequate to put such calculi to the test of practice. Each laboratory doubtless has its own graded repertoire of tasks with which to challenge its local evolving ICS. Our schedule[6], designed to fill the next 2–3 years, is concerned with six types of task: first, as far as vision is concerned we wish to develop a machine that will identify single objects (definitions provided by the programmer) placed within the field of vision, and learn to identify single objects (by generalizing from examples); second, manipulating a hand to move to any accessible prescribed position and pick up an isolated object, and also an object from a group. The third task is that of world modelling to enable the machine to "know" relative locations of objects and "self", and to update the internal model on the basis of sensory input; to integrate several views of an object and tactile information. The fourth is simple planning by simulation (graph traversing[23]), and is concerned with planning a route for "self" from one location to another, avoiding contact with obstacles and planning movements of the hand to pick up an object from a set and move it without disturbing other objects, also executing plans and reforming them if they fail, or are about to fail. The fifth task is that of higher level planning (theorem proving[12]) whereby, given a set of world axioms, means of achieving simple states are designed, for example "Go to a cube", "Put a ball into a cup". The sixth task we have set ourselves comes under the heading of generalization (induction): to learn general statements about simple events from specific occurrences, e.g. " 'Put cup onto ball' implies failure", "'Put ball onto cup' implies success"; to generalize from several similar specific observations, for example "'Put anything onto ball' implies failure"; and eventually to generalize to qualified sets, e.g. "'Put anything flat onto cube' implies success".

Educational Aids for Young Children

There are similarities between some elements of our schedule and the Stanford-Binet tests for infants. This circumstance justified a speculative postscript to the earlier list of industrial uses. It is generally agreed that an important application for advanced computer systems will be in educational technology. It is also already apparent that very young children, for example of primary school age, are in some ways the most rewarding subjects, because the teacher to child ratio is too low fully to satisfy the young child's appetite for continual responsiveness. Anyone who has watched 6 year olds wrestle absorbedly, through the complexities of the teletype, with computer-supplied arithmetic homework cannot help being struck by the motivating power of the interactive terminal. What about even younger age-groups or mentally handicapped older children? Something can be done using the cathode ray display and voice output, permitting communication between child and machine in pictures and words. This possibility is being investigated in our laboratory among others. But just as the human teacher supplements pictures and words with direct demonstrations, by manipulation, for example, of cuisenaire rods for arithmetic, of buttons and beads for sets, of cups and sand and liquid for conservation laws and so on, so it may turn out that when computer terminals can be equipped with adequate "hand-eye" capability these too will be pressed into service as teachers' aids.

The possibility of such a development by the end of the 1970s deserves serious attention. It is particularly attractive for the research worker who likes to have some specific application in mind, because the subject matter of infant teaching has a certain relevance to the intellectual content of artificial intelligence research—namely the explication of real-world phenomena in terms of basic logical and mathematical concepts. To the lay onlooker, however, there may seem to be something de-humanizing, even psychologically dangerous, in the exposure of the very young to interaction with machines. I shall not trespass on the province of the educational psychologist, beyond saying that in our own work with small children at Edinburgh[24] this criticism has indeed been encountered in some quarters; but it has been conspicuously lacking from three specific categories of person—(1) the teachers, (2) the children's parents, and (3) the children themselves.

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Differential Binding Properties of Cytochrome c: Possible Relevance for Mitochondrial Ion Transport*

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Similarities between the binding properties of cytochrome c and the permeability properties of mitochondria suggest that cytochrome c may be a transport carrier of the inner mitochondrial membrane. Cytochrome c could be the basis of a mechanism by which mitochondrial activities are regulated by the metabolic state of the cell. Variations in the structure of cytochromes c from different species may result from evolutionary selection for particular ion-binding properties.

THE chief difficulty in understanding the evolutionary variations of the primary structure of cytochrome *c* (refs. 1–3) has been the lack of observable functional differences between the proteins of different species. For example, when properly deionized samples are used, the proteins from the cow, pig, sheep, horse, man, chicken, turkey, pigeon, tuna and the moth, *Samia cynthia*, all yield the same first-order rate constant for their reactions with bovine cytochrome *c* oxidase over a wide range of cytochrome *c* concentrations (unpublished results of L. Smith), even though their amino-acid sequences differ from that of the bovine protein by up to twenty-seven residues out of a total chain length of 104. If such is the case for all biological functions of the protein, then species variations can only result from random processes, such as the evolutionary fixation of selectively neutral variants, and consequently one would expect to observe polymorphism with respect to cytochrome *c* structure in suitably chosen populations of a single species. Such molecular heterogeneity has not been found in the small numbers of individual humans and horses examined[2], but because the protein changes particularly slowly in the course of evolution[1–5], it is likely that a much larger sample would be needed to provide a statistically significant answer to the question of whether such polymorphism occurs[3].

Calculations of Fitch and Markowitz[6], based on statistical phylogenetic trees for cytochrome *c* (ref. 7), have shown that out of the seventy amino-acid positions that have varied in the course of evolution in more than the thirty cytochromes *c* of known structure[2], only about ten positions can sustain variations in any one mammalian species without resulting in the evolutionary disappearance of that line of descent. The number of these "concomitantly variable codons" or "covarions" is a quantitative expression of the stringency of structure-function requirements of the protein. Their paucity in the case of cytochrome *c* indicates that a large portion of both surface and internal residues in this protein is required to be precisely as they are in any one mammalian species. Such a situation is unlikely to reflect a wide latitude of permissible structural variations, all of which would be functionally indistinguishable. It is also difficult to see how more than 90 per cent of the protein could be invariant in any one mammalian species if the only surface requirements are the provision of suitable areas for interaction with cytochrome *c* oxidase, cytochrome *c* reductase, and possibly the mitochondrial inner membrane, the only known functions of the protein. The separate requirement for the third type of interaction has not been demonstrated[8].

Finally, the availability of a 2·7 Å resolution model of ferric horse cytochrome *c* (unpublished results of R. E. Dickerson, T. Takano, P. Eisenberg, O. Battfay, L. Samson and E. M.) has made it possible to locate in the tertiary structure the positions at which evolutionary residue replacements have occurred in the proteins of different taxonomic groups of species. Remarkably, it seems that these are well defined regions for each such group (unpublished results of E. M., W. M. Fitch and R. E. Dickerson). For example, the cytochromes *c* of mammalian species vary within a semicircular band in front, above and behind the haem, leaving both lateral segments of the protein largely invariant (Fig. 1). The insect and plant protein variations are distributed quite differently from those of mammalian cytochromes *c*. Thus, to the extent that neutral mutations may take place, they cannot all be fixed in the same areas in the proteins from different taxonomic groups, indicating again that there must be widespread selective pressures on cytochrome *c* structure and that these are expressed at different sites in the molecule in different groups of species.

Because the classical electron transport functions of cytochrome *c* do not seem to provide the basis for clear cut differences in activity among the proteins from different species which could be related to the degrees of phylogenetic divergence[9–11], we were led to search for other possible physiological functions for the protein. It could still be argued, of course, that not all aspects of the electron transport properties of the cytochromes *c* of different species have been as well examined as the reactions with cytochrome *c* oxidase mentioned above, and that selective effects might be obtained even with degrees of functional difference below the sensitivity of our *in vitro* tests[2]. These are not attractive possibilities, however, in view of the arguments we have presented, which point to particularly stringent structural requirements and presumably correspondingly stringent selective pressures.

Ion-binding Properties

The one property previously shown to exhibit large variations among the cytochromes *c* of different species is the binding of various inorganic anions by the ferric form of the protein[12]. For example, two chloride ions are bound to one molecule. In a particular set of conditions, the ratios of the areas under the electrophoretic boundaries corresponding to one and two bound ions, which presumably reflect the relative binding affinities at each of the two chloride sites, were found to be 0·8, 1·8, 6, 9 and 32 for the screw worm fly, human, hog, pigeon and *S. cynthia* cytochromes *c* respectively[12]. An examination of the binding properties of the ferric and ferrous forms of horse cytochrome *c* for numerous ions by measurement of electrophoretic mobilities gave the results shown in Table 1.

These results indicate a correlation between the ion binding properties of cytochrome *c* and the transport properties[13,14] of mitochondria. There are four classes of substances involved in mitochondrial transport. Some, such as ammonia, penetrate the mitochondrial matrix

* This material was presented by E. M. as part of the Fourth Keilin Memorial Lecture to the Biochemical Society, in Cambridge, on July 8, 1970.

Table 1. ELECTROPHORETIC MOBILITY OF HORSE HEART CYTOCHROME *c* TOWARDS THE CATHODE IN THE PRESENCE OF VARIOUS IONS

	Added ions	Electrophoretic mobility at 1° C, $cm^2/s/V \times 10^5$ Ferricytochrome *c*	Ferrocytochrome *c*
1	None	6·1	4·6
2	Cl^-	3·7; 4·2	4·6
3	Fumarate	2·6; 3·1	3·5
4	Maleate	2·5	2·2
5	Maleate and Cl^-	4·1	—
6	Na^+	6·1	4·7; 5·2
7	K^+	6·1	4·6; 5·1
8	Ca^{2+}	6·1	5·1; 5·4
9	Mg^{2+}	6·1	5·1; 5·4
10	P_i	4·3	2·6
11	P_i	4·4	2·7
12	Succinate	6·2	4·1
13	Succinate and P_i	2·2	—
14	Citrate	1·5	1·3
15	α-Ketoglutarate	1·9	2·9
16	Glutamate	4·9	3·9
17	Aspartate	4·9; 5·5	3·5
18	ADP	3·3; 4·1	—

The electrophoretic mobilities were measured with the model H Spinco electrophoresis-diffusion apparatus in the 2 ml. cells, as described[12]. Unless otherwise stated, all solutions contained 0·407 to 1·017 mM horse heart cytochrome *c* (prepared according to E. M. and Walasek[27] and freed from bound ions by electrodialysis to the isoionic point[12]), 0·10 M Tris-cacodylate buffer (*p*H 7·2) and 0·05 M of the added ions brought to *p*H 7·2 with Tris or cacodylic acid, as required. In experiments 4 and 5 the solutions contained 0·15 M Tris-maleate (*p*H 7·0) and 0·10 M of the same buffer with 0·05 M NaCl, respectively. The solution in experiment 11 contained 0·011 M Tris-phosphate and 0·10 M Tris-cacodylate (*p*H 7·2). The solution in experiment 12 contained 0·05 M Tris-succinate (*p*H 7·2), while that in experiment 13 was the same with the addition of 0·011 M Tris-phosphate. In experiment 18, ADP was 3·8 mM. In all cases the ferricytochrome *c* solutions were dialysed at 1° C against the various buffers. The ferrocytochrome *c* solutions were obtained by reduction with Tris-ascorbate and equilibrated against the various buffers by passage through a 1 × 50 cm column of 'Sephadex G-25' (Pharmacia). Where two electrophoretic boundaries were observed both electrophoretic mobilities are listed.

independently of any transport phenomena, apparently in the unionized form. Others, such as chloride ions, fumarate and maleate, are incapable of penetrating the inner mitochondrial membrane or do so only at relatively high *p*H[13,15,16]. Such impermeant ions were all found to bind strongly to ferricytochrome *c* and much less or not at all to the reduced protein. Of these, maleate showed the strongest apparent binding to ferrocytochrome *c* (Table 1, experiments 1 and 4); even so, the change of mobility towards the cathode of the reduced protein (4·6 to 2·2 $cm^2/s/V \times 10^5$) was considerably less than that of the ferric form (6·1 to 2·5 $cm^2/s/V \times 10^5$). Maleate has been shown to stimulate the entry of isocitrate and ketoglutarate into mitochondria[15] and therefore its status as a strict impermeant is in some doubt. It was also shown that Cl^- competes with maleate for the same binding site or sites (Table 1, experiments 2–5). The third class consists of cations, such as K^+, Na^+, Ca^{2+} and Mg^{2+}, which are capable of being accumulated in the mitochondrial matrix against a concentration gradient by the expenditure of chemical energy. These all bind to ferrocytochrome *c* but not to the ferric protein (Table 1, experiments 6–9), and from the relative areas under the two boundaries it was estimated that K^+ bound only slightly better than Na^+, whereas Ca^{2+} was clearly more strongly bound than Mg^{2+}. Such relative affinities may be reflected in the relative rates of mitochondrial transport of these cations[13]. Equilibrium dialysis experiments have confirmed that Ca^{2+} binds to reduced but not to oxidized cytochrome *c*.

The last class of transported ions consists of a large variety of anionic substances, which flow in and out of the mitochondrial matrix following the concentration gradient, if suitable "counterions" are present. Counterions are presumably fixed to the same sites in the translocating material as the anion studied or moved in response to the *p*H change. No energy expenditure is involved in these movements. Pressman[13] lists ten groups in this last class, each group consisting of the set of permeant anions, the particular counterions which are effective for this group, and in several cases inhibitors which specifically prevent the movement of this group of substances. A translocating material or exchange-diffusion carrier is assumed to exist for each group, for the rate of flow is proportional to the concentration gradient only up to a certain level, above which the mechanism seems to be saturated. Representatives of seven of these ten groups of substances were found to bind to cytochrome *c* in both oxidation states (Table 1, experiments 10–18). Moreover, like the transport of succinate which occurs only in the presence of inorganic phosphate[13], the binding of succinate to cytochrome *c* also takes place only in the presence of phosphate (Table 1, experiments 11–13). The mitochondrial transport of phosphate is blocked by several mercurials[17–19]. Mersalyl, a typical compound of this group, was found not to influence the effect of phosphate on the electrophoretic mobility of cytochrome *c* in either oxidation state. Nevertheless, gel filtration experiments with ^{32}P-phosphate, similar to those commonly used to measure the interaction of micromolecular substances with proteins[20], demonstrated that mersalyl causes a considerable increase in the binding of phosphate to the protein. This might explain the inhibition of mitochondrial transport of the ion because sufficiently firm binding would preclude adequate dissociation from the carrier. Another group of substances depending on an exchange-diffusion carrier consists of ATP and ADP[13,14,21]. In mitochondrial transport the counterions for ATP and ADP can only be ADP and ATP; atractyloside prevents the movement of both nucleotides; and AMP and other nucleotides are transported extremely slowly if at all[14,21]. It was shown, by equilibrium dialysis, that ADP binds to the protein in both oxidation states, in a stoichiometric ratio of roughly two molecules of the nucleotide per molecule of the ferric protein and with an equilibrium constant of the order of 10^3, indicating that there is weak binding; that ATP also binds to cytochrome *c* with approximately one molecule of nucleotide binding per molecule of ferricytochrome *c*; that ADP competes with ATP for the same site; and that atractyloside prevents the binding of ATP. Measurements of electrophoretic mobility confirmed that ADP binds to ferricytochrome *c* (Table 1, experiment 18). The occurrence of two boun-

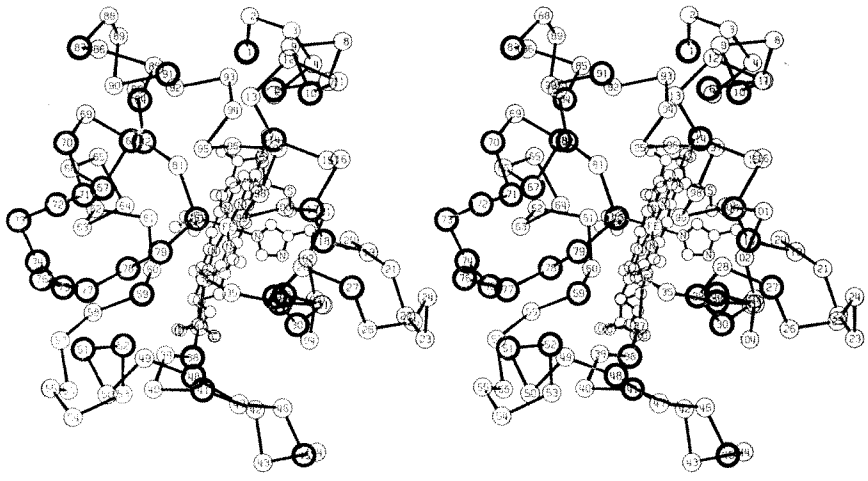


Fig. 1. Stereoscopic α-carbon diagram of the horse heart cytochrome *c* molecule in the ferric form, according to Dickerson *et al.* (unpublished results). Those residues that are invariant among all the over thirty cytochromes *c* of known primary structure are emphasized by heavy circles; those that have varied among the proteins of mammals are 11, 12, 15, 33, 35, 44, 46, 47, 50, 58, 60, 62, 83, 88, 89, 92, 100, 103 (a total of eighteen). Those that represent variations in the cytochromes *c* of primates are 11, 12, 15, 46, 47, 50, 58, 62, 83, 89 (a total of ten), according to Fitch *et al.* (unpublished results). The amino-acid sequences of the cytochromes *c* are given in refs. 1–3.

daries, both of which migrated slower than the free protein, also confirmed the presence of at least two ADP binding sites on cytochrome *c*.

Model for Cytochrome *c* as an Ion Carrier

On the basis of this correlation between the transport properties of mitochondria and the binding properties of ferro and ferricytochrome *c* for typical non-penetrating anions, for the cations which can be accumulated in mitochondria, and for representatives of some of the groups of anions which can undergo exchange-diffusion processes through the inner mitochondrial membrane[13], it is suggested that cytochrome *c* is a carrier for some of the substances which require specific translocation mechanisms to move in or out of the mitochondrial matrix. A diagrammatic representation of the proposed model is given in Fig. 2. This shows that cytochrome *c* reduction takes place near the outer surface of the inner membrane, and that, on reduction, cations such as Ca^{2+} are bound to the protein, whereas any non-permeant anions such as Cl^- that are bound to the ferric form of the protein are discharged. Similarly, near the inner surface of the inner mitochondrial membrane oxidation of cytochrome *c* occurs by reaction with cytochrome *c* oxidase; permeant cations are discharged whereas impermeant anions such as Cl^- are bound. Substances which move by exchange diffusion, such as ADP and ATP, are shown to be bound by both oxidation states of the protein. Clearly, such substances would not penetrate into the mitochondrial matrix either if the binding affinity for the carrier is too low—as may be the case for succinate in the absence of phosphate (Table 1)—or if the binding is too strong and no substance capable of competing for the same carrier site or "counterion" is present. In that case the movement of the substance down its concentration gradient will be slow, for it depends on the weak dissociation from the carrier at the low concentration side of the membrane. Such may be the case for citrate and ketoglutarate (Table 1) in the absence of counterions. Similarly, counterions may have several possible roles. They could merely bind to the carrier and prepare a site suitable for the binding of the transported material, such as may occur with phosphate and succinate (Table 1). Alternatively they could react with the same carrier site as the ion transported and, if the binding affinity to the carrier is within a useful range of that of the transported ion, net exchange would be greatly speeded up in the presence of appropriate counterions in the compartment into which the anion must flow. Counterions could also bind to different carrier sites and merely move out of the mitochondrial matrix in response to the electrochemical gradient generated by the inflow of the transported anion[13]. Finally, inhibitors of ion transport may act by either inhibiting binding to the carrier (atractyloside and ATP or ADP) or causing too firm a binding (mercurials and inorganic phosphate). Clearly, other modes of action are also possible.

The mechanical details represented in Fig. 2 should not be taken literally. In the first place, the arrows connecting the reduced and oxidized forms of cytochrome *c* need not necessarily represent physical translation of the molecule from one side of the membrane to the other. Rather, the "cytochrome *c* pool" could possibly contain a number of molecules of cytochrome *c* in local oxido-reduction and ion-binding equilibria, the movement of individual protein molecules being limited largely to the rotations necessary to bring the appropriate electron and ion donating and accepting sites in contact. Such an arrangement would also be useful in enhancing the specificity of transport. Indeed, if, for example, the carrier protein has binding affinities for two ligands L_1 and L_2 which are in a ratio of 1·1, and if on average each ligand molecule sequentially binds four times to carrier molecules during its transport through the membrane the overall effective, ratio of the binding affinities of L_1 to L_2 will be $(1{\cdot}1)^4$.

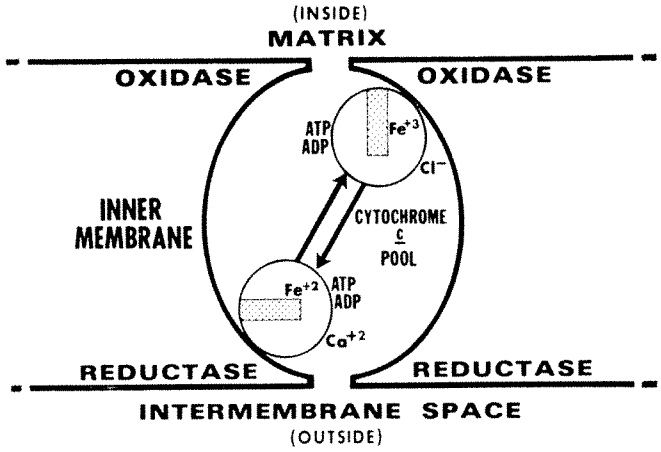


Fig. 2. Diagram of the proposed model for the ion-carrier function of cytochrome *c* in the mitochondrial inner membrane. The smaller circles in the "cytochrome *c* pool" represent cytochrome *c* in the reduced (lower circle) and oxidized forms (upper circle). The cross-hatched areas represent the haem prosthetic group of the protein and are placed to indicate that the orientation of cytochrome *c* with respect to the membrane surfaces is not the same near the outer (reductase area) and inner (oxidase area) surfaces. Ca^{2+}, Cl^-, ATP and ADP represent the corresponding ions bound to the cytochrome *c* surface, Ca^{2+} being an example of the class of cations which can accumulate in the mitochondrial matrix through the expenditure of chemical energy, Cl^- an example of an impermeant anion, and ATP and ADP examples of anions which are translocated by exchange-diffusion processes.

Such a mechanism could also account for the very large effect of temperature on the rates of transport of some substances across the mitochondrial membrane so well documented in the cases of ADP and ATP[21]. Second, though the oxidase and reductase sites are shown in Fig. 2 near the inner and the outer surfaces of the membrane, respectively, this is not essential. All that is necessary is that at the reductase site the external fluid is available for contact with the cytochrome *c* while at the oxidase site the internal fluid is similarly available, and that at each site the carrier molecules are properly oriented to carry out the exchanges postulated here.

It should be emphasized that the model depicted in Fig. 2 does not present any explicit picture of the coupling of the energy-yielding mechanism with cation transport. Moreover, the evidence presented for this scheme is entirely indirect, consisting only of a correlation between the binding properties of cytochrome *c* for various ions, and the transport properties of the mitochondrial inner membrane in which cytochrome *c* is located. Even that evidence is incomplete, for only some seventeen substances and one cytochrome *c* have been examined out of the several dozen ions and the several varieties of mitochondria studied for their transport phenomena. Direct evidence could in principle be obtained by finding which carrier systems are depressed in mitochondrial preparations from which the original cytochrome *c* has been removed, and by showing that when the cytochrome *c* is replaced by the protein from another species the ion transport properties are now those of he re-incorporated cytochrome *c*, not those of the original mitochondria. Differences in the transport properties of mitochondria possibly suited to such experiments have been reported for Ca^{2+} accumulation in mammalian and yeast mitochondria[13,22,23], and for succinate transport in mammalian and fly mitochondria[16,24]. These and similar experiments are predicated on the assumption that one can obtain mitochondria sufficiently depleted of cytochrome *c* to make the rate or inhibition of transport of carried molecules critically dependent on the amount of the presumed carrier, within the ranges measurable by available techniques. Such tests are being attempted and X-ray crystallography is being used to locate the more tightly bound ions on the protein surface.

Metabolic and Evolutionary Consequences

Finally, if the above hypothesis is correct, cytochrome *c* may act as a mitochondrial probe of the metabolic state of the cell outside the organelle and may possibly represent the controlling device which integrates the activities of the mitochondrion with the energy metabolism of the rest of the cell. The controlling influences of the ATP/ADP ratio[25] and of citrate[26] have been amply documented and need no elaboration here. It is yet to be determined whether other ionic substances possibly transported via cytochrome *c* also function in metabolic control systems. The present hypothesis should also lead to predictions concerning the transport characteristics of the mitochondria of different species on the basis of the binding properties of their cytochrome *c* and, in general, cytochrome *c* binding studies should make it possible to determine the action of many substances on mitochondrial transport. From the evolutionary point of view, it is particularly interesting that as the cytochromes *c* of different species have quantitatively very different binding properties for some impermeant anions[12], it is likely that such differences will extend to other substances bound by the protein. Thus as the transport of such ions across the mitochondrial membrane is of metabolic importance, the differences in the structures of the cytochromes *c* would have definite functional repercussions. One could then hope eventually to understand in detail how the structures of the different cytochromes *c* are adapted to the metabolic activities of the particular species in which they reside.

We thank Dr A. Schejter for making available and discussing his studies of the effect of specific ion binding on the oxidation-reduction reaction of cytochrome *c*, Drs W. M. Fitch, I. M. Klotz, H. Lardy, L. Lorand and E. C. Slater for discussion of various sections of this work and numerous suggestions, and Drs R. E. Dickerson, W. M. Fitch and L. Smith for permitting citation of their unpublished work.

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Stereochemistry of Cooperative Effects in Haemoglobin

by

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The oxygenation of haemoglobin is accompanied by structural changes in the subunits triggered by shifts of the iron atoms relative to the porphyrin and, in the β-subunits, also by the steric effect of oxygen itself. The oxygen-free form is constrained by salt-bridges which are broken by the energy of haem–haem interaction with the release of H^+. 2,3-Diphosphoglycerate may add to the constraints by being stereochemically complementary to a site between the β-chains; this complementarity is lost on oxygenation.

Haem–Haem Interaction and the Problem of Allostery

I WISH to propose a stereochemical mechanism of haem–haem interaction which is based partly on a comparison of the atomic models of native deoxy and methaemoglobin of horse[1–3], and partly on an X-ray study of a haemoglobin derivative in which the transition from the quaternary structure of oxy- to that of deoxyhaemoglobin was inhibited (my unpublished results). For a description of the anatomy of the haemoglobin molecule and of the change in quaternary structure that accompanies its reaction with ligands, I refer the reader to previous publications[1,4,5]. The basic features of the molecule are shown in Figs. 1 and 2. I start by summarizing the nature of haem–haem interaction and then go on to describe relevant structural and other observations. I then explain the proposed mechanism, discuss its implications for the size of the functional unit of haemoglobin, and finally apply it to the theory of allostery. In the following article I shall deal with the stereochemical mechanism of the Bohr effect and of the regulatory function of 2,3-diphosphoglycerate.

Nature of Haem–Haem Interaction

This is best explained by analogy with the biblical saying "To him who hath shall be given"[6]. Suppose we have two haemoglobin molecules *A* and *B*, *A* holding three molecules of oxygen already and *B* holding none; to which of them will an approaching free oxygen molecule stick? The chances are 70 to 1 in favour of *A*. If *Y* is the fractional saturation of haemoglobin with oxygen, *p* its partial pressure and *K* and *n* are constants, then the oxygen equilibrium curve can be roughly represented by Hill's equation

$$Y = \frac{Kp^n}{1 + Kp^n}$$

which corresponds to the equilibrium $Hb_n + nO_2 \rightleftharpoons Hb_n(O_2)_n$, Hb representing the haemoglobin concentration in equiva-

lents of iron. Hill's equation fits the experimental data best for a Hill's constant $n \sim 2{\cdot}7$; this value is independent of pH and temperature. More accurately, the oxygen equilibrium curve can be represented by Adair's equation which contains four separate equilibrium constants for the reactions of successive haems with oxygen[7,8]. Monod, Wyman and Changeux[9] have shown that on the basis of simplifying assumptions two arbitrary constants give reasonable agreement with observation, and have used haemoglobin as a model of an allosteric enzyme.

Wyman pointed out the main features which any model of haem–haem interaction would have to satisfy[10]. These are illustrated by his logarithmic plot of the oxygen equilibrium curve which has a slope of unity at very low and very high oxygen concentrations and a slope of about three in the middle range, corresponding to Hill's constant. The total free energy interaction ΔF can be calculated from the perpendicular distance between the two asymptotes and normally lies between 2·5 and 3 kcalories per iron atom. Wyman writes: "In principle, the interaction energy might equally well be interpreted as a stabilizing energy between the unliganded or the liganded haems. In the former case we might say, figuratively, that the first oxygen enters the molecule with more difficulty than the second owing to the necessity of breaking up a pre-existing partnership between the haems; in the latter case the second oxygen enters more easily owing to the decoying effect of the first"[10]. We now know that the former alternative is correct, because the oxygen affinity of isolated α and β subunits corresponds to that of the fully combined oxyhaemoglobin, showing that the low oxygen affinity of deoxyhaemoglobin must be the result of constraints imposed on this form. This is confirmed by its structure.

The physiological purpose of haem–haem interaction lies not so much in ensuring an increased oxygen affinity as successive molecules of oxygen combine, as in making the affinity fall as successive oxygen molecules dissociate. This is because the partial pressure of oxygen in the tissues is not much lower than in the lungs. If haemoglobin had a hyperbolic oxygen equilibrium curve, not more than a small fraction of the oxygen carried by it would be drawn off, with the result that man would asphyxiate even if he breathed pure oxygen. The Bohr effect and 2,3-diphosphoglycerate both increase the efficiency of respiratory transport further by lowering the oxygen affinity of haemoglobin in the tissues.

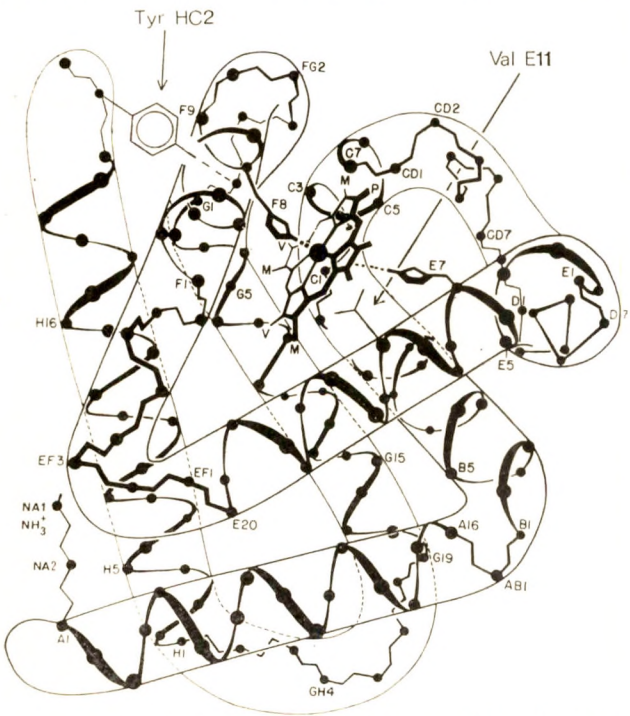


Fig. 1. Diagrammatic sketch showing the course of the polypeptide chain in the β subunit. In going from the amino to the carboxyl end, helical regions are denoted A to H; non-helical residues at the amino end NA, and at the carboxyl end HC. Non-helical regions between helices are denoted as AB, BC and so on. The haem lies in a pocket between helices E and F. The conformation of the α subunit is closely similar, except for the addition and deletion of certain residues.

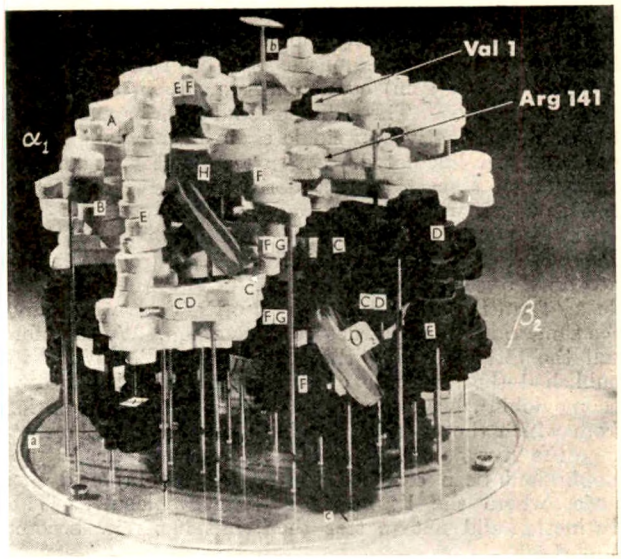


Fig. 2. View of the oxy or methaemoglobin molecule showing the α-chains in white, the β-chains in black and the haems in grey. The sign in the centre indicates the two-fold symmetry axis which runs down the water-filled central cavity. The terminal regions of the α-chain are near at the top, those of the β-chains near the bottom of the molecule. In the deoxy form, the cross-links between the α-chains lie in the central cavity; those between lysine $40\alpha_1$ and histidine $146\beta_2$ lie near the letter H of the label HS in the lower centre of the molecule. The label HS indicates the position of the reactive cysteins 93β. On deoxygenation, the FG region of the α_1-chain turns away from, and that of the β_2-chain turns towards, the observer.

Movements of Iron Atoms relative to the Porphyrin

X-ray studies of iron porphyrin compounds have shown that the length of the bond from the iron atom to the nitrogen atoms of the porphyrin ring varies between 2·061 Å in high spin ferric and 1·99 Å in low spin ferric compounds[11,12]. In metal-free porphyrin the distance from the centre of the ring to the nitrogen atoms is 2·01 Å, smaller than the Fe–N distance in high spin, and larger than that distance in low spin compounds. In six-coordinated low spin ferric porphyrin complexes, therefore, the iron atom tends to lie within 0·05 Å of the plane of the four nitrogen atoms[12]. In five-coordinated high spin ferric compounds, on the other hand, the iron atom is forced to lie out of that plane by distances of between 0·38 and 0·47 Å (ref. 11) (Table 1).

No crystal structures of ferrous porphyrins have been published, but the radius of ferrous iron in low spin compounds is believed to be the same, within 0·02 Å, as that in low spin ferric compounds, so that the iron atom in low spin ferrous compounds should also lie within 0·05 Å of the plane of the nitrogen atoms. The radius of the high spin ferrous iron, on the other hand, exceeds that of the high spin ferric by 0·12 Å (ref. 13). If we add this to the Fe–N distance of 2·061 Å in the five-coordinated high spin ferric form, we obtain an Fe–N distance of 2·18 Å. This would place the iron atom 0·83 Å out of the plane of the four nitrogen atoms.

Of ferrous haemoglobin or myoglobin derivatives, the deoxy form is high spin and five-coordinated, so that we should expect the iron atoms to lie about 0·8 Å out of the plane of the four nitrogen atoms, while oxy and carbonmonoxy forms are both low spin and six-coordinated so that the iron atoms should lie in that plane. All ferric derivatives are six-coordinated, the acid and fluoride met derivatives being high spin and the remainder

low spin. In agreement with expectation, the iron atoms in (high spin) acid metmyoglobin and meterythrocruorin lie 0·3 Å from the plane of the porphyrin ring[14,15], while those in a (low spin) cyanomethaemoglobin of lamprey lie in the plane[16]. Three-dimensional Fourier analyses of the deoxy derivatives of single chain haemoglobins have not yet been calculated. Difference Fourier analyses of deoxy with respect to met derivatives give conflicting results which will be discussed elsewhere.

The quaternary structure of mammalian haemoglobin depends only on the coordination of the iron atom but not on its valency or spin state. The structure of the deoxy form is unique, whereas all other derivatives have the same quaternary structure in common. We therefore call the latter simply "the liganded form". When we published the structure of that form we called it oxyhaemoglobin[1], because this was the state of the solution from which the crystals had been prepared, but by the time the X-ray data were collected, most of the haemoglobin had been oxidized to the (high spin) acid met state, where the ligand is a water molecule. This distinction did not matter then, but has now become important.

When Dr H. Muirhead and I measured the atomic coordinates of methaemoglobin using a plumb line and an electron density map drawn on the scale of 1 Å = 5 mm we did not believe our resolution of 2·8 Å to be sufficient to determine the displacement of the iron atoms out of the plane of the porphyrin ring[1]. But by redrawing the maps on a scale of 1 Å = 2 cm and viewing them in Dr F. M. Richards's reflecting box[17] it was clear that we had underestimated the information contained in them. Dr W. Bolton and I had no difficulty in fitting the porphyrin exactly to the contours. On measuring the perpendicular distance from the plane of the porphyrin ring to the observed iron peak, we found it to be 0·3 Å in both the α and β subunits, in exact agreement with the displacement of the iron atom observed in metmyoglobin[14] and meterythrocruorin[15]. Performing the same experiment with the electron density maps of deoxyhaemoglobin, on the other hand, revealed a displacement of 0·75 Å in both the α and the β subunits, in close agreement with the value of 0·83 Å calculated above. Both displacements are on the side of the proximal histidine (F8). In these measurements we kept the porphyrin ring flat. When we tried to make it slightly domed, as it is in haemin chloride[18], the displacement of the iron atoms from the plane of the four nitrogens came out smaller but their displacements from the mean plane of the light atoms remained the same.

According to R. J. P. Williams, theory predicts that the length of the bond linking the iron atom to Nε of the proximal histidine will also increase by 0·1–0·2 Å in going from a low spin to a high spin ferrous derivative. According to J. L. Hoard it should remain unchanged[11]. Our X-ray data are indecisive. Depending on its exact value, the distance of the proximal histidine from the plane of the porphyrin ring should increase by 0·75–0·95 Å. Such a shift, occurring in the transition from oxy to deoxyhaemoglobin, would be sufficient to force drastic changes in the contacts between the porphyrin ring and the tightly packed amino-acid side chains of the surrounding globin. Hoard first predicted that the reaction with oxygen should be accompanied by a substantial movement of the iron atom relative to the porphyrin ring, and that this could lead to cooperative movements in the protein framework of the haemoglobin molecule[30].

Room in the Ligand Pocket

Haem is attached to globin by a covalent bond extending from the iron atom to Nε of the proximal histidine F8. On the distal side, not directly linked to the iron atom, lie histidine E7 and valine E11 (Fig. 1). The ligand comes to lie between the iron atom and these two residues. Further removed from the iron atom 60 atoms of the globin are in van der Waals contact with the porphyrin ring (Fig. 2 of ref. 1).

We first select the smallest ligand that produces full haem–haem interaction. This is a hydroxyl ion, with a van der Waals radius of 1·5 Å, which becomes attached to the iron atom in the oxidation of deoxy to methaemoglobin at alkaline *p*H. A hydroxyl ion, placed at the expected distance of 2·1 Å from the iron atom in either the α or the β subunits of the liganded form or in the α subunit of the deoxy form, can be accommodated without making short contacts with neighbouring amino-acid side chains. In the β subunits of the deoxy form, on the other hand, the γ methyl of valine E11(67) would come to lie within 2·5 Å of the hydroxyl ion, which is 1 Å less than the sum of the van der Waals radii, so that there is not enough room for even the smallest ligand, let alone an oxygen molecule. Direct comparison of the electron density maps of the β-chains confirmed that the distance between the prophyrin ring and valine E11 shrinks by about 1 Å in going from the met, or oxy, to the deoxy form. Similarly, the difference electron maps of deoxy minus met-BME-haemoglobin, to be described, show a striking movement of valine E11 towards the haem group. These observations demonstrate that in the deoxy form of the β-chains the valines E11 must move relative to the porphyrin ring before the iron atoms can react, and that in going to the oxy form the distance between the porphyrin and helix E widens to make room for ligands. The width of the pocket in the α-chain, on the other hand, shows no measurable change, and has room for ligands in both forms.

Role of the C-terminal Residues

In oxy or methaemoglobin the C-terminal residues of all four chains have complete freedom of rotation, and the penultimate tyrosines have partial freedom in the sense that they spend only a small fraction of their time in their bound position, between helices F and H. The experimental evidence for this statement will be reviewed in the following article. In deoxyhaemoglobin, on the other hand, each of the C-terminal residues is doubly anchored by salt-bridges. The α-carboxyl of arginine HC3(141)α_1 is linked to the α-amino group of valine NA1(1)α_2, and its guanidinium group to aspartate H9(126)α_2. The α-carboxyl of histidine HC3(146)β_1 is linked to the ε-amino group of lysine C5(40)α_2 and its imidazole to aspartate FG1(94)β_1 (see Figs. 1 and 2 of the following article). All four penultimate tyrosines are firmly anchored in pockets between helices F and H, partly by van der Waals contacts with the helices and partly by hydrogen bonds between their OH groups and the carbonyls of valines FG5 (refs. 2 and 3). The conformation is such that the tyrosines cannot be displaced from

Table 1. DIMENSIONS OF IRON PORPHYRIN COMPLEXES (Å)

Compound	Centre of ring to N_{porph}	Fe–N_{porph}	Fe–axial ligands	Out of plane displacement	Fe Valency	Spin
Porphyrin[11]	2·005–2·006					
Chlorohaemin[15]	2·008	2·062	Cl at 2·218	0·475	3+	High
Acid metmyoglobin, meterythrocruorin and methaemoglobin			Nε at 1·9	0·3	3+	High
			H_2O at 2·1			
Deoxyhaemoglobin		**~2·18 (calc)**	**Nε at 1·9–2·1?**	**0·75**	**2+**	**High**
Bis(imidazole)-αβγδ-tetraphenyl-porphinate Fe(III)Cl (ref. 12)	1·989	1·989	Nε at 1·957 and 1·991	<0·01	3+	Low
Cyanomethaemoglobin of lamprey[16]				<0·1	3+	Low
CO erythrocruorin[15]				~0·05	2+	Low

their pockets without also displacing the C-terminal residues and rupturing their salt-bridges. I shall show this property to be vital for the entire mechanism of interaction between the subunits (Fig. 3).

Enzymatic removal of the C-terminal residues affects haem–haem interaction. Amputation of histidine HC3(146)β has the smallest effect, reducing Hill's constant from 2·7 to 2·5 (ref. 19). But Dr J. Greer pointed out to me that this does not mean that the salt-bridge of its carboxyl group with lysine C5(40)α is unimportant, because the α-carboxyl group of the histidine which normally forms the salt-bridge can be replaced in the truncated haemoglobin by the α-carboxyl of tyrosine HC2(145). Displacement of the C-terminal histidine by N-substituted maleimides, attached to cysteine F9(93)β, reduces Hill's constant to about 2·0 (ref. 31). In these derivatives the salt-bridge with lysine C5(40)α is not made, but the remaining structure of the α and β-chains is left undisturbed in both the oxy and deoxy forms. Removal of arginine HC3(141)α diminished Hill's constant to 1·7; removal of both the histidine and the arginine reduces Hill's constant to near unity as well as inhibiting most or all of the Bohr effect (ref. 19 and unpublished results of J. V. Kilmartin, J. Wootton and J. A. Hewitt).

Why should removal of these residues inhibit interaction? Because they are free in oxyhaemoglobin, they clearly serve no function there. Even in deoxyhaemoglobin their positions are such that they could exert no direct influence on the conformation of the haem groups or their immediate environment. We are driven to conclude that their importance for haem–haem interaction lies in the constraining crosslinks they form between the subunit in deoxyhaemoglobin.

This is confirmed by X-ray studies of the derivatives just described which were crystallized by Dr J. V. Kilmartin. Human Des-His-146β and Des-Arg(141)α-deoxyhaemoglobin each crystallize isomorphously with normal human deoxyhaemoglobin. Des-His-(146)β + Des-Arg-(141)α deoxyhaemoglobin, on the other hand, crystallizes in a form closely related to normal oxyhaemoglobin. This shows that in the absence of the constraining salt-bridges the quaternary deoxy structure is unstable, even if all four chains are in the deoxy state.

Conformational Changes within the α and β Subunits

Before the quaternary structure of the haemoglobin molecule can change from the deoxy to the oxy form, the reaction of the iron atoms with oxygen or other ligands may cause the tertiary structure of the individual subunits to alter, so leading to the rearrangement of the subunits. These initial changes cannot easily be observed on electron density maps of the fully liganded or the fully deoxygenated forms. I therefore tried to detect them in a haemoglobin derivative whose quaternary structure is locked in the oxy form.

The lock is *bis*-maleimido-methyl ether (BME), a bifunctional reagent which inhibits all cooperative effects in haemoglobin by linking cysteine F9(93) to histidine FG4(97) in the same β-chain[20–22]. The reagent, which lies in the $\alpha_1\beta_2$ contact, also blocks the entry of the penultimate tyrosines HC2(145) of the β-chains into their pockets between helices F and H, and causes small changes in the remainder of the β-chains and the C-helix of the α-chains[22]. Difference electron density maps of met minus deoxy BME-haemoglobin should therefore show the initial changes undergone by the subunits on reaction with ligands.

Crystals of horse met and deoxy BME-haemoglobin are isomorphous with crystals of the native methaemoglobin, so that the phase angles of the X-ray reflexions should be approximately the same. The crystals had been grown in the acid met form. I reduced them, collected X-ray data to 3·5 Å resolution and, with the help of Dr J. Greer, calculated difference Fourier syntheses.

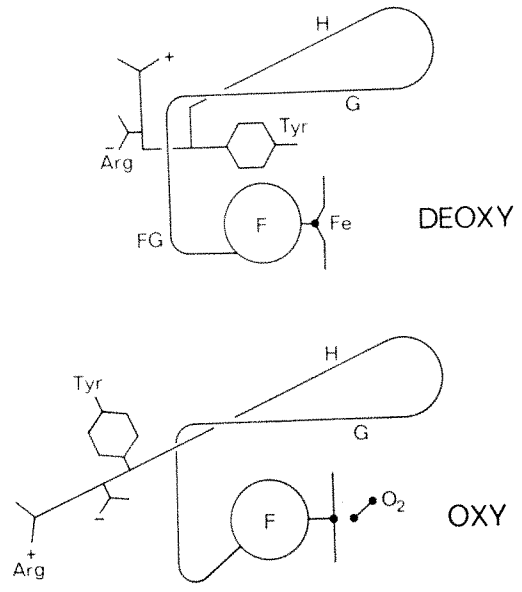


Fig. 3. Diagrammatic sketch showing the change in tertiary structure of the haemoglobin subunits on reaction with ligands. Movement of the iron atom into the plane of the porphyrin ring causes a movement of helix F towards helix H, which expels tyrosine HC2 from its pocket between the two helices.

When acid methaemoglobin is reduced, the haem-linked water molecule is removed and shows up as a positive peak in a difference map of met minus deoxy. The strength of that peak is a measure of the fraction of the iron atoms in the crystal that have been reduced. I first used ferrous citrate and found it had reduced most of the iron atoms in the β subunits, but very few of those in the α subunits. I next used $Na_2S_2O_4$ which reduced all the iron atoms in the β subunits and most of those in the α subunits. Ligands must be removed from the β-chains more easily than from the α-chains.

Difference electron density maps of met with respect to deoxymyoglobin and erythrocruorin reveal no significant changes in the conformation of the globin chain or movements of the haem relative to the globin. By contrast, the difference maps of met minus deoxy BME-haemoglobin show striking changes.

In the α-chains, the positive peak representing the entry of the haem-linked water molecule is matched by a negative one of the same magnitude representing the expulsion of tyrosine HC2(140) from its pocket between helices F and H. There is a further negative peak in the position of the amide group linking tyrosine HC2(140) to arginine HC3(141), corresponding to the fixed position which that amide normally takes up in native deoxyhaemoglobin. There is no positive peak corresponding to this amide, however, showing that it is rotating as freely in BME met as in native methaemoglobin. There are no peaks corresponding to either the side chain or the carboxyl group of arginine HC3(141), showing that these rotate freely in both forms of BME-haemoglobin.

There is a pair of positive and negative peaks indicating that helix F moved inwards, towards the centre of the molecule, narrowing the pocket between it and helix H. This looks like the movement responsible for the expulsion of tyrosine HC2 (Fig. 3). Finally, there are pairs of positive and negative peaks on either side of the propionic acid side chains of the haem group, showing that its inclination is becoming less steep in the liganded form (Fig. 2).

In the β-chains the entry of tyrosine HC2(145) into its pocket between helices F and H is blocked by the BME group, but the difference Fourier indicates a similar inward movement of helix F, leading to a narrowing of the pocket between it and helix H, as in the α-chain, evidently designed to expel the tyrosine if it were there.

The most prominent feature is a negative peak in the position of valine E11(67), showing the widening of the space between the porphyrin ring and helix E on ligand binding already referred to. There are also peaks near the haem group (when viewed as in Fig. 18 of refs. 4, 5) showing that its tilt becomes less steep in the liganded form, that is, its iron atom has moved away from valine E11.

The $\alpha_1\beta_2$ contact shows signs of strain as though on dissociation of ligand the residues were trying to move towards the deoxy conformation; that is, a set of positive and negative peaks which correspond to a movement towards the observer of the β_2 subunit in Fig. 2. It also looks as though the entry of the tyrosine HC2(140)α_1 into its pocket pressed on the indole ring of tryptophan C3(37)β_2, causing it to tilt over and to press on proline C2(36)β_2, and thus helping to push the β-chain in the required direction (see Fig. 15 of refs. 4, 5).

These results show that on binding of ligand to the α subunit, helix F moves so as to expel tyrosine HC2(140) from its pocket. A similar movement of helix F occurs in the β-chain. On removal of ligand, residues at the $\alpha_1\beta_2$ contact show signs of strain moving them towards the deoxy formation, even though the required sliding at the contact is inhibited by the BME.

I then examined the atomic models of oxy and deoxyhaemoglobin to see if the change in the width of the tyrosine pocket is detectable there, and found that, on going from deoxy to oxy, its width had shrunk by 1·3 Å in the α and by 2 Å in the β subunits. Model building shows that the tyrosines do not fit into the pockets of the oxy form without making short contacts with atoms of helices F and H.

The differences in quaternary structure are as previously described[23], with small shifts of not more than 1 Å at the pair of subunit contacts $\alpha_1\beta_1$ and $\alpha_2\beta_2$, and large relative shifts of up to 7 Å at the contacts $\alpha_1\beta_2$ and $\alpha_2\beta_1$. Apart from the salt-bridge just mentioned between histidine HC3(146)β_1 and lysine C5(40)α_2, a small increase in the number of van der Waals interactions seems to occur on oxygenation; the number of hydrogen bonds at either pair of contacts probably remains unchanged. Neither of the two forms contains any direct links between the two β-chains, but it will be shown in the following article that these are furnished in the red cell by 2,3-diphosphoglycerate.

At 5·5 Å resolution we had detected no changes in the tertiary structures of the individual chains. At 2·8 Å resolution various small shifts have become apparent, some distances between residues in neighbouring helices having changed by as much as 2–3 Å, which suggests that each subunit undergoes a small concerted change in tertiary structure. This possibility was first suggested by Dr A. D. McLachlan after he had built space filling models of myoglobin and of the two haemoglobin chains. He found the side chains in the interior of the myoglobin molecule to be tightly packed, which made the model rigid, while those in the haemoglobin subunits seem more loosely packed, giving the helices some freedom of movement. The observed changes will be described elsewhere.

Where is the force that changes the quaternary structure applied ? The evidence is overwhelmingly in favour of the contacts $\alpha_1\beta_2$. During the change of quaternary structure the contacts $\alpha_1\beta_1$ remain largely passive, whereas large movements occur at $\alpha_1\beta_2$. All but one of the mutations replacing residues at the $\alpha_1\beta_2$ contact diminish haem–haem interaction, whereas mutations at the $\alpha_1\beta_1$ contact do not. Nearly all residues at the $\alpha_1\beta_2$ contact are invariant throughout the vertebrates (except lamprey), while many of those at the $\alpha_1\beta_1$ contact are variable. The BME difference Fourier shows the beginnings of the changes that would make the contact jump from the oxy to the deoxy conformation.

The contact $\alpha_1\beta_2$ is dovetailed, so that the CD region of one chain fits into the FG region of the other (see Fig. 8 of ref. 1 or Fig. 14 of refs. 4, 5). During the change of quaternary structure the two subunits rotate relative to each other so that the dovetailing of CDβ with FGα remains much the same, whereas that of CDα with FGβ changes. The change works like this: in oxyhaemoglobin a knob consisting of the side chain of threonine C3(38)α fits into a notch made up of the main chain of valine FG5(98)β. In deoxyhaemoglobin that same notch is occupied by the side chain of threonine C6(41)α —that is, the one protruding from the next turn of the C helix. At the same time the hydrogen bond linking aspartate G1(94)α to asparagine G4(102)β in oxyhaemoglobin is replaced by a hydrogen bond between tyrosine C7(42)α and aspartate G1(99)β in deoxyhaemoglobin[3]. If there were an intermediate quaternary structure, there could be no dovetailing, because knobs would fit against knobs; nor could any hydrogen bond be made to stabilize it. On stereochemical grounds its existence therefore seems unlikely. Another type of intermediate would be one where the $\alpha_1\beta_2$ contact, say, has clicked to the oxy conformation and the $\alpha_2\beta_1$ contact has remained in the deoxy conformation. In such an asymmetric intermediate all four contacts would be severely stressed, which would make it very unstable. But in view of the high speed of the reaction of haemoglobin with oxygen the occurrence of such an intermediate cannot be excluded.

Spectroscopic evidence favours the existence of an intermediate, but not necessarily one with a quaternary structure different from the oxy and deoxy forms. McConnell and his group find the ESR spectra of spin-labelled oxy and deoxyhaemoglobin to be markedly different[24]. Their spin label is attached to cysteine F9(93)β which lies near the tyrosine pocket and the $\alpha_1\beta_2$ contact, so that the label would sense changes in the conformation of the β and the α-chains as well as shifts at that contact (Fig. 2). If haemoglobin existed in only two alternative conformations, then the ESR spectra at successive stages of oxygenation should exhibit common isosbestic points. Because this is not the case with one of the labels used, the authors argue that there must be an intermediate structure. I shall suggest what type of structure this might be.

We must now consider the tertiary structure of the individual subunits which changes on uptake of ligands, as the BME experiment has proved. On uptake of oxygen, the tertiary structures of the individual subunits click to the oxy conformation, but the quaternary structure may remain in the deoxy conformation until several of the subunits have reacted with oxygen; consequently, subunits in the oxy conformation will be constrained in the quaternary structure of deoxy, or vice versa. Will these constrained subunits take up an intermediate conformation ?

The BME difference Fourier should tell, because it shows deoxy subunits locked in the quaternary structure of oxy. When I compared that difference Fourier with the actual differences between the native oxy and deoxy structures, I found it contained many, but not all, of the expected pairs of positive and negative peaks. This suggests but does not prove intermediate tertiary structures. Direct comparisons of the oxy and deoxy structures are still at too early a stage to judge whether intermediate tertiary structures are probable or not on stereochemical grounds. So the X-ray evidence is not yet clear.

Ogawa and Shulman have examined NMR spectra of mixed hybrids in which one pair of chains is kept locked in the liganded form as the cyanomet derivative, and the other pair is free to be oxygenated or deoxygenated. They find that deoxygenation of $(\alpha O_2\beta^+CN)_2$ in phosphate buffer did not show any effect on the paramagnetically shifted proton resonances of the β-haem and its immediate environment, while marked changes can occur in the spectrum of the α-haem on deoxygenation of $(\alpha^+CN\beta O_2)_2$. They interpret this result to mean that the former derivative remains in the quaternary oxy structure throughout

the reaction, while the latter may change to the quaternary deoxy structure, where the liganded α-chains are subject to strains extending from the subunit boundaries right to the haem groups[25]. The magnitude of these strains seems to be too small, however, to contribute significantly to the internation energy between the haems.

In my proposed mechanism I shall assume that each subunit can take up only two alternative stable tertiary structures, the deoxy and the liganded form. But these structures may be subject to strains when subunits are confined in the "wrong" quaternary structure.

Mechanism of the Conformational Change

I shall discuss first the conformation changes in the individual subunits that follow the binding of ligand; next, the change in quaternary structure caused by them; and finally, the probable order of the steps of the reaction with ligands.

What is the trigger that sets the conformational changes in motion? The distances between the haem groups are far too large (25–37 Å) for electromagnetic interactions to be effective, which suggests that the trigger is stereochemical. It was not clear, however, whether the changes are triggered by some deformation of the haem group or by the ligand prising the haem pocket apart. Consider first the α subunit. Because a ligand finds ample room in its haem pocket in both the oxy and the deoxy form, and the width of the pocket does not change significantly, the latter mechanism can be safely excluded. We have seen that the transition from deoxy to oxyhaemoglobin involves a movement of the proximal histidine towards the plane of the porphyrin amounting to 0·75–0·95 Å. The transition from deoxy to acid methaemoglobin involves a smaller movement, of 0·45–0·65 Å, in the same direction. Movements of this magnitude should be sufficient to set the changes in tertiary structure of the reacting subunit in motion, because the iron atom is rigidly linked to histidine F8 and the porphyrin is in contact with about sixty atoms of the globin which is flexible and can give. The way it gives can be seen in the difference electron density maps of BME deoxy versus methaemoglobin, which showed, among other things, that helix F moves towards the centre of the molecule and expels tyrosine HC2(140) from its pocket between helices F and H (Fig. 3). The expelled tyrosine must pull arginine HC3(141) with it, thus breaking its salt-bridges with the opposite α subunit and releasing Bohr protons (see Fig. 2 of the following article).

We may now turn to the β subunits. Here the trigger is clearly composite. Before the ligand can reach the iron atom, it must prise open the haem pocket (or rather, wait until thermal vibrations provide the activation energy needed to open it); reaction with the haem then moves the iron into the plane of the porphyrin ring. The accompanying changes in tertiary structure have one feature in common with those observed in the α-chain: helix F moves towards the centre of the molecule, narrowing the pocket between it and helix F and presumably expelling tyrosine HC2(145). The validity of this vital point is supported by much indirect evidence concerning the inhibitory effects of reagents which either remove that tyrosine or prevent its entry into the pocket, but it will have to be checked by direct experiment. The expelled tyrosine must pull histidine HC3(146) with it and break its salt-bridge to aspartate FG1(94) (see Fig. 1 of the following article). The salt-bridge to lysine C5(40)α may or may not have been broken already before the β-chain could have reacted with a ligand.

We shall now examine the likely order of the steps involved in the reaction with ligands and the ensuing conformational changes (Fig. 4). In deoxyhaemoglobin, the α subunits have room for ligands, but the β subunits do not, so that the α subunits are likely to react first. The argument is not rigorous, for relative rates of combination with oxygen of the different subunits must depend on the total activation energy needed to change their tertiary structure from the deoxy to the oxy state—which cannot be readily predicted. Low ligand affinity of the β subunits in the deoxy tetramer is not supported by kinetic data, but further work is needed to test this point. In any case the cooperative mechanism I propose would not be affected if the order of steps turned out to be the reverse of the one given below or if it proved to be random.

Reaction of $Fe\alpha_1$ with oxygen causes tyrosine HC2(140)α_1 to be expelled from its pocket and the links between arginine HC3(141)α_1 and the α_2 subunit to be broken, with the release of Bohr protons. $Fe\alpha_2$ reacts next, followed by the expulsion of its tyrosine and the rupture of the links between the C-terminal arginine and the α_1 subunit, with further release of Bohr protons. By now four of the six salt-bridges constraining the deoxyhaemoglobin tetramer will have been broken, with a resulting change in the allosteric equilibrium constant in favour of the quaternary oxy structure. For illustrative purposes I shall assume that this is of a magnitude sufficient to change the quaternary structure to the oxy form. (In fact, the change might occur at any stage in the reaction depending on external factors such as *p*H and the concentrations of CO_2 and organic phosphates.) The $\alpha_1\beta_2$ and the $\alpha_2\beta_1$ contacts now give way and the tetramer clicks to the oxy form, breaking the remainder of the constraining salt-bridges, that is, the ones between lysines C5(40)α and histidine HC3(146)β, and the ones between DPG and the two β subunits. This step leads to the liberation of DPG but does not release Bohr protons.

We now have an intermediate with the quaternary structure of oxyhaemoglobin; the two-fold symmetry of the molecule is conserved; the haem and its immediate environment in the α subunit have the oxy conformation, and those in the β-chains have the (strained) deoxy conformation; that is, the tyrosines HC2(145)β are still in their pockets, keeping the ligand sites obstructed by valine E11(67). A structure of this type could be the intermediate sensed by McConnell's spin label. The change of quaternary structure has halved the activation energy needed to expel the tyrosines HC3(146)β from their pockets, because it has broken the salt-bridges between the C-terminal histidines and the α subunits, so that only the internal salt-bridges between the C-terminal histidines and aspartates of the same β-chain now remain to hold the tyrosines in place, with a resulting increase in oxygen affinity (see Fig. 1 of the following article).

The iron atoms of the β-chains now react with ligand, each reaction being accompanied by the expulsion of a tyrosine and the rupture of one of the salt-bridges just mentioned, causing release of the last Bohr protons.

The essence of the mechanism may be summarized as follows. The haem group is so constructed that it amplifies a small change in atomic radius, undergone by the iron atom in the transition from the high spin to the low spin state, into a large movement of the haem-linked histidine relative to the porphyrin ring. This movement, and the widening of the haem pocket required in the β chain, triggers small changes in the tertiary structure of the reacting subunits, which include a movement of helix F towards helix H and the centre of the molecule. Exactly how leverage from the iron atoms pushes helix F in that direction is not yet clear, but this movement can be observed to narrow the pocket between helices F and H so that the penultimate tyrosine is expelled. The expelled tyrosine pulls the C-terminal residue with it, rupturing the salt-bridges that had held the reacting subunit to its neighbours in the deoxy tetramer. The rupture of each salt-bridge removes one of the constraints holding the molecule in the deoxy conformation and tips the equilibrium between the two alternative quaternary structures some way in favour of the oxy conformation. In this conformation the oxygen affinity is raised because the subunits are no longer constrained by the salt-bridges to

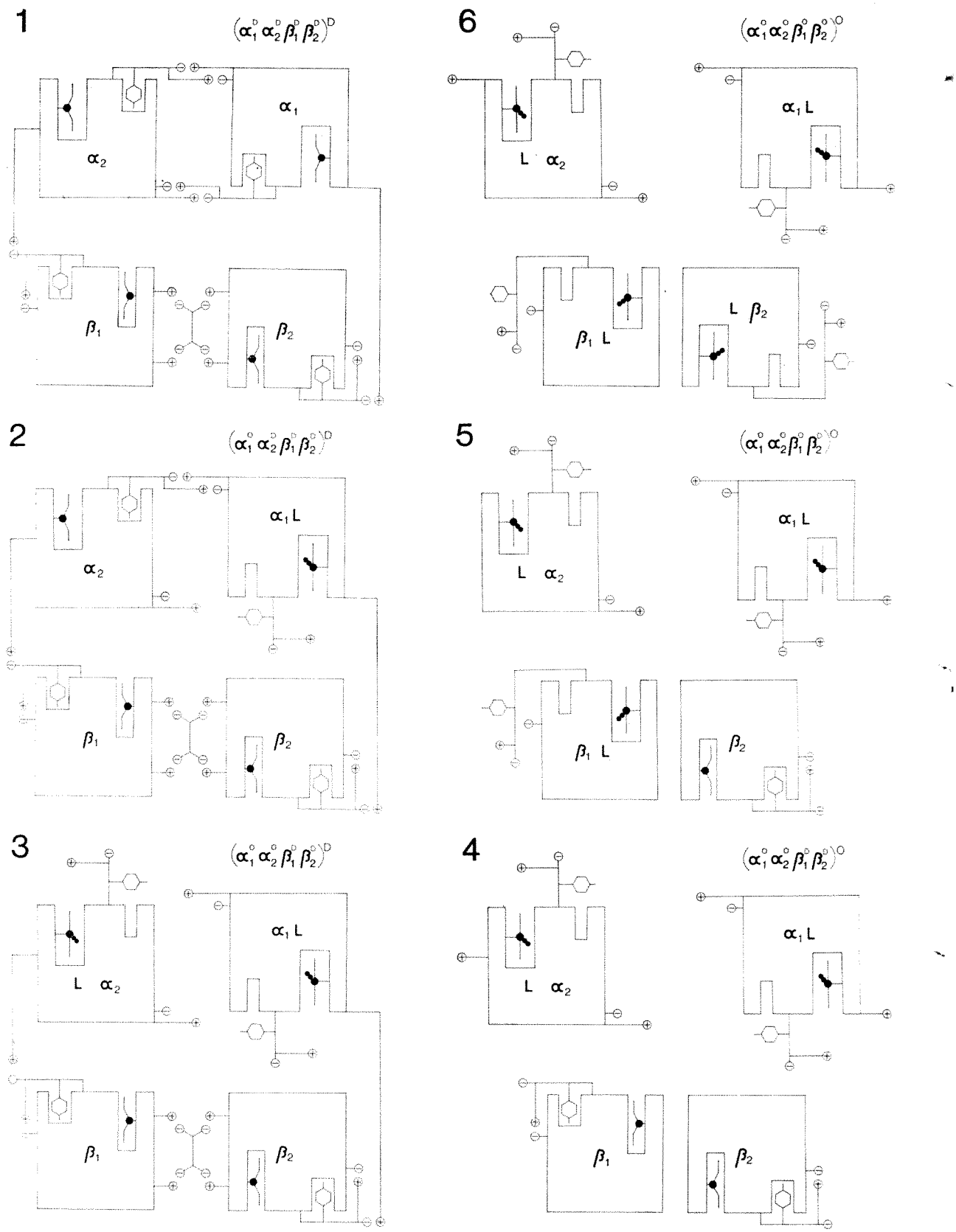


Fig. 4. Diagrammatic sketch showing one possible sequence of steps in the reaction of haemoglobin with oxygen. **1**, Deoxyhaemoglobin with all salt-bridges intact and with one molecule of DPG clamped between the two β-chains. At steps 1–2 and 2–3, the α-chains are oxygenated. The tyrosine pockets are narrowed, the tyrosines expelled and the salt-bridges with the partner α-chains are broken. In step 3–4 the quaternary structure clicks to the oxy form, accompanied by the expulsion of DPG, and the breakage of the salt-bridges between subunits α_1–β_2 and α_2–β_1. Note that the internal salt-bridges of the β-chains are still intact and their haem pockets too narrow to admit ligands. At steps 4–5 and 5–6, the β subunits react in turn, accompanied by widening of the haem pockets, narrowing of the tyrosine pockets and rupture of the internal salt-bridges from the C-terminal histidines to aspartates 94. Note that the change of quaternary structure could in fact take place at any stage of the reaction, and the cooperative mechanism is independent of the sequence in which the individual subunits react.

maintain the tertiary deoxy structure. The following article shows that it also leads to the liberation of Bohr protons, in agreement with the finding that their release is exactly proportional to the amount of oxygen taken up, and that the two reactions are synchronous[26,27]. I have suggested that the subunits react in the order α_1, α_2, β_1, β_2, but this is not certain and my cooperative mechanism would work equally well in whichever sequence the individual subunits reacted.

In haemoglobins where one pair of subunits, either α or β, is unreactive, the other, reactive, pair may or may not be able to actuate the change in quaternary structure: this will depend on the conformation of the unreactive pair and other factors. The literature contains instances where reaction with ligands of either the α or the β subunits alone apparently can or cannot change the quaternary structure of the tetramer. The reasons are not clear without X-ray analysis of each separate case.

Nature of Interaction Energy

Where in this large molecule should one look for that energy? Conceivably the reaction of one haem with oxygen might exercise a direct steric effect which raises the oxygen affinity of its neighbours. Nuclear magnetic resonance spectra offer an extremely sensitive probe for small changes in the environment of one haem caused by the binding of oxygen to another. Studies with mixed hybrids show no evidence that such changes occur unless there is a change in quaternary structure, which led Shulman and his colleagues to suggest that the energy of interaction arises at the boundaries between the subunits[25,36].

Bolton and Perutz found fewer van der Waals interactions at contacts between the α and β subunits in deoxy than in oxyhaemoglobin, and about the same number of hydrogen bonds. Consequently, neither of these is likely to provide the stabilizing interaction that constrains the subunits in the quaternary deoxy form. The most obvious constraints are the six salt-bridges made by the C-terminal residues which link the contacts $\alpha_1\alpha_2$, $\alpha_1\beta_2$ and $\alpha_2\beta_1$. This is borne out by the discovery that enzymatic removal of the four C-terminal residues makes the quaternary deoxy structure unstable and inhibits haem–haem interaction. The bond energy per salt-bridge may reasonably be estimated as 1–2 kcal/mole. For six salt-bridges this gives a total interaction energy of between 6 and 12 kcal/mole of haemoglobin, that is, a value of the same order as the observed interaction energy of 12 kcal/mole. If it is only the salt-bridges which constrain the deoxy tetramer, then part of the energy released by the reaction of the haem groups with oxygen must be expended to break them. The change in conformation of the haem group that leads to the expulsion of the tyrosine provides the required stereochemical mechanism.

Do the changes in the free energy of the subunit contacts in going from the deoxy to the oxy tetramer account for all the interaction energy or for only part of it? This question is important for an understanding of the mechanism, because the answer should tell us whether direct interaction occurs between the β-chains, say, if they react with oxygen after the tetramer has clicked to the oxy conformation. We have seen that haem–haem interaction is absent when the transition to the deoxy structure is inhibited as in BME haemoglobin and haemoglobin from which the C-terminal residues have been removed. Another system is haemoglobin M Iwate (His F8(87)$\alpha\rightarrow$Tyr)[28] where the α-chains are unreactive and the tetramer is locked in the deoxy conformation[29]. The β-chains have a low affinity for oxygen, and their reaction with oxygen produces no haem–haem interaction or Bohr effect, showing again that these effects are absent without a change of quaternary structure. The biochemical data on other systems offer no firm evidence that haem–haem interaction ever occurs without a change of quaternary structure, which suggests that the changes in the free energy of the subunit contacts do seem to account for all the energy of interaction. On the other hand, the abnormal haemoglobins include unexplained instances of very weak haem–haem interaction where the change of quaternary structure does take place.

Dimers or Tetramers as the Functional Units

It has been suggested that $\alpha\beta$ dimers exhibit all the cooperative effects characteristic of the haemoglobin tetramer[32]. These dimers have the structure $\alpha_1\beta_1$, so that the contact $\alpha_1\beta_2$ remains open. There is no reason why an oxyhaemoglobin dimer should not function as well as a tetramer, for the terminal residues are free, and the subunits are not constrained by the $\alpha_1\beta_2$ contact. On the other hand, the deoxy form can exhibit a low oxygen affinity only in the tetrameric form, for dissociation into dimers of whatever type would remove the constraining salt-bridges on which the energy of interaction depends. This conclusion may seem to contradict the finding that haemoglobin solutions show full cooperativity in conditions where the haemoglobin is apparently split into $\alpha\beta$ dimers. Kellett, Midgarden and Schachman, however, have recently discovered that only the liganded form of haemoglobin is split, whereas the deoxy form remains tetrameric in all conditions formerly believed to produce dimers (see ref. 35). They estimate the dissociation constants of the two forms to differ by a factor of at least 10^4. For comparison, the stabilizing free energy of either 6 or 12 kcalories for the extra six salt-bridges in the deoxy form would lower the dissociation constant by a factor of 5×10^5 and 2×10^9 respectively—sufficient to make dissociation of the deoxy form unobservable. This suggests that all cooperative effects observed on binding of haem ligands take place in the tetrameric molecule while it has the quaternary deoxy structure; splitting into dimers may occur at very low haemoglobin concentrations or at high concentrations of neutral electrolytes, but only after the salt-bridges have been broken, when normally the quaternary structure would click to the oxy conformation.

Implications for Allostery

For purposes of comparison haemoglobin may be regarded as an enzyme made up of two different pairs of subunits with oxygen as the substrate, haem as the coenzyme, and H^+ and DPG as the allosteric effectors. Let α and β denote the subunits in the free conformation and α^S and β^S their conformation when substrate is bound. $[\ ]^T$ represents the unreactive and $[\ ]^R$ the reactive quaternary structure. Then for illustrative purposes, the reaction scheme of Fig. 4 can be rewritten as

$$\left[\alpha_2\beta_2\right]^T \underset{+2S}{\rightleftharpoons} \left[\alpha_2^S\beta_2\right]^T \rightleftharpoons \left[\alpha_2^S\beta_2\right]^R \underset{+2S}{\rightleftharpoons} \left[\alpha_2^S\beta_2^S\right]^R$$

The mechanism of haem–haem interaction contains features of several of the theories that have been advanced to account for interaction between ligand sites in enzymes. Monod's model postulates "the existence of only two (or at least two) stable states that are accessible to allosteric oligomers. These states differ by the distribution and/or energy of intersubunit bonds, and therefore also by the conformational constraints imposed on the subunits. As a result, the affinity of one (or several) of the stereospecific sites is altered when a transition occurs to the other state"[9]. These words ring prophetically if we look at the mechanism in terms of the quaternary structure.

Monod *et al.* assumed all the subunits in the quaternary T-state to be in the unreactive and those in the quaternary R-state in the reactive form, no matter whether they were liganded or not. In their discussion they envisaged the possibility that this assumption might prove too

simple and this is indeed the case. The haemoglobin subunits change their tertiary structure in response, not to the change in quaternary structure, but to the binding of ligand, as predicted by Koshland's sequential model[34]. Yet Koshland's model implies that the change in tertiary structure of each subunit directly affects the ligand affinity of its neighbours, and there is no evidence of this happening in haemoglobin. Rather, all—or nearly all—the interaction energy arises through the step by step release of the constraints on the unreactive quaternary structure, which changes the equilibrium in favour of the reactive quaternary structure and diminishes the work required to change the tertiary structure of each subunit from the unreactive to the reactive form (see Fig. 6 of the following article).

In Koshland's terms[35], the change in tertiary structure of the α subunits would be caused by induced fit in response to a change in conformation of the coenzyme on reacting with substrate; that of the β subunits in response to both the change of conformation of the coenzyme and the steric adaptation of the active site to the substrate.

How would allosteric effectors influence the reactivity? In haemoglobin both hydrogen ions and DPG lower the reactivity; the former strengthen the salt-bridges constraining the inactive or T state by increasing the fraction of molecules carrying positive charges on one α amino and two imidazole groups (see the following article); DPG may increase the free energy of interaction by introducing additional salt-bridges specific for the T-state, to which it is stereochemically complementary but has either no affinity or a lower one for the R-state.

The cooperative mechanism outlined here explains most of the important properties of haemoglobin, even though stereochemical details are still lacking. It is of a kind that could not have been guessed without knowing the structure of the two forms in atomic detail and without further information which the inhibited BME-haemoglobin provided. Even then it could be worked out only by combining the structural information with many other results.

It is remarkable that there should be such an exceedingly complex, subtle and elegant instrument of respiratory transport, exploiting a difference in atomic radius of 13 per cent between the covalent and ionic forms of iron.

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The Bohr Effect and Combination with Organic Phosphates

LIKE haem–haem interaction, the Bohr effect results from the change in structure which the haemoglobin molecule undergoes on combination with oxygen (see preceding article). The stereochemical consequences of this change have been studied in atomic models derived from electron density maps of haemoglobin at high resolution[1–3]. The models show that the change in structure alters the environment of three pairs of weak bases so that they would tend to take up hydrogen ions on discharge of oxygen. The α-amino groups of valine 1α and the imidazole groups of histidines 146β are free in oxyhaemoglobin, but come close to carboxyl groups in the deoxy form[4,5]; the imidazole groups of histidines 122α approach guanidinium groups in the oxy and carboxyl groups in the deoxy form. A survey of all other ionizable groups in the structure of oxy and deoxyhaemoglobins suggests that none of them are likely to change their state of ionization significantly on binding of oxygen near neutral *p*H.

Early Ideas concerning the Bohr Effect

The Bohr effect is produced on combination of O_2, CO or NO with the ferrous form of haemoglobin (deoxyhaemoglobin) or on oxidation of deoxyhaemoglobin to the ferric form (methaemoglobin) but not on combination of methaemoglobin with ligands. In other words, it is observed only in those reactions involving a change in quaternary structure from the deoxy form, in which the iron atoms are five coordinated, to the oxy or liganded form in which they are six coordinated. It is observable either as a change in *p*H on binding of haem ligands or as a change in affinity of the haem iron to ligands as a function of *p*H. At a *p*H above 6·0 haemoglobin takes up protons on release of haem ligands; this is known as the alkaline Bohr effect and reaches its maximum of 0·7 protons per haem ligands at physiological *p*H.

The alkaline Bohr effect has a two-fold function. On liberation of oxygen haemoglobin neutralizes the protons released in the blood by the uptake of CO_2, thereby facilitating its transport from the tissues to the lungs. Acid *p*H lowers the affinity of haemoglobin for haem ligands, which helps the release of oxygen in tissues rendered acid by bicarbonate and lactic acid. Below *p*H 6·0 the Bohr effect is reversed, protons being liberated on release of haem ligands. Because *p*Hs below 6 are not likely to occur *in vivo*, however, this reverse, or acid, Bohr effect may have no physiological significance.

Wyman showed that the Bohr effect could be attributed to ionizable amino-acid side chains with a pK close to 7·0 which might either release or take up H^+ as a result of oxygenation of the iron atoms[6]. He determined the heat of dissociation of the Bohr protons as 6,500 calories per equivalent, and showed that this is characteristic for histidine. Further analysis based on the variation of proton released or taken up as a function of pH on oxygenation led him to suggest that each haem interacts with two histidines, one on each side of the haem, a scheme that had already been proposed by Conant[7]. To the histidine responsible for the alkaline Bohr effect he ascribed a pK of 7·8 in deoxy changing to 6·2 in oxyhaemoglobin; to that responsible for the acid Bohr effect a pK of 4·9 in deoxy and 5·7 in oxyhaemoglobin. Coryell and Pauling arrived at the same conclusion on the basis of magnetic susceptibility measurements and introduced the term "haem-linked acid groups"[8]. The environment of the haems first predicted by Conant was verified when the structure of myoglobin was solved, but the mystery of the Bohr effect was deepened because the tertiary structures of myoglobin and of each of the haemoglobin chains proved closely similar, yet myoglobin was known to show no Bohr effect[9]. This meant that the haem-linked histidines could not be responsible for it and that the term "haem-linked acid groups" was a misnomer. When the uptake of haem ligands was found to be accompanied by a marked change in quaternary structure[10] it seemed possible that this might alter the environment of ionizable groups other than the haem-linked ones in the required way. We were able to identify two of the groups concerned[4,5], but it took electron density maps of both the oxy and deoxy forms at high resolution before we could see all the groups and determine the exact stereochemical mechanism which changes their state of ionization in the two forms of haemoglobin.

C-terminal Residues

In the liganded form of haemoglobin the C-terminal arginines of the α-chains and histidines of the β-chains rotate freely. The penultimate tyrosines seem to rotate freely for most of the time; they spend the remainder in a slot between helices F and H. Several pieces of evidence support this picture. The C-terminal residues are invisible in the electron density maps of oxyhaemoglobins; only traces of the penultimate tyrosines are to be seen. Because model building showed various ways in which the C-terminal groups could be held down by salt-bridges[1], I thought at first that this freedom might be an artefact, arising from the weakening of salt-bridges by the 2 M ammonium sulphate solution in which the crystals were suspended.

Fortunately, crystals of methaemoglobin can be prepared free of salt; these are isomorphous with those of oxyhaemoglobin precipitated by salt. X-ray reflexions of the salt-free crystals were collected on a diffractometer to a resolution of 3·5 Å and a difference Fourier synthesis of salt-free minus salty haemoglobin was calculated. If the C-terminal residues had been held down in fixed positions in salt-free crystals they should have shown up there as positive peaks, but there was no sign of these. In fact, the difference map was featureless, except for a negative peak on the dyad axis, possibly representing a negative ion trapped in the internal cavity. I had to conclude therefore that the C-termini were equally free in the absence of salt.

Other evidence supporting freedom of rotation of the penultimate tyrosines of the β-chains comes from work on a haemoglobin carrying spin labels at the reactive SH-groups 93β (ref. 11). The spin label oscillates rapidly between two alternative conformations, in one of which it is free and in the other immobilized. X-ray analysis showed that in the immobilized conformation the spin label takes the place of the penultimate tyrosine in the pocket between helices F and H, implying that the tyrosine itself oscillates rapidly between a free and an immobilized position and that these oscillations are synchronized with those of the spin label[12].

When we proposed that the contribution of the β-chains to the alkaline Bohr effect arose through the freedom of the C-terminal histidines in oxy and the linkage of their imidazoles to aspartates 94 of the same β-chains in deoxyhaemoglobin[4], we were unable to explain why this link was maintained only in the deoxy form, even though no steric obstacles appeared to stand in its way in the oxy form. The expulsion of the penultimate tyrosines already described and the existence of an unsuspected salt-bridge of lysine C5(40)α with histidine HC3(146)β now provide the explanation. Fig. 1*a* shows the freedom of the C-terminal residues in oxyhaemoglobin.

Consider now the transition to the deoxy structure. In oxyhaemoglobin lysine C5(40)α_1 thrusts its side chain far out into the solution. During deoxygenation the α_1-chain turns relative to the β_2-chain by 13·5°. The ε-amino group of lysine C5(40)α_1, being furthest removed from the axis of rotation of the α_1-chain, is shifted by 7 Å into the $\alpha_1\beta_2$ contact, where it can form a salt-bridge with the C-terminal carboxyl group of the β_2-chain. This salt-bridge keeps tyrosine 145β_2 fixed in its pocket between helices F and Hβ_2 and allows the further salt-bridge between the imidazole of histidine 146β_2 and aspartate 94β_2 to be formed (Fig. 1*b*). The electron density maps of deoxyhaemoglobin at high resolution show the interatomic distances between the acidic and basic groups to have the correct length (~3 Å) for the salt-bridges[2,3].

These findings suggest that histidine HC3(146)β contributes to the Bohr effect by a change in pK of its imidazole, arising through the shift from its free position in oxyhaemoglobin to its fixed position in deoxyhaemoglobin. In oxyhaemoglobin the pK of the imidazole must be slightly raised by the negative field of the histidine's own free carboxyl group. In deoxyhaemoglobin the field of the C-terminal carboxyl group is compensated by lysine 40α, but the imidazole becomes linked to aspartate 94β. This apparently produces a much larger increase in pK than the more distant interaction with its own carboxyl group in oxyhaemoglobin. We do not know of any comparable model situations which would allow us actually to predict the changes in pK, but it should soon be possible to titrate the histidines directly by NMR spectroscopy. The recently observed absence of half the alkaline Bohr effect in des-histidine(146)-haemoglobin confirms our scheme[13].

The first hint of the involvement of the C-terminal arginines of the α-chains was the observation that enzymatic removal diminished the alkaline Bohr effect[14], which has recently been confirmed[13]. This leaves a Hill's coefficient of 1·8, indicating that the change of quaternary structure still accompanies the reaction with oxygen[13]. Because the pKs of guanidinium groups and α-carboxyl groups are 12·5 and 3·4 respectively, neither group could itself lose or gain protons at neutral pH, which implies that their contribution must arise from interaction with other ionizable groups. The next advance came with the discovery that blocking of the α-amino groups of valine 1α with cyanate inhibited about one-quarter of the Bohr effect[5].

The electron density maps of oxyhaemoglobin at 2·8 Å resolution showed the valines 1α to be free, and the maps of deoxyhaemoglobin at 5·5 Å resolution showed bridges connecting the C-terminus of one α-chain with the N-terminus of its partner chain. These observations led us to propose that the pKs of the α-amino groups are normal in oxyhaemoglobin, but are raised in the deoxy form through linkage of the α-amino groups of one chain to the α-carboxyl groups of their partner chains. At that stage our hypothesis could not be proved, however, and in any case it was uncertain whether it could account for the entire share contributed to the Bohr effect by the α-chains. Our new results confirm the interaction between these

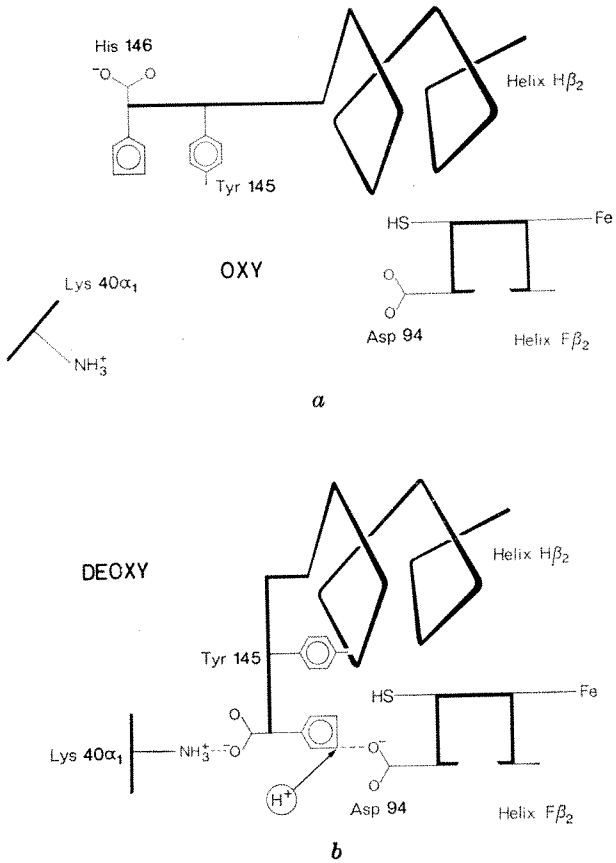
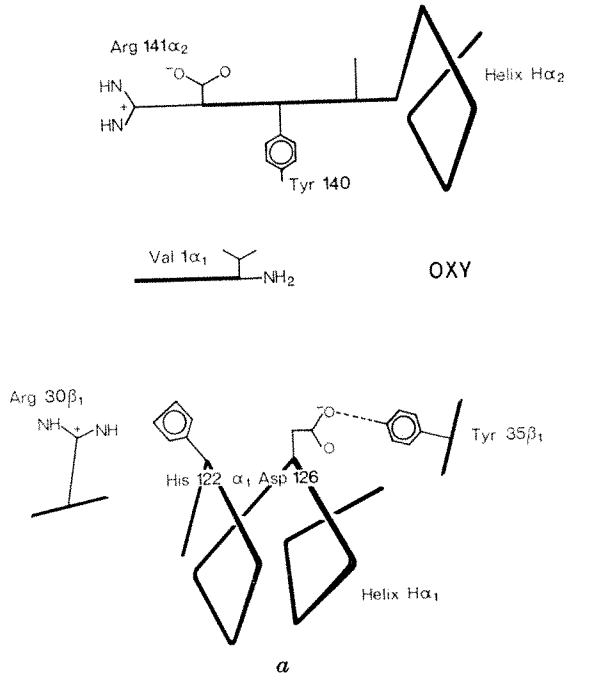


Fig. 1. Change in conformation of C-terminal residues of β-chain on deoxygenation: *a*, in oxyhaemoglobin histidines 146β_2, aspartates 94β_2 and lysine 40α_1 are free and far from each other. Access to the SH groups is free; *b*, in deoxyhaemoglobin, formation of a salt-bridge between aspartate 94 and the imidazole of histidine 146 leads to ionization of the latter. The salt-bridge is cemented by a further bridge between the C-terminal carboxyl of the β_2-chain and lysine 40α_1. Access to the SH groups is restricted.

terminal residues, and suggest the possibility of a further residue contributing to the Bohr effect.

The electron density maps of deoxyhaemoglobin at high resolution show that the C-terminal arginine of each α-chain forms two salt-bridges with its partner chain[2,3]. One bridge extends from the guanidinium group α_2 to aspartate H9(126)α_1 and the other from the α-carboxyl group α_2 to a cluster of three ionizable groups composed of valine NAl(1), aspartate A4(6) and lysine H10(127)α_1 (Figs. 2 and 3). The precise constellation of this cluster is not resolved but can be guessed with the help of stereochemical rules. Aspartate 6 is bound only to lysine 127. The α-carboxyl group of arginine 141 must have one of its oxygens pointing towards valine 1 rather than towards lysine 127, for otherwise the O–N distance would be too short. The electron density peak for valine 1 does not resolve its isopropyl side chain from its α-amino group, which leaves one free to rotate this residue as desired. On electrostatic grounds, however, the α-amino rather than the isopropyl group would tend to face the α-carboxyl group, resulting in the arrangement of Fig. 3. The proximity of the positively charged lysine 127 would weaken the effect of the negatively charged carboxyl on the *pK* of the α-amino group of valine 1, even though the lysine's charge is compensated by that of aspartate 6. Perhaps for this reason, the ionization of the α-amino groups may be only partial, so that it may not account for the α-chain's entire contribution to the Bohr effect.

We come now to a second possible contribution from the α-chains. Formation of links extending from the guanidinium group of the arginine HC3(141)α_2 to aspartate H9(126)α_1 causes a rearrangement in a cluster of polar groups which includes histidines H5(122)α_1. In oxyhaemoglobin the centre of the imidazole of histidine H5(122)α_1 lies within 5·5 Å of the guanidinium group of arginine B12(30)β_1 and is separated by 4·5 Å from the centre of the carboxyl group of aspartate H9(126)α_1. In deoxyhaemoglobin these distances are altered to 7·5 Å and 3 Å respectively (Fig. 4). This shift would suggest that the *pK* of histidine 122 is lowered by the proximity of a positive charge in oxy and raised by the proximity of a negative one in deoxyhaemoglobin. On oxygenation of



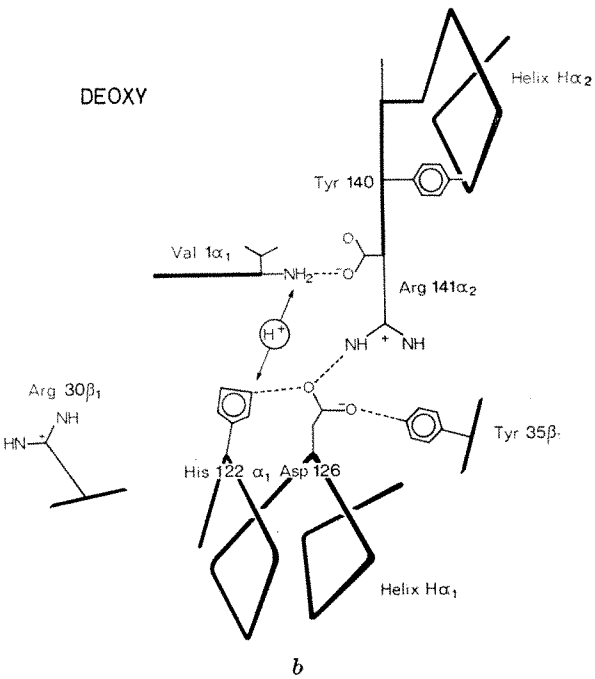


Fig. 2. Change in conformation of terminal regions of the α-chain on deoxygenation: *a*, in oxyhaemoglobin valine 1α_1 and arginine 141α_2 are free, histidine 122α_1 is close to arginine 30β_1, and aspartate 122α_1 is hydrogen bonded only to tyrosine 35β_1; *b*, in deoxyhaemoglobin valine 1α_1 is in contact with the carboxyl group of arginine 141α_2. Aspartate 126α_1 forms close contacts with histidine 122α_1, tyrosine 35β_1 and arginine 30α_2.

Feα, the expulsion of tyrosine HC2(140) would rupture the links of the C-terminal arginines with valine 1 and aspartate H9. Clearly, this would liberate the Bohr protons bound to the α-amino groups of valine 1, but it is not yet clear why this would also affect the conformation of histidine H5 relative to its two neighbours. Their movements seem to depend on the change in quaternary structure.

It may be hard to test the part played by histidine 122α chemically unless a mutant can be found in which it is replaced by a neutral residue. Similarly the relative contributions of valine 1α and histidine 122α may prove difficult to assess exactly unless at least one of these groups can be titrated directly.

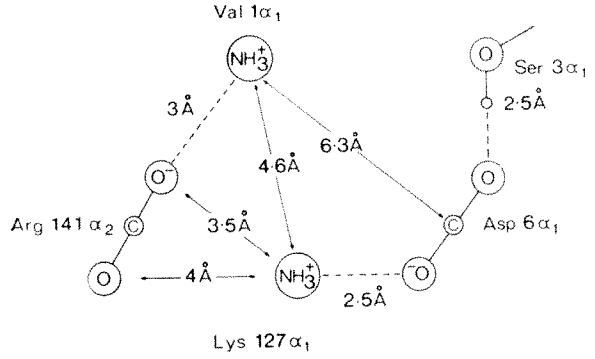


Fig. 3. Distances between members of the cluster of ionizable groups near valine 1α in deoxyhaemoglobin.

There are no changes in the conformation of the haem-linked histidines which would lead one to expect a change in their *pK*s. The proximal histidines F9 are linked to iron and would not be ionized; a contribution from the distal histidines is excluded by the presence of a full Bohr effect in haemoglobin Zürich in which histidine E7β is exchanged for an arginine[15]. A survey of all the other ionizable groups in oxy and deoxyhaemoglobin shows that most of the salt-bridges in the molecule are left undisturbed by the reaction of the haem group with ligands; the few that are disturbed involve ionizable groups with *pK*s far outside the range of physiological *p*H. Small changes in the general pattern of ionizable groups on the surface of the haemoglobin molecule do occur as a consequence of the change in quaternary structure that accompanies oxygenation. Some attempts are being made to use these as the basis for the interpretation of the Bohr effect, but theoretical reasons suggest that their contribution is small and experimental evidence confirms this[13]. We therefore conclude that no groups other than the ones described here contribute significantly to the alkaline Bohr effect.

The Acid Bohr Effect

The acid Bohr effect is observed at *p*H 6·0 and requires ionizable groups that change their *pK* from about 5·7 to 4·9 in going from oxy to deoxyhaemoglobin[6]. I could not find any groups that change their environment in the way required, however, and this made me wonder if there was not a different explanation. Horse oxyhaemoglobin crystals undergo a drastic lattice change at *p*H 5·9, the monoclinic angle decreasing from 111° to 98° (ref. 16). Patterson projections indicated at first that this lattice transition left the internal structure of the haemoglobin molecules unaltered[17], but isomorphous replacement with paramercuribenzoate showed that this was not true: a difference Patterson proved that the lattice transition was accompanied by a reduction of the distance between the reactive cysteines from 30 Å to 25 Å (ref. 18). (On deoxygenation this distance increases from 30 Å to 37 Å; that is, the changes are in the opposite direction.) Such a

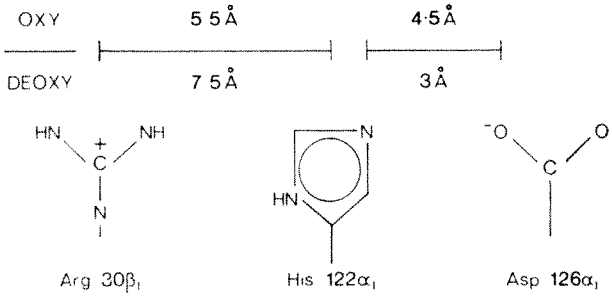


Fig. 4. Distances between histidine 122α and its neighbours in oxy and deoxyhaemoglobin.

reduction cannot occur without a change in quaternary structure which would affect the oxy and deoxy forms differently. I suggest that this is the cause of the acid Bohr effect: its details remain to be investigated. Incidentally, there is a further lattice change below *p*H 5·4, with a reduction of the angle from 98° to 85°, which may be the expression of a further change in quaternary structure[16].

SH Groups, the Spin Label, Invariant Residues

The reactivity of the SH groups at cysteine 93 increases in going from the deoxy to the oxy form and is linearly proportional to the degree of oxygenation[19]. This is readily accounted for by the changed conformation of the C-terminal histidines. In the oxy form they leave the SH groups accessible, while in the deoxy form they restrict access to it (Fig. 2). Oxygenation of each β-chain is accompanied by expulsion of tyrosine HC2 and the rupture of the restricting salt-bridges, thus accounting for the linear relationship between the two phenomena.

A similar mechanism accounts for the changed immobilization of the spin label attached to cysteine 93β. In deoxyhaemoglobin the tyrosine pocket is occupied, so that the spin label is free. In oxyhaemoglobin the tyrosine pocket is open, for part of the time, so that the spin label can be immobilized there. The transition from the free to the immobilized conformation is therefore proportional to the number of β chains which have taken up oxygen, as has been observed[20].

As far as can be judged from amino-acid sequences so far determined, all the residues involved in our scheme for the alkaline Bohr effect are present in all mammalian haemoglobins; they are also present in the α-chain of chicken, the only bird haemoglobin sequenced so far[21]. In the α-chains of carp the N-terminal serine is acetylated and could not contribute to the Bohr effect[22]; but histidine 122, aspartate 126 and arginine 141 are all present.

Chemical evidence bearing on our scheme will be described by Kilmartin and Wootton[13]; the temporal sequence in which the different residues liberate their hydrogen ions during the reaction with oxygen was discussed in the preceding article where it was shown that the combination of each atom of iron with ligand leads to the expulsion of the penultimate tyrosine from its pocket, and that this, in turn, breaks the salt-bridge formed by the C-terminal residue, with the liberation of a Bohr proton. This makes the liberation of Bohr protons synchronous with the uptake of haem ligands.

Model for the Binding of Organic Phosphates

2,3-Diphosphoglycerate (DPG) is present in the red cells of humans and many other species, and lowers the oxygen affinity of haemoglobin in a physiologically advantageous way[23]. It increases the free energy of haem–haem interaction but does not alter the Bohr effect. According to several investigators it binds specifically to deoxyhaemoglobin in the ratio of 1 mole/mole with a binding constant at neutral *p*H and physiological salt concentration of $1{\cdot}3 \times 10^5$, corresponding to a free energy of binding of

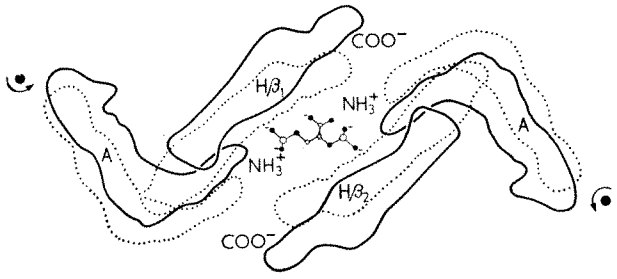


Fig. 5. Projection of the electron density maps of oxy (. . .) and deoxyhaemoglobin (———) at 5·5 Å resolution, showing the N and C-terminal portions of the β-chains grouped around the two-fold symmetry axis which is normal to the plane of the paper. In relation to Fig. 2 of the preceding article, the molecule is viewed from the top and the parts seen here are near the bottom. The DPG molecule has been tentatively placed in the central cavity, centred approximately on the two-fold axis (in fact the carboxyl group makes it unsymmetrical). The picture shows that its phosphates are close to the N-termini of the β-chains in deoxyhaemoglobin. On oxygenation the N-termini move apart and the H helices close up, thus expelling the DPG. The circular arrows indicate the positions of the axes around which the β subunits rotate on deoxygenation.

7 kcalories/mole. It also binds to free β-chains (β_4) in both oxy and deoxy states, suggesting that the binding site is on the β-chains. In certain conditions the binding is less specific and not stoichiometric, so that DPG binds to both the oxy and the deoxy forms, but the deoxy form has the higher affinity[24].

The affinity of DPG for haemoglobin is lowered by salt, showing that the binding is electrostatic, and is totally inhibited at the salt concentrations used to precipitate our crystals of deoxyhaemoglobin. For this reason we have not yet tried to determine its binding site by X-ray analysis, but fortunately Bunn and Briehl have obtained biochemical data which go far towards pinpointing that site[25]. They measured the effect of DPG on the oxygen affinities of normal human haemoglobin A and various other haemoglobins, including a minor human adult component (A_{1C}) in which the N-termini of the β-chains are covalently bound to a hexose by a Schiff base linkage; foetal human haemoglobin (F_{II}); and a minor component of human cord blood (F_I) in which the N-termini of the β-chains are acetylated. DPG increased the partial pressures of oxygen needed to produce half saturation with oxygen by a factor of 2·1 in haemoglobin A, 1·2 in haemoglobin A_{1C} and F_{II}, and unity in F_I, showing that the α-amino groups of the β-chains are involved in the binding of DPG. There is, however, clearly another nearby group which is responsible for the difference between haemoglobin A and haemoglobin F_{II}. A survey of the haemoglobin model and of the differences between the amino-acid sequences of the human β and γ-chains showed that of the residues which differ, 143(H21)β, which is histidine in adult and alanine in foetal haemoglobin, is the only likely one in the neighbourhood of the N-terminal amino groups.

When the atomic model of deoxyhaemoglobin was completed, Dr W. Bolton and I placed a model of DPG on the two-fold symmetry axis in the central cavity to see if binding to these residues would be likely on stereochemical grounds. We found that it fitted neatly so that its charged groups were within hydrogen-binding distance of the following pairs of residues of the β-chains: valine NA1(1), lysine EF6(82) and histidine H21(143). The position is shown in Fig. 5.

What happens on oxygenation ? The distance between the α-amino groups increases from 16 Å to 20 Å, so that they can no longer make contact with the phosphates of the DPG, and the helices H close up so that DPG is expelled from the central cavity. In oxyhaemoglobin this particular binding site is closed, therefore, but at this stage we cannot be sure that oxyhaemoglobin does not contain an alternative site, with a weaker binding constant, to which DPG can become attached.

Sheep haemoglobin has no affinity for DPG. The explanation is this: in its β-chain residues NA2 are deleted, which would make the non-helical segment at the amino end more than 3 Å shorter, and increase the distance between the two α-amino groups to about 22 Å, so that they could not reach the two phosphate groups of DPG.

DPG is thus seen to be stereochemically complementary to a constellation of six positively charged groups in the β-chains which face the central cavity of the haemoglobin molecule. DPG has two negatively charged groups with an average pK_a of 2·8 and three with an average pK_a of 7·1, whereas the three pairs of positively charged protein groups facing them have pK_as of 10·5, 6·8 and 7·6 respectively. Thus the charges on the DPG could be just compensated by those of the protein. This also explains why DPG does not alter the Bohr effect. On dissociation, the number of protons bound by DPG would roughly equal the number lost by the two α-amino groups and two histidines. An explanation of this general nature for the absence of a Bohr effect has been advanced[26].

ATP, ADP and AMP have binding constants with haemoglobin lower than DPG by one, two and three orders of magnitude respectively. The steric reasons for this lie in the progressive shortening of the distances between the terminal phosphate groups which weakens their interaction between the two rigidly spaced α-amino groups of the β-chains. (For fully stretched chains the distances between the terminal phosphates are 6·5 Å in 2,3-DPG, 5·5 Å in ATP and 2·8 Å in ADP.)

Pyridoxal phosphate binds to deoxyhaemoglobin in the ratio of 1 mole/mole by forming a Schiff base linkage with the α-amino group of one of the β-chains[27]. Its physiological effect is similar to that of 2,3-DPG, of which it is a competitive inhibitor. Model building shows that its phosphate group would lie near the two-fold symmetry axis of the molecule, within hydrogen bonding distance of the ε-amino groups of the two lysines EF6(82)β. The proximity of the phosphate group to the two-fold axis would inhibit the binding of a second molecule of pyridoxal phosphate. The binding site coincides with that of 2,3-DPG.

Discussion

The influence of DPG may consist simply in the stabilization of the quaternary deoxy structure where it may form the equivalent of four additional salt-bridges cross-linking the β subunits, but it probably has no direct influence on the tertiary structure of the individual subunits. Hydrogen ions, on the other hand, influence the tertiary structure of the β-chains and the quaternary structure directly by stabilizing the deoxy conformation of each. This is clearly seen by reference to Fig. 4 of the preceding article and Figs. 1 and 2. By increasing the fraction of the α-amino groups of valine 1α and imidazoles of histidine 122α that carry positive charges, H^+ directly opposes the rupture of the salt-bridges between the α-chains; the activation energy needed to expel the tyrosines from the pockets is thereby increased and, consequently, the movement of the iron atom towards the plane of the porphyrin ring which is needed for their reaction with oxygen is opposed. Similarly, in the β-chains, H^+ increases the fraction of the C-terminal histidines which carry positive charges, thereby strengthening the salt-bridges with aspartate 94 and opposing the expulsion of the tyrosines even if the quaternary structure had already clicked to the oxy conformation. The only salt-bridges not affected by H^+ near neutral *p*H are those between the C-terminal carboxyl groups of the β-chains and lysine 40α and those involving DPG.

This study has perhaps conveyed a too mechanistic picture of the cooperative effects involved in respiratory transport. In fact we should imagine a dynamic system, in which both the tertiary structure of each subunit, and the quaternary structure of the haemoglobin molecule as a whole, oscillate rapidly and continuously between the

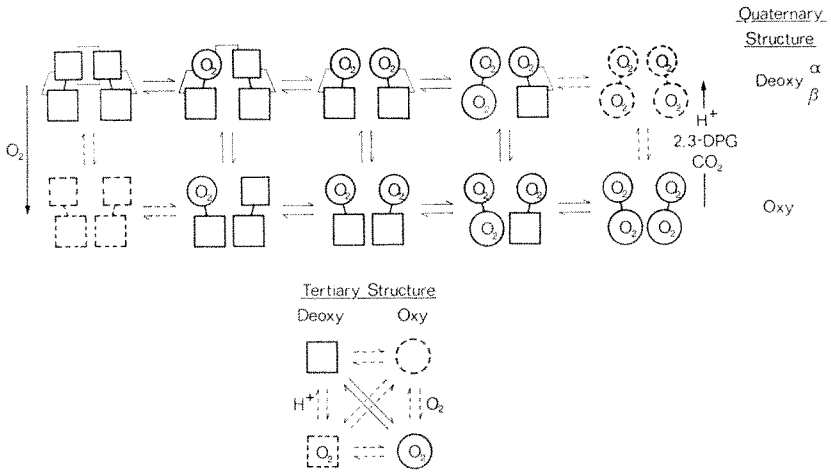


Fig. 6. Diagrammatic sketch of the allosteric mechanism of haemoglobin. The smaller subunits represent α, the larger β subunits. The clamps between them represent salt-bridges. Forms that may be too unstable to be observed have been drawn in broken lines. Note that the sequence of reaction of the individual subunits is unimportant.

oxy and the deoxy conformations. The concentrations of the different ligands alter the equilibrium between these two conformations of the various components rather than switching any of them completely to one or the other conformation (Fig. 6). This also means that the transition between the two quaternary structures could occur at any stage in the reaction, depending on the concentration of H^+, CO_2 and DPG, and on other factors, and that the mechanism would work equally well regardless of the sequence of reaction of the subunits.

I thank Dr W. Bolton for preparing the salt-free methaemoglobin crystals and Dr J. Greer for calculating the difference Fourier.

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Mathematical Model of Clonal Selection and Antibody Production

by
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Equations are developed to describe the effects of interaction between antigen and target cell. The model shows promise in predicting the constants of simple experimental systems.

When an animal is injected with a foreign substance, called an antigen, it may respond by producing antibodies, which are protein molecules that bind specifically to the antigen and hasten the elimination of the antigen from the animal[1,2]. Many features of such immune responses have been explained by the clonal selection theory[3,4] in which it is assumed that, in an adult animal, there are target cells that are capable of recognizing an antigen and being stimulated by the antigen to proliferate and produce antibodies. This clonal proliferation will lead to an increased population of target cells so that, if the animal is exposed a second time to the same antigen, the secondary response can be much more rapid and vigorous.

These notions are supported by several lines of evidence. (1) The antibody-like protein molecules which are produced by a patient with multiple myeloma are homogeneous but differ from one case to the next in their amino-acid sequences[1,2]. If it is assumed that multiple myeloma results from the uncontrolled proliferation of a single clone, starting from one target call, then all the cells in

a clone produce the same antibodies but different clones produce different antibodies. (2) The specificity of antibodies is determined by their amino-acid sequences. It is possible to denature antibodies and on renaturation their specificity and activity are recovered[5–7]. (3) The target cells seem to carry antibody-like surface receptors that combine specifically with antigen (see, for example, refs. 8, 9). Binding of antigen to surface receptors could be the mechanism whereby antigen is recognized by those cells that are able to produce the corresponding antibodies. (4) Small lymphocytes have been triggered into an active state *in vitro* by a variety of substances[10]. It is therefore tempting to believe that small lymphocytes are the target cells. Several cell types may actually be involved in triggering a primary immune response (see, for example, refs. 11–13), but the details are not yet clear.

In view of this and other evidence which supports a clonal selection theory of antibody formation, I have attempted to devise a quantitative mathematical model of clonal selection and antibody production for use in the planning and interpretation of experiments. In this article I will outline the model, report some calculations and encouraging agreement with experiment, and discuss future possibilities. (A more complete description of this work will be published elsewhere[14].) I have attempted to keep the model as simple as possible but retain enough biological content so that quantitative comparisons can be made with experiments. At present the model is restricted to the production of humoral antibodies in an adult animal. Moreover, the response to only one kind of antigen at a time is treated and the origin of the target cells and their diversity is not considered. Although it is necessary to introduce some arbitrary, but hopefully reasonable, assumptions into the model, these assumptions could, in principle, be tested by experiment and the resulting model shows agreement with experiments and suggests novel interpretations of data.

Outline of the Model

The model will be developed for a special case in which all the antibodies that are produced in response to a particular antigen are identical. A general model for heterogeneous antibody production in response to a single antigen will be sketched here for use in the comparisons with experiment.

The four kinds of cells considered in the model are indicated in Fig. 1. Target cells are initially present and capable of combining with antigen and thereby being transformed into proliferating cells which secrete antibody and rapidly divide. In the presence of sufficient antigen the proliferation continues, but when the free antigen concentration decreases to a low level, the proliferating cells divide unsymmetrically to become plasma cells, terminal antibody secreting cells, and memory cells which become, or are, functionally identical with target cells. The target, proliferating, and memory cells have antibody-like surface receptors which can combine specifically and reversibly with antigen and the number or fraction of the sites that are bound to antigen determines

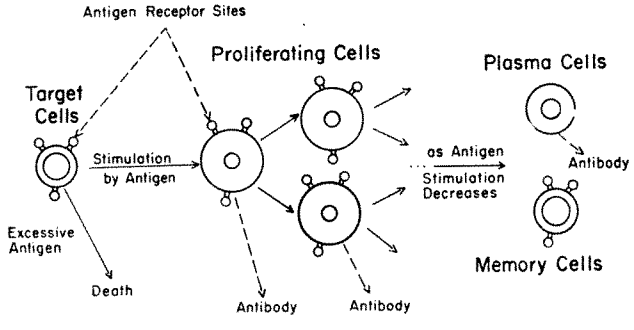


Fig. 1. Cells in the model.

the mitotic activity of a cell. Thus, if one or more sites are occupied on a target cell, it will be transformed to a proliferating cell in a mean time, T_1, though if nearly all the sites are occupied, the target cell is assumed to be killed, so that a possibility of inducing tolerance (that is, destruction of target cells) by exposure to very high antigen doses is built into the model. If many sites on a proliferating cell are occupied by antigen it will divide symmetrically in a mean time, T_2, while if less than one site is occupied, on the average, the cell will divide unsymmetrically to produce a plasma cell and a memory cell.

The binding of a cell receptor site to antigen is taken to be characterized by an intrinsic association constant, k', and the binding of antigen to antibody has the association constant, k. Conditions of chemical equilibrium can be worked out[14] for the interaction of antigen molecules having a single combining site per molecule (univalent) with antibodies having two sites per molecule (bivalent) and cells having many sites, m per molecule (multivalent). When multivalent antigens are considered, they are described by their effective number of independent antigenic sites per antigen molecule. The essential assumption of the model is that the average fraction r', and number, R' (where $R'=mr'$) of receptor sites, which are bound to antigen on a cell, determine its mitotic activity. Equations of chemical equilibrium are used to determine these quantities as functions of time.

It is assumed that the concentrations of the four kinds of cells and of antigen and antibodies are governed by ordinary differential equations in which some of the coefficients are functions of r' or R'. Let N_1, N_2, N_3 and N_4 denote the concentrations of target, proliferating, plasma and memory cells. The population of target cells will be depleted if these cells are binding antigen and we write

$$\frac{dN_1(t)}{dt} = -F(R')\frac{N_1(t)}{T_1} \qquad (1)$$

where T_1 is the mean life of a stimulated target cell (for transformation to the proliferating state or death) and thus $0 \leq F(R') \leq 1$. When $R' \ll 1$, there is only a small chance that a receptor site is occupied and stimulation is unlikely. In this case, $F(R') \ll 1$. When, on the other hand, many sites are bound to antigen, $R' \gg 1$ and it is natural to let $F(R') \simeq 1$. A simple but arbitrary choice for $F(R')$ which is used in all the following work is $F(R') = R'/(1+R')$.

Transformed target cells form the source of proliferating cells. Let this source be written $G(R')N_1/T_1$ so that G/F is the fraction of cells leaving the target state that reach the proliferating state. To allow for the induction of tolerance by high concentrations of antigen, I have assumed that G/F is small when nearly all sites are occupied; in particular, $G/F = (1-r') \equiv (1-R'/m)$.

In addition, the division of proliferating cells, with a mean life, T_2, will change the population. Let the fraction of such cells that on division remain proliferating be $(1+H(R'))/2$; the fraction that becomes plasma cells and memory cells is then $(1-H(R'))/2$. Division of a proliferating cell therefore leads, on average, to $1+H(R')$ new proliferating cells, together with $1-H(R')$ other cells. The net gain or loss of proliferating cells per division is then $H(R')$, and

$$\frac{dN_2(t)}{dt} = G(R')N_1(t)/T_1 + H(R')N_2(t)/T_2 \qquad (2)$$

According to our assumptions, when most sites are occupied (that is, when $R' \gg 1$) $H \simeq 1$ while when a site is seldom occupied (that is, when $R' \ll 1$) $H \simeq -1$. A simple choice for H with these properties is $H = (R'-1)/(R'+1)$.

Division of proliferating cells will serve as the source

of plasma and memory cells. If equal numbers of the latter are produced,

$$\frac{dN_3}{dt} = \frac{1 - H(R')}{2} N_2/T_2 - N_3/T_3 \quad (3)$$

and

$$\frac{dN_4}{dt} = \frac{1 - H(R')}{2} N_2/T_2 \quad (4)$$

where, in equation (3), T_3 is the natural lifetime of a plasma cell. (The corresponding lifetimes of target, proliferating and memory cells have been taken to be infinite in the above equations. They are assumed finite but long in the numerical work to be reported below.) According to equation (4), memory cells simply accumulate as inert cells which do not interact with antigen. To let them be equivalent to target cells, the source of memory cells $(1-H)N_2/2T_2$, is treated as a source of target cells and added to the right side of equation (1). Then N_1 will include both the target cells and the memory cells.

The differential equation for Ab(t), the concentration of antibodies, has two source terms arising from production of antibodies, by proliferating and plasma cells. If c_2 and c_3 are the rates at which a proliferating cell and a plasma cell produce antibodies, these source terms are $c_2N_2(t)$ and $c_3N_3(t)$. An antibody is assumed to have a natural lifetime T_5' while an antibody bound to antigen is taken to have a much shorter lifetime, T_5, for destruction or elimination from the system. If r is the fraction of antibody sites bound to antigen, then antibodies with two sites per molecule will be bound to antigen with the probability $2r-r^2$. Therefore,

$$\frac{d[Ab]_t}{dt} = c_2N_2(t) + c_3N_3(t) - \{(2r - r^2)/T_5 + 1/T_5'\}\,[Ab]_t \quad (5)$$

The rate of change of the antigen concentration, $d[Ag]_t$, will be the result of a possible source of antigen S_t, together with an elimination with mean life T_6 and an accelerated elimination of antigen, bound to antibodies, [bound Ag_t], with lifetime T_5, thus

$$\frac{d[Ag]_t}{dt} = S_t - \frac{[Ag]}{T_6} - [\text{bound } Ag_t]/T_5 \quad (6)$$

These six differential equations (1) to (6), together with the conditions of chemical equilibrium (which determine r, r' and R', and [bound-Ag] from N_t, [Ab] and [Ag]) and the definitions of F, G and H, constitute the equations of our model. They are to be solved subject to initial conditions on N_1, N_2, N_3, N_4, [Ab] and [Ag] for a specified antigen source S_t. Usually, a population of target cells, $N_1(0)$, is postulated, the initial concentrations of other cells and antibodies are set to zero, antigen is introduced either as an initial condition, Ag(0), or as a source, S_t, and the response of the system is determined as a function of time. Because many of the coefficients of the differential equations are functions of R' or r, which in turn depend on N_t, [Ab] and [Ag], the differential equations are non-linear and the solutions have been found by numerical methods.

So far, we have assumed that the antibodies produced in response to an antigen are all identical. In fact, even the antibodies produced in response to a simple antigen are heterogeneous, differing in association constants for binding antigen by orders of magnitude. Moreover, measurements of the association constants (affinity) and their variations with time are among the most relevant for comparison with the calculations.

In the clonal selection theory, heterogeneity of antibodies is said to arise from heterogeneity of the target cells. The model has therefore been generalized by dividing the populations of cells and antibodies into a finite number of groups. Let each group have an index $j(j = 1, 2, \ldots J)$ such that all quantities pertaining to that group have a subscript j. For example, $N_{1,j}$ is the number of target cells in group j; k_j' is their affinity for antigen, R_j' is the mean number of bound receptor sites per cell in group j, and so on. Cells (and antibodies) in the different groups have different affinities for antigen but a target cell in a particular group is assumed to produce other cells and antibodies exclusively in that group. Certain quantities are, for simplicity, assumed to be independent of j, namely, m, c_2, c_3 and all lifetimes.

With these assumptions, the foregoing equations have been generalized to allow for a multigroup representation of cell and antibody heterogeneity[14]. The resulting system of equations is solved by the program Abigail 2, on the Los Alamos digital computer, Maniac II. The program determines, at each time, the concentrations of the groups of cells, antibodies and antigen and an effective association constant, $\bar{K}$, for the population of antibodies (and free antibodies). Because of the wide range of association constants, a logarithmic scale of association constants is used in the multigroup calculation (that is, $k_{j+1} = \text{const} \times k_j$) and $\bar{K}$ is determined as $\bar{K} = \exp(\overline{\log k})$. It has been shown[14] that the resulting values of $\bar{K}$ are almost equivalent to the "average association constant, K_0" used in the immunological literature[1].

Comparison with Experiments

The model may be used to predict the response of a system, in terms of cellular proliferation and antibody production, to a prescribed exposure to antigen. In making quantitative comparisons with experiments on antibody production it is important to have experimental information on both the concentration and affinity of the antibodies or at least to know how the antibody assay depends on both these quantities. In a series of careful experiments on rabbits[15-17], antibody–antigen association constants in various conditions were measured. The antigen was bovine γ-globulin to which 2,4-dinitrophenyl (DNP) groups had been attached, and the anti-DNP antibodies were analysed. I have chosen constants in the model in an attempt to simulate this experimental system. The following values were selected[14]: $c_2 = c_3 = 7 \times 10^6$ h^{-1} (ref. 18); $T_1 = T_2 = 10$ h; $T_5 = 10$ h, $T_6 = 100$ h, $T_5' = 200$ h (ref. 1); $T_3 = 50$ h (ref. 19); $m = 10^3$. Cells and antibodies in the same group were assumed to have the same affinity for antigen, that is, $k_j = k_j'$ for $j = 1, 2, \ldots J$. The immunological system of a rabbit was taken to be a volume of 0·2 l., and an antigen molecule was assumed to have ten independent and identical DNP sites per molecule.

One thousand[18] target cells were taken, normally distributed (in log k') in seventeen groups between $k_1' = 3{\cdot}9 \times 10^3$ and $k_{17}' = 2{\cdot}6 \times 10^8$ l./mole, such that three cells were in groups 1 and 17. This choice was based on observations[15] of antibodies with association constants as high as 10^8 but with strong fluctuations between individual rabbits, suggesting few target cells of such high affinity for antigen. In many conditions, however, antibody affinities between 10^5 and 10^6 l./mole were found, suggesting a more abundant supply of corresponding target cells.

Antigen has been put into the model system, either as an initial condition or as a prolonged source. Experimentally, the antigen was injected with Freund's complete adjuvant which produces a slow absorption of the antigen by the animal[1]. Such a source was taken to be a linear function of time declining to zero at 10^3 h.

Before making detailed comparisons with the experiment, some general features of the computed response are considered. The concentration of free antigen, L, selects certain groups of cells for proliferation. A high concentration of antigen kills cells of high affinity and stimulates those of low affinity while a low concentration of antigen stimulates only cells of high affinity. Roughly, groups for which $10^{-2} \lesssim k_j'L \lesssim 1$ will proliferate most rapidly[14]. If the free antigen concentration, L, is nearly constant for a

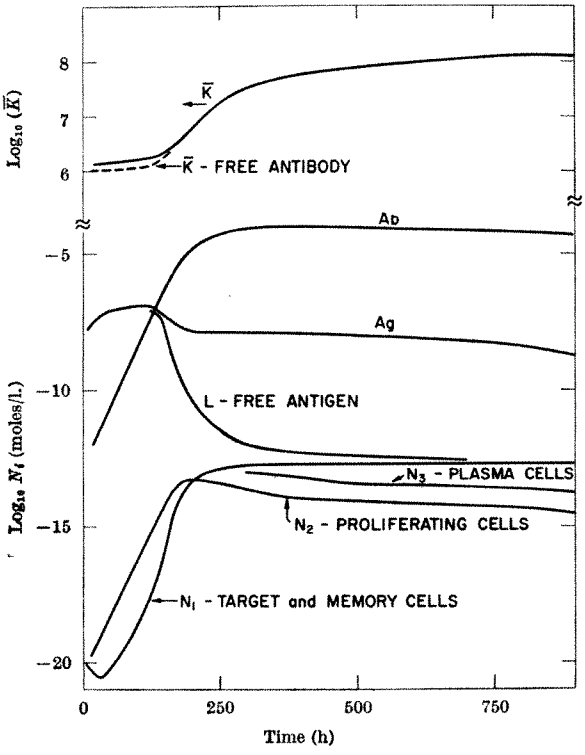


Fig. 2. Calculated concentrations and effective antibody association constant, $\bar{K}$, for a prolonged (1,000 h) source of antigen, containing 3 mg presented to the standard target cells. Parameters, given in the text, were chosen to represent DNP-bovine, γ-globulin as antigen in a rabbit.

long period of time, the antibodies will tend to have association constants reflecting this selective proliferation, that is, $10^{-2} \lesssim \bar{K}L \lesssim 1$.

The response to a typical prolonged source of antigen is shown in Fig. 2. Here, memory cells were identical to target cells so that the combined population first declines, because of the depletion of target cells, and later increases as a result of unsymmetric division of proliferating cells. At early times, the concentrations of proliferating cells and antibodies increase more or less exponentially. When the concentration of antibodies reaches the concentration of antigen, [Ab] = [Ag], the antibodies combine with much of the antigen and the concentration of free antigen begins to decrease to a low level. Cellular proliferation thereupon diminishes and the antibody population begins to decline. Note, however, that while the free antigen concentration is decreasing, the effective antibody affinity, $\bar{K}$, is increasing by about two orders of magnitude. This results from selective proliferation of those groups of cells with highest affinity for antigen.

Many features of the computed response are in agreement with experiment. The exponential increase of antibody concentration followed by a plateau and gradual decline are in general accord with experiments. The calculated increase of $\bar{K}$ from about 10^6 to 10^8 moles/l. is in excellent agreement with experiments[15], though this is partly the result of the design of the target cell affinity spectrum, $N_{1,j}(0)$. The computed variation of $\bar{K}$ is somewhat more rapid than observed.

A series of these experiments have been done with different doses of antigen, both as an initial condition (to simulate intravenous injection) and as a prolonged source (to simulate injection with adjuvant) and results are compared in Table 1. During the response to antigen as an initial condition, the antibody affinity, $\bar{K}$, does not increase much in time (compare column 3 of Table 1*A* giving $\bar{K}$ at an early time with column 5 giving the final value). The reason for this small increase is that after [Ab] = [Ag], the antigen is rapidly eliminated from the system and the free antigen concentration decreases rapidly so that there is only a short time during which the selective proliferation of high affinity cells takes place. This is in contrast to the result with a prolonged source (see Fig. 2 and Table 1*B*) where the free antigen concentration remains at a low but mildly stimulating level for a long time. Thus, if antigen is given as an initial condition it is possible to obtain high concentrations of low affinity antibodies or low concentration of high affinity antibodies. Only if the antigen is administered as a prolonged source is it possible to obtain a large amount of high affinity antibodies. This feature furnishes an explanation of the well known efficacy of adjuvants[1].

For the high doses in Table 1, cells in various high affinity groups are killed (see the last column) by the postulated mechanism of high dose tolerance. The prolonged source leads to lower antigen concentrations, destruction of fewer groups and, for this reason also, to higher affinity antibodies. It can be seen, however, that the responses to the highest doses of antigen in Table 1 predict an unreasonable amount of cellular proliferation and antibody production. I infer that when a rabbit is given such a large injection of antigen, its antibody producing system may be "overloaded" in that cellular crowding and similar effects will retard the proliferation[14].

The early values of $\bar{K}$ (third column of Table 1*B*) are in excellent agreement with the earliest values[15] measured for doses of 5–250 mg. This is shown in Fig. 3 where experimental[15] and calculated[14] values of $\bar{K}$ are compared for free antibodies. For the higher doses the measured value of $\bar{K}$ did not increase appreciably with time (in contrast to the results in Table 1*B*); this may be the result of overloading of the experimental system. Experimental[17] and calculated threshold doses to produce a barely detectable level of antibodies (50–70 μg/ml.) are in agreement at around 30 μg.

In Table 1*B*, it is seen that very small doses are leading to killing of high affinity cells. This unexpected effect is suggestive of tolerance produced by very low doses of antigen[20,21] and has been examined with care. It turns out to be the result of a detail of the numerical program[14] which does not have a clear biological counterpart. There is, however, an obvious mechanism for including low dose tolerance in the model. Consider target cells exposed to a low concentration of antigen such that $R' \ll 1$ and few target cells will be stimulated in a time T_1. The resulting proliferating cells will be weakly stimulated and will most likely divide only once. Very small clones, consisting of exactly two cells, will result. In the model it was assumed that equal numbers of memory and plasma cells arise from the proliferation, but if on average more plasma

Table 1. RESPONSE OF STANDARD TARGET CELLS TO VARIOUS DOSES OF ANTIGEN

(*A*) Antigen as initial condition or sudden injection

	When [Ab] = [Ag]			Final (300 h) conditions		
Dose	Time (h)	$\bar{K}$ l./mole	[Ab] moles/l.	$\bar{K}$ l./mole	N_1 moles/l.	No. of cell groups killed
300 mg	205	$4{\cdot}5\times10^{4}$	$8{\cdot}9\times10^{-4}$	$1{\cdot}4\times10^{5}$	$2{\cdot}7\times10^{-12}$	8
30 mg	170	$1{\cdot}2\times10^{5}$	$1{\cdot}6\times10^{-4}$	$4{\cdot}0\times10^{5}$	$4{\cdot}7\times10^{-13}$	6
3 mg	145	$4{\cdot}0\times10^{5}$	$2{\cdot}7\times10^{-5}$	$1{\cdot}3\times10^{6}$	$7{\cdot}8\times10^{-14}$	4
300 μg	120	$1{\cdot}4\times10^{6}$	$4{\cdot}0\times10^{-6}$	$4{\cdot}8\times10^{6}$	$1{\cdot}2\times10^{-14}$	2
30 μg	105	$5{\cdot}3\times10^{6}$	$5{\cdot}9\times10^{-7}$	$1{\cdot}8\times10^{7}$	$1{\cdot}8\times10^{-15}$	1
3 μg	100	$2{\cdot}2\times10^{7}$	$8{\cdot}0\times10^{-8}$	$7{\cdot}2\times10^{7}$	$2{\cdot}4\times10^{-16}$	0
0·3 μg	100	$7{\cdot}5\times10^{7}$	$9{\cdot}1\times10^{-9}$	$1{\cdot}9\times10^{8}$	$2{\cdot}3\times10^{-17}$	0
0·03 μg	140	$8{\cdot}9\times10^{7}$	$1{\cdot}1\times10^{-10}$	$2{\cdot}0\times10^{8}$	$2{\cdot}4\times10^{-20}$	0
(*B*) Prolonged (1,000 h) source of antigen: final conditions at 1,000 h						
300 mg	190	$3{\cdot}2\times10^{5}$	$3{\cdot}8\times10^{-3}$	$1{\cdot}9\times10^{7}$	$2{\cdot}0\times10^{-11}$	3
30 mg	160	$6{\cdot}8\times10^{5}$	$3{\cdot}9\times10^{-4}$	$3{\cdot}8\times10^{7}$	$2{\cdot}0\times10^{-12}$	2
3 mg	140	$1{\cdot}9\times10^{6}$	$3{\cdot}8\times10^{-5}$	$1{\cdot}4\times10^{8}$	$2{\cdot}2\times10^{-13}$	0
300 μg	120	$6{\cdot}6\times10^{6}$	$4{\cdot}1\times10^{-6}$	$1{\cdot}6\times10^{8}$	$2{\cdot}2\times10^{-14}$	0
30 μg	110	$2{\cdot}6\times10^{7}$	$4{\cdot}4\times10^{-7}$	$1{\cdot}7\times10^{8}$	$2{\cdot}2\times10^{-15}$	0
3 μg	120	$9{\cdot}5\times10^{7}$	$5{\cdot}0\times10^{-8}$	$2{\cdot}1\times10^{8}$	$2{\cdot}4\times10^{-16}$	0
0·3 μg	200	$9{\cdot}3\times10^{7}$	$4{\cdot}2\times10^{-9}$	$1{\cdot}3\times10^{8}$	$1{\cdot}6\times10^{-17}$	1
0·03 μg	$>10^{3}$	—	9×10^{-10}	5×10^{6}	$7{\cdot}9\times10^{-21}$	3
(*C*) Secondary response. Both primary and secondary sources are prolonged (1,000 h) 3 mg doses						
3 mg	<20	$\sim6\times10^{7}$	6×10^{-8}	2×10^{8}	$4{\cdot}4\times10^{-13}$	0

Cell parameters simulate DNP-bovine γ-globulin as antigen in a rabbit. Column 6 represents the final concentration of memory cells plus target cells.

cells were made, then the net effect of producing these small clones would be to deplete the number of target plus memory cells. For example, if the proliferation produced three times as many plasma cells as memory cells, it would be possible to prescribe a low antigen concentration as a function of time which would reduce any initial population of target cells (plus the memory cells) to zero. This could be done without substantially affecting the response to high doses, in which very large clones are made.

Secondary responses may be simulated with the Abigail 2 program by using the memory cells from a first exposure to antigen as the target cells for the second exposure. Extensive cellular proliferation will result from the first exposure to moderate doses of antigen as shown in Table 1 and in Fig. 4. Moreover, when the first exposure is the consequence of a prolonged source of antigen, the memory cells will have high affinity for antigen and, during the secondary response, the antibodies will at all times have high affinity for antigen. The secondary response will be quicker than the primary response because of the larger number of target cells. An example is given in Table 1*C* for a 3 mg prolonged source of antigen. These qualitative features of the secondary response, quickness and high affinity antibodies, are in agreement with experiment[16,17] but comparable quantitative results do not seem to have been reported. Threshold doses in secondary responses have been compared with experiment with inconclusive results[14].

Usefulness of the Model

Considerable agreement is found between model calculations and experiments with simple antigens. How might the model be used for the planning and interpretation of experiments? First of all, the model suggests that it is useful to measure or have in mind both association constants and concentration of the quantities involved in an immune response. A concentration of free antigen will select certain cells for proliferation according to their affinity for antigen and the resulting antibodies will reflect this selective proliferation. The response will also depend on the affinity spectrum of the target cells, that is, the number of target cells as a function of affinity for antigen, and by studying the response of a system to

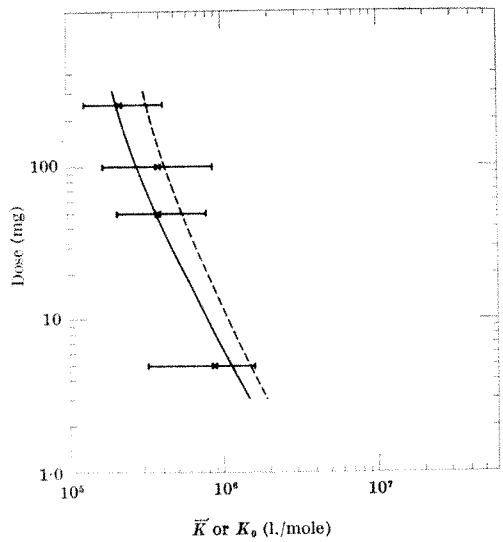


Fig. 3. Early antigen-antibody association constants. The measured value was found from a Sips plot, 2 weeks after injection of the indicated dose with Freund's complete adjuvant (ref. 15); mean and extreme values are shown with four or five rabbits for each dose. $\bar{K}$ was computed at the time when [Ag] = [Ab] and is representative of $\bar{K}$ at earlier times. —×—, Experimental values of K_0; - - -, computed $\bar{K}$ for total antibody; ———, computed $\bar{K}$ for free antibody only.

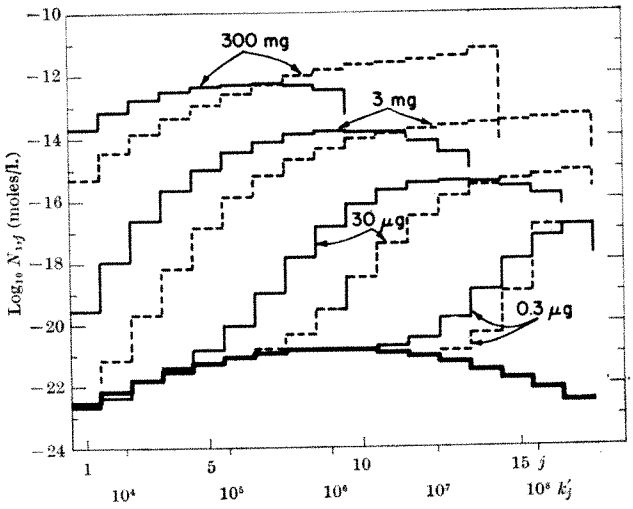


Fig. 4. Affinity spectrum for target and memory cell populations. The concentration of cells per group is shown as a function of the group index, j, and the antigen-receptor association constant or affinity, k_j'. ———, Initial target cell population: same all curves; ———, final population for rapid presentation of antigen as initial condition; - - -, final population for prolonged (1,000 h) source of antigen.

antigen it is possible to deduce some characteristics of the target cells and perhaps their origin. In particular, one can distinguish between virgin target cells and those selected by previous exposure to antigen, by both the quickness of the response and the affinity of the early antibodies[14].

If enough is known about the spectrum of target cells, then according to the model it is possible to tailor an antigen concentration to achieve special effects such as high dose tolerance, low dose tolerance or the selective proliferation of a single clone of cells, so as to produce nearly homogeneous antibody. The latter might be achieved by exposing a system having few target cells of high affinity to a prolonged low concentration of antigen. An ability selectively to produce homogeneous antibody could be of great help in elucidating the origins of antibody diversity. If, for example, two related animals were induced to produce identical antibodies, this would indicate an inheritance of the information prescribing the variable portion of the antibody.

The present model attempts to give a unified and quantitative interpretation of diverse immunological data; it suggests connexions and interpretations which might otherwise remain obscure. For example, we have already noted that certain high doses of antigen ($\gtrsim$30 mg of DNP bovine γ-globulin in rabbits) may lead to overloading of the immunological system in the sense that crowding of cells and similar effects change the response. A mechanism of low dose tolerance has also been suggested, namely, production of very small clones in which less than half the cells are memory cells. As a final example, I offer the speculation that heavy (19S) antibodies are produced by proliferating cells while light (7S) antibodies are produced by plasma cells. In the calculations, most antibodies early in the primary response come from proliferating cells; later in the primary response and at nearly all times in the secondary response the antibodies are largely of plasma cell origin. These time behaviours are analogous to those observed for the 19S and 7S antibodies[1]. In addition, it has been reported[22] that heavy and light antibodies have common genetic determinants in their variable portions, suggesting a clonal relationship of the producing cells.

Clearly many of the assumptions of the model are quite arbitrary, for example, the number of sites per cell, and the functions F, G and H which determine the response of the cells to antigen. Quantitative experiments on the

interactions between cells and antigen would therefore be valuable for confirming or denying the basic assumptions of the model. Progress has already been made in separating cells that bind particular antigens[23,24] and it is hoped that these antigen-cell interactions may be quantitatively characterized in the near future.

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Reconstitution of 50S Ribosomal Subunits from Dissociated Molecular Components

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50S ribosomal subunits of *Bacillus stearothermophilus* have been reconstituted by incubating their RNA and protein components at high temperatures.

30S RIBOSOMAL subunits have been reconstructed *in vitro* from their molecular components for ribosomes from several different species of bacteria[1,2]. This has made it possible to determine the parts that individual molecular components play in the function of the ribosomes[2-4] and to describe in some detail the events leading to the assembly of the 30S subunit from its parts[5,6,10].

The 50S subunit of the ribosome has been less tractable. It is much more complex than the 30S subunit, and contains two RNA molecules—with sedimentation coefficients of 23S and 5S—and about 35 distinct protein components (ref. 25 and C. G. Kurland, personal communication). This structural complexity could account for the difficulty of reconstituting 50S subunits *in vitro*; only partial reconstructions from protein deficient "core" particles and split proteins have been achieved[7-9]. None of these experiments revealed a functional requirement for 5S RNA. We now report that using *Bacillus stearothermophilus* 50S subunits, we can reconstitute "50S" particles with considerable protein-synthesizing activity from dissociated RNA and protein components. Using this system, there is a partial requirement for 5S RNA in the reconstitution of functional 50S particles.

Reconstitution of 50S Subunits

One striking characteristic of the 30S reconstitution reaction is its strong temperature dependence. An Arrhenius activation energy of about 40 kcalories/mole has been obtained by measuring the rate of reconstitution of *Escherichia coli* 30S subunits at various temperatures[10]. 50S reconstitution might have a much higher free energy of activation and therefore require a higher incubation temperature to achieve a measurable rate of assembly *in vitro*. This would explain the lack of success in attempts to reconstitute *E. coli* 50S subunits. To permit higher incubation temperatures without inactivating the ribosomes, we decided to use a thermophilic bacterium, *B. stearothermophilus*. Reconstitution of 30S subunits from this organism has previously been studied[2].

RNA ("RNA50") and protein ("TP50") fractions were prepared by treating purified 50S ribosomal subunits with urea-LiCl as described in the legend to Fig. 1. Usually, more than 90 per cent of RNA was recovered in the RNA fraction and only a few per cent of RNA was found in the protein fraction. The amount of residual protein in the RNA50 fraction was about 2 to 3 per cent of that in an equivalent amount of 50S subunit. The residual proteins in "RNA50" fractions were analysed by standard polyacrylamide gel electrophoresis at *p*H 4·5 (ref. 11) after digestion of RNA with ribonuclease. Two weak bands were observed, both of which had counterparts with much higher intensities in the control gel made with an equivalent amount of TP50 fraction. Thus extraction of these two proteins by the urea-LiCl method was not complete, and the RNA fraction used for the reconstitution probably consists of a mixture of free RNA molecules and RNA molecules associated with these two proteins. Whether the reconstitution of functional 50S particles to be described below is really from free RNA or from RNA molecules with these proteins still tightly bound is not established. Fig. 1*a* shows the sedimentation pattern of the "RNA50" preparation analysed on a sucrose gradient. Intact 23S rRNA was a single peak. About 70 per cent of the 5S RNA was present in the protein fraction (TP50) and about 30 per cent in the "RNA50" fraction.

In many earlier experiments, the protein preparation was simply diluted with the reconstitution buffer and then mixed with the RNA preparation and heated at 60° C for 45 min as described in the legend to Fig. 1. The standard conditions for reconstitution were determined from several experiments to be described below. After incubation, particles were recovered and analysed by sucrose density gradient centrifugation in the presence of a small amount of reference ^{32}P-50S subunits. Fig. 1*b* shows that most of the original 23S ribosomal RNA (rRNA) then sedimented in a single peak which coincided with the peak of the reference ^{32}P-50S subunits. Thus the

addition of the 50S protein fraction to the RNA preparation converted most of the 23S rRNA to particles sedimenting at 50S.

Biological activity of the reconstituted particles was determined by using a poly U-directed polyphenylalanine synthesizing system[12] in the presence of 30S ribosomal subunits, and comparing this with the activity of control (undissociated) 50S subunits which were treated in the same way. The activity of the control 50S ribosomal subunits varied with the preparation, but remained about the same in different experiments as long as the same preparation was used. Table 1 and Fig. 2 show that reconstituted particles have definite incorporation activity. Neither the protein fraction alone nor the RNA fraction alone showed any significant activity (Table 1, experiments 1, 2 and 3). The degree of restoration of synthetic activity varied from 15 per cent to about 60 per cent depending on the experiment. One possible source of variation might be variable inactivation of the components during the incubation (60° C, 45 min). The presence of small amounts of urea in the reconstitution mixture in earlier experiments (final concentration 0·02 to 0·2 M) may be responsible for this inactivation. The reconstitution activity was higher when dialysed TP50 fraction was used. In addition, kinetic experiments with dialysed TP50 fraction suggested less inactivation during the incubation at 60° C over extended periods. For the sake of convenience, however, many earlier experiments to optimize conditions for reconstitution were done using undialysed protein preparations. The degree of reconstitution after 30 min incubation at 60° C was essentially constant at different urea concentrations used in these experiments (between 0·02 and 0·2 M urea). At 0·4 M or higher urea concentration, reconstitution was definitely impaired.

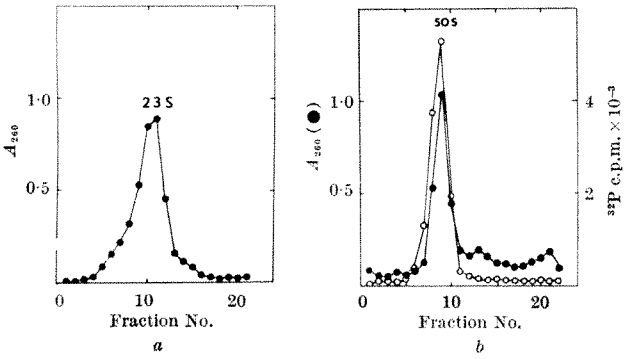


Fig. 1. Sucrose gradient sedimentation pattern of "RNA50" used for the reconstitution (*a*) and particles recovered from the reconstitution mixture (*b*). RNA and proteins used for the reconstitution were prepared as follows: purified 50S ribosomal subunits in TMAI buffer (about 200 to 500 A_{260} units per ml.) were mixed with an equal volume of 8 M urea-4 M LiCl solution and kept in an ice bath for 40 to 50 h. (In other experiments, ribosomes were treated with an equal volume of 8 M urea-4 M LiCl solution plus 0·6 volume of 10 M LiCl solution.) The mixture was centrifuged (10,000 r.p.m. for 20 min), and the supernatant was used as total 50S ribosomal proteins (TP50). Precipitated RNA was washed with a mixture of 8 M urea-4 M LiCl solution and TMAI (1 : 1), and then dissolved in H_2O. Composition of TMAI is as follows: 10^{-2} M Tris-HCl (*p*H 7·8)–10^{-2} M $MgCl_2$–3×10^{-2} M NH_4Cl–6×10^{-3} M mercaptoethanol. Reconstitution was done as follows: 10 A_{260} equivalents of 50S proteins (in about 50 μl.) prepared as above were diluted with 5 ml. of the standard reconstitution buffer: 10^{-2} M Tris-HCl (*p*H 7·5)–2×10^{-2} M $MgCl_2$–0·3 M KCl–6×10^{-3} M mercaptoethanol. 1 A_{260} equivalent is defined as the amount of protein which can be obtained from 1 A_{260} unit of ribosomes. RNA prepared as above (10 A_{260} units in 30 to 100 μl.) was added. The mixture was heated at 60° C for 45 min, and then cooled in ice. 0·65 volume of ethanol (precooled at −20° C) was added and the mixture was kept for about 1 h at −20° C. The reconstituted ribosomal particles precipitated in this way were recovered by centrifugation and dissolved in 0·2 ml. of TMAI. Aliquots were used for analyses of incorporation activity and/or sedimentation pattern of the reconstituted particles. In *a*, about 5 A_{260} units of RNA preparation were mixed with a small amount of reference ^{32}P-labelled 50S ribosomal subunits and put on a sucrose gradient (5–20 per cent in TMAI buffer, 4·6 ml.) and centrifuged at 45,000 r.p.m. for 100 min in the cold. Fractions were collected from the bottom of the tube, suitably diluted and both A_{260} and radioactivity were measured. The peak of ^{32}P-50S was at fraction 6. In *b* the reconstituted "50S" particles (about 10 A_{260} units) were mixed with a small amount of ^{32}P-labelled 50S ribosomal subunits (less than 0·5 A_{260} unit), put on a sucrose gradient (10–30 per cent in TMAI buffer, 16 ml.) and centrifuged at 22,500 r.p.m. for 18 h in the cold. Fractions were collected and measured for both A_{260} and ^{32}P.

Table 1. POLYPEPTIDE SYNTHETIC ACTIVITY OF 50S PARTICLES RECONSTITUTED FROM DISSOCIATED RNA AND PROTEIN COMPONENTS

Exp.	Components present during reconstitution	c.p.m.	50S activity of control per cent 50S	Per cent of standard reconstitution
1	TP50+"RNA50"	1,273	50	(100)
	TP50	55	2	4
	(Control 50S)	(2,540)	(100)	—
2	TP50+"RNA50"	792	30	(100)
	TP50+"23S RNA"	907	35	115
	TP50	53	2	7
	(Control 50S)	(2,610)	(100)	—
3	TP50+"RNA50"	1,017	18	(100)
	TP50+"RNA30"	120	2	12
	"RNA50"	0	0	0
	(Control 50S)	(5,550)	(100)	—
4	TP50+"RNA50"	2,545	61	(100)
	TP50+"RNA50(phenol)"	793	19	31
	TP50+"RNA50(phenol)"+30S	1,390	33	54
	(Control 50S)	(4,170)	(100)	—

TP50: Total proteins prepared from 50S by urea-LiCl method; "RNA50": RNA fraction prepared from 50S by urea-LiCl method; "RNA30": RNA fraction prepared from 30S by urea-LiCl method; "RNA50(phenol)": total RNA (23S and 5S) prepared from 50S by phenol extraction method.

Reconstitution procedures are described in the legend to Fig. 1. Reconstituted "50S" particles were recovered by alcohol precipitation. Aliquots containing 1·4 A_{260} units of particles were assayed for poly U-directed phenylalanine incorporation activity in the presence of 1·0 A_{260} unit of *E. coli* 30S ribosomal subunits according to the method described previously[23]. Undissociated *B. stearothermophilus* 50S ribosomal subunits treated in the same way were used as control. The treatment (that is, heating in the reconstitution mixture at 60° C for 45 min and alcohol precipitation after cooling) resulted in reduction of activity of control 50S ribosomal subunits to 40 per cent of the original activity. This reduction took place almost entirely during the alcohol precipitation step; and the heating step itself did not cause any significant reduction. Because the degree of activity reduction was the same in each set of experiments, the alcohol precipitation could be used without introducing uncontrollable variations. In experiment 2, "23S RNA" was prepared as follows: "RNA50" prepared by the urea-LiCl method was centrifuged on a sucrose density gradient. The A_{260} of aliquots was measured, peak fractions containing 23S RNA (see Fig. 1*a*) were pooled, and RNA was recovered by alcohol precipitation. In experiment 4, "RNA50(phenol)" was prepared as follows: 50S ribosomal subunits (in TMAI) were treated with phenol (saturated with 0·15 M NaCl–0·015 M sodium citrate) in the presence of 0·17 per cent bentonite. Phenol extraction was repeated six times. RNA was finally precipitated with 2 volumes of alcohol, dissolved in H_2O and dialysed overnight against H_2O. The reconstitution was done as described before except that 5 A_{260} units of purified *B. stearothermophilus* 30S ribosomal subunits were added in the 5 ml. reconstitution mixture as indicated. After incubation at 60° C for 45 min, the reaction mixtures were cooled in ice. Five A_{260} units of 30S subunits were added to the tubes which did not have 30S subunits. The reconstituted particles as well as added 30S subunits were then recovered by the alcohol precipitation method.

Table 1 also compares the activities obtained with several different RNA preparations. First, RNA ("RNA50") prepared by the urea-LiCl method can be purified by sucrose gradient centrifugation followed by alcohol precipitation. The 23S rRNA obtained from the peak region after sucrose gradient centrifugation was as active as the starting RNA preparation (Table 1, experiment 2). This result is not surprising, for we have found that the protein fraction contains most of the 5S RNA.

In contrast to "RNA50" prepared by the urea-LiCl method, RNA prepared by phenol treatment of 50S subunits was only weakly active (Table 1, experiment 4, and Fig. 2). The difference between "RNA50" and "RNA50 (phenol)" does not seem to result from incomplete removal of proteins from "RNA50". Several RNA preparations made by repeated phenol extractions were analysed for residual proteins by the ribonuclease digestion-polyacrylamide gel electrophoresis technique. This "RNA50 (phenol)" also contained the same two protein bands in amounts comparable with those in the "RNA50" preparation. The difference in activity between two kinds of preparation may be due to difference in their conformation. "RNA50" was prepared in the presence of 5×10^{-3} M Mg^{2+}, whereas "RNA50(phenol)" was prepared by repeated extraction of 50S with phenol saturated with a solution of NaCl (0·15 M) and sodium citrate (0·015 M). The former showed a slightly higher sedimentation coefficient (2–3S) than the latter in several conditions. In polyacrylamide gel electrophoresis, the former moved slightly faster than the latter. These results suggest that "RNA50" has a more compact structure than RNA prepared by the phenol method.

Table 1 (experiment 3) also shows that RNA (16S rRNA, called "RNA30" here) prepared from 30S subunits by the same urea-LiCl method cannot replace RNA

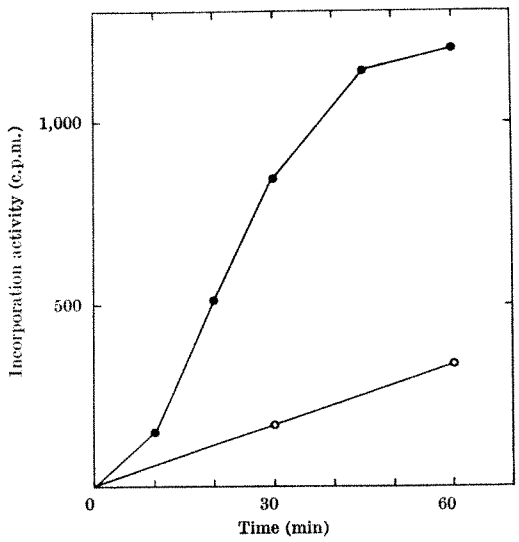


Fig. 2. Time course of reconstitution. Reconstitution procedures were as described in the legend to Fig. 1. After various times of incubation at 60° C, the reconstitution reaction was stopped by cooling, and particles were recovered by alcohol precipitation. Aliquots containing 1·5 A_{260} units of RNA were analysed for the incorporation activity. Closed circles: with "RNA 50"; open circles: with RNA prepared by phenol extraction method.

(23S rRNA) from 50S subunits. The same "RNA30" was fully active in 30S reconstitution (see Fig. 4). The 30S subunit preparation which was used to prepare RNA ("RNA30") for the experiment in Table 1 contained a small amount of 50S subunits (about 10 per cent of the total amount). This contamination can account for the weak activity of "RNA30" when tested with TP50. This shows that the requirement for RNA is specific.

Efficiency of Reconstitution

We have examined the effect of several factors on the efficiency of reconstitution by measuring the degree of reconstitution at times when reconstitution was still proceeding (usually after 30 min incubation, see Fig. 2). Thus the degree of reconstitution measured roughly reflects the rate of reconstitution, although precise information on kinetics of the reconstitution cannot be obtained from these results.

Fig. 3 shows the effect of KCl concentration on reconstitution. As in the case of reconstitution of *E. coli* 30S subunits (optimum ionic strength about 0·37, ref. 10), there is a sharp ionic strength optimum (about 0·33) for the reconstitution of 50S particles. If the ionic strength of the reconstitution mixture is below 0·1 or above 0·8, very little reconstitution takes place. The optimum ionic strength is about the same in these two different reconstitution systems, namely, *E. coli* 30S and *B. stearothermophilus* 50S reconstitutions.

The high ionic strength is probably needed to prevent non-specific RNA–protein or protein–protein interactions, as it is with *E. coli* 30S reconstitution[5,10]. The inhibition by higher ionic strength may be caused either by a weakening of specific interactions by high salts or by the formation of an inactive, highly compact ribosomal RNA structure.

Fig. 4 shows the effect of the temperature of incubation. The degree of reconstitution after incubation for 30 min was compared at different temperatures. For comparison, the effect of temperature on the reconstitution of 30S subunits from the same organism was examined in identical reconstitution conditions. The degree of reconstitution of 30S subunits after 5 min incubation at various temperatures is shown in Fig. 4. There is a marked difference in temperature dependence between 30S and 50S ribosome reconstitution. At 50° C, 30S reconstitution is more than 50 per cent complete in 5 min, whereas 50S reconstitution is barely detectable even after 30 min incubation.

The optimum temperature for 50S ribosome reconstitution is about 60° C. At 65° C, the efficiency of reconstitution drops, presumably because of heat inactivation of ribosomal components, intermediates or finished reconstituted "50S" particles. Thus 60° C was selected as the standard incubation temperature.

Cells of *B. stearothermophilus* multiply with a doubling time of about 15 min at 60° C. Thus although 50S ribosomal subunits can be assembled *in vitro*, their rate of assembly is much slower than the rate of assembly *in vivo* or that of 30S subunits under identical *in vitro* conditions.

Effect of 30S Subunits on Reconstitution

Genetic studies in our laboratory strongly suggest that the assembly of 50S ribosomal subunits *in vivo* is aided by participation of 30S ribosomal subunits or their precursor particles (ref. 13, and unpublished results of Nashimoto and Nomura). We therefore added purified 30S ribosomal subunits in an amount equivalent to that of the "RNA50" present in the system, incubated the mixtures for various times at 60° C, and compared the incorporation activity of the resultant particles with that of the control particles reconstituted in the absence of 30S subunits. 30S subunits were added to the control sample after the incubation. They frequently gave a significant stimulation of reconstitution, but the effect was not reproducible. A more reproducible stimulatory effect was observed when RNA50 prepared by the phenol extraction method was used instead of "RNA50" prepared by the urea-LiCl method. An example is shown in Table 1 (experiment 4). Even with phenol-prepared RNA, however, the stimulatory effect of 30S subunits was not always observed. The reason for this variability as well as the significance of the apparently positive stimulatory effect of 30S in many experiments is not clear. Thus we still do not know whether the coupling of 50S subunit biosynthesis with 30S biosynthesis inferred from *in vivo* work can be demonstrated *in vitro*.

5S RNA and 50S Reconstitution

The 50S ribosomal subunit contains one molecule of 5S RNA[14]. The role of 5S RNA is unknown. We have

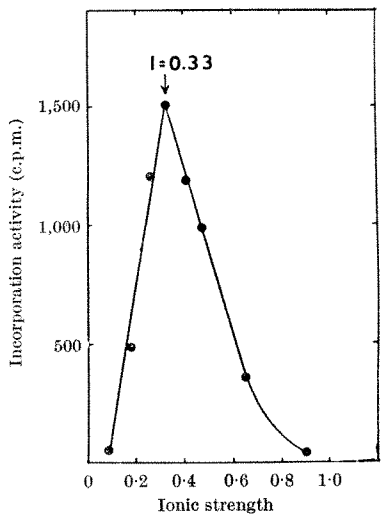


Fig. 3. Effect of ionic strength on reconstitution. 10 A_{260} units of "RNA50" were mixed with the equivalent amount of 50S proteins in 5 ml. of reconstitution buffer which had the standard concentration of all components (see the legend to Fig. 1) except KCl. KCl concentration was varied and the final ionic strength of the reconstitution buffers is shown on the abscissa. The mixtures were incubated at 60° C for 30 min and the reconstituted particles were recovered by alcohol precipitation as described in the legend to Fig. 1. Aliquots of the particle containing 1·5 A_{260} units were assayed for their activity in poly U-directed ^{14}C-phenylalanine incorporation.

done preliminary fractionation of the present reconstitution system in order to see whether 5S RNA is required for 50S function.

Analysis of ^{32}P-labelled 50S subunits showed that the principal portion (about 70 per cent) of 5S RNA is present in the protein fraction obtained by the urea-LiCl method. The protein fraction was dialysed against urea-Tris buffer containing 0·2 M KCl and then fractionated by DEAE-cellulose column chromatography as described in Table 2. Most of 5S RNA was eluted by 1 M KCl (fraction C), but not by 0·3 M KCl. Reconstitution experiments were then performed using separated fractions (Table 2). In the absence of fraction C, only weak 50S activity was observed after reconstitution. Addition of fraction C to the system increased the degree of reconstitution by a factor of about two. Analysis of RNA in fraction C by polyacrylamide gel electrophoresis[24] showed that it contains 5S RNA in addition to a very small amount of high molecular weight RNA and 4S RNA. Both *E. coli* 5S RNA and *B. stearothermophilus* 5S RNA purified by a standard gel filtration method[15] could replace fraction C in the stimulation of reconstitution (Table 2). This shows that 5S RNA is an important component of the 50S subunit. 5S RNA is also present in the "RNA50" fraction used in the reconstitution, and this may explain why we could not get a higher stimulatory effect of 5S RNA.

Using protein-deficient particles, Monier and his co-workers demonstrated specific reassociation of 5S RNA with ribosomal particles[16–18]. But their reconstituted "48S particle" did not have incorporation activity. In the present reconstitution system, we also studied incorporation of 5S RNA into reconstituted particles. Radioactive 5S RNA was prepared from ^{32}P-labelled 50S subunits in the following way: the protein fraction obtained by the urea-LiCl method was fractionated by DEAE-cellulose column chromatography as described in Table 2, and fraction C was extracted with phenol. RNA recovered by alcohol precipitation was further purified by sucrose gradient centrifugation to remove a small amount of high molecular weight RNA. This ^{32}P-5S RNA preparation was about 95 per cent pure as judged by polyacrylamide gel electrophoresis. Contamination by ^{32}P-transfer RNA was very small. This ^{32}P-5S RNA was added to the usual reconstitution mixture containing non-radioactive 5S RNA. The amount of ^{32}P-5S RNA added was about equal to that already present in the reconstitution mixture. After incubation, reconstituted particles as well as any residual RNA were recovered by alcohol precipitation and analysed by zonal sedimentation on a sucrose gradient in the presence of reference ^{3}H-50S subunits. As shown in Fig. 5, about 55 per cent of ^{32}P-5S RNA co-sedimented with the reconstituted particles in a single peak which coincided with the peak of reference ^{3}H-50S subunits. We conclude that externally added ^{32}P-5S RNA was incorporated into 50S particles. This incorporation seems to be quantitative—that is, every reconstituted "50S" particle has about one 5S RNA molecule in addition to one 23S rRNA molecule.

Table 2. FRACTIONATION OF 50S RIBOSOMAL PROTEINS AND STIMULATORY ACTIVITY OF 5S RNA

Exp.	Fractions added together with "RNA50"	50S activity c.p.m.	50S activity Per cent	Stimulation by "5S"
1	A	83	5	—
	A+B	279	16	(1·0)
	A+B+C	447	26	×1·6
	A+B+*E. coli* 5S	623	36	×2·2
	(Control 50S)	(1,746)	(100)	
2	A+B	229		(1·0)
	A+B+C	542		×2·4
	A+B+*B. stear.* 5S	461		×2·0

A, 0·2 M KCl eluate fraction obtained from TP50 in DEAE-cellulose column chromatography, 10 A_{260} equivalents per tube; B, 0·3 M KCl eluate fraction, 10 A_{260} equivalents per tube; C, 1 M KCl eluate fraction containing 5S RNA, 0·35 A_{260} units per tube; *E. coli* 5S, pure *E. coli* 5S RNA, 2·0 A_{260} units per tube; *B.s tear.* 5S, pure *B. stearothermophilus* 5S RNA, 1·8 A_{260} units per tube.

About 140 A_{260} equivalents of TP50 fraction prepared as described in the legend to Fig. 1 were dialysed overnight against the buffer containing 4 M urea–0·01 M Tris, *p*H 7·8–0·2 M KCl–10^{-3} M $MgCl_2$, and then applied to a small DEAE-cellulose column (1·0 cm × 1·3 cm). The column was washed with the same buffer, and materials which came through were called fraction A. The column was then eluted with the same buffer but containing 0·3 M KCl (fraction B), and finally with the buffer containing 1 M KCl (fraction C). Fraction C was dialysed overnight against H_2O. The reconstitution mixture contained, in a final volume of 5·7 ml. 10 A_{260} units of "RNA50", and in addition, fractions A, B, C and pure *E. coli* 5S RNA or *B. stearothermophilus* 5S RNA as indicated. Final ionic strength was adjusted to 0·33 and final urea concentration to 0·2 M. After incubation at 60° C for 45 min, the reconstituted particles were recovered by alcohol precipitation and aliquots containing about 1·4 A_{260} units of RNA were assayed for poly U-directed phenylalanine incorporation activity.

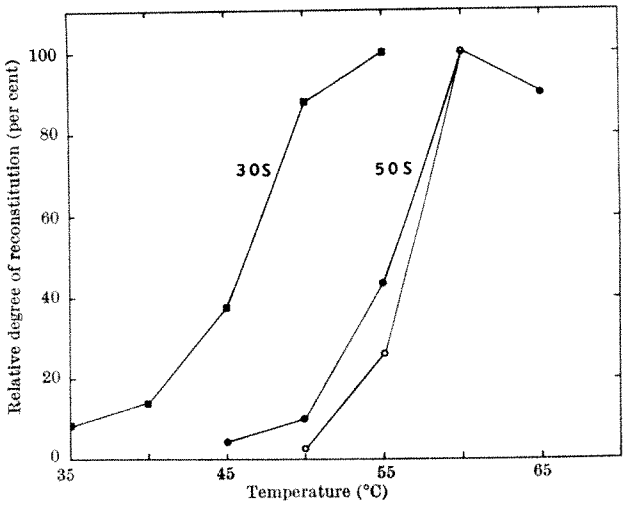


Fig. 4. Effect of temperature on reconstitution. Reconstitution of 50S subunits was done in two ways at different temperatures. The incubation time was 30 min in both cases. In one case (○) reconstitution was done as described in Fig. 1. In the other case (●), reconstitution was done using dialysed TP50 preparation as follows. The reconstitution mixture contained 4·8 A_{260} units of "RNA50" and 8·0 A_{260} equivalents of dialysed TP50 in the final volume of 0·48 ml. Other components in the reaction mixture were the same as in the first method. After incubation for 30 min at 60° C, aliquots containing 1 A_{260} unit of particles (100 μl.) were taken and the polypeptide synthesizing activity of reconstituted particles was assayed directly (without alcohol precipitation) at 37° C using the poly U-directed phenylalanine incorporation system. In both cases, the activity obtained at 60° C (1,330 c.p.m. and 463 c.p.m., respectively) was taken as 100 per cent. Reconstitution of 30S subunits was done using the same conditions as used for 50S reconstitution in the second case above (■). Both RNA and proteins were prepared from the purified 30S subunits. Reconstitution mixtures were incubated at the indicated temperatures for 5 min. The incorporation activity (assayed by poly U-directed phenylalanine incorporation in the presence of 50S subunits) obtained at 55° C was taken as 100 per cent which corresponded to 53 per cent of the activity of the control 30S subunits treated in the same way.

Significance of *in vitro* 50S Reconstitution

Although 50S subunits are probably reconstituted from completely protein-free RNAs, the RNA fraction used for the reconstitution contained a small amount of two different proteins. It is therefore possible that the presence of these proteins bound to 23S rRNA is important for reconstituting functional 50S particles. Physical conversion of 23S rRNA to particles sedimenting at 50S is almost quantitative. In this case, protein-free 23S rRNA is certainly converted to "50S" particles, which may or may not be functional. Incorporation of protein-free 5S RNA molecules into "50S" particles is also quantitative. The present reconstitution system will be useful in the study of the physical and functional roles of the molecular components of 50S subunits, as exemplified by our preliminary experiments on the role of 5S RNA.

50S subunits can be reconstituted in the absence of any other external macromolecular components, which demonstrates that the information for their assembly is contained in the structure of their molecular components, as is the case with 30S subunits. The *in vitro* assembly reaction in the present system, however, is too slow to account for the efficient ribosome assembly reaction which takes place *in vivo*. Perhaps our *in vitro* conditions are not fully optimized. Alternatively, the *in vivo* assembly reaction may be much faster because it uses immature submethylated precursor 23S RNA[20–22] (and precursor

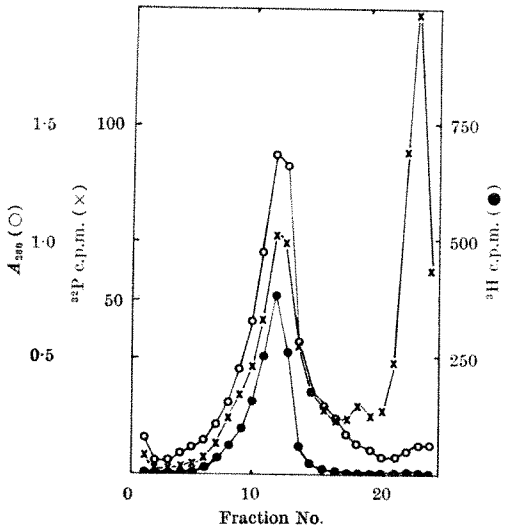


Fig. 5. Incorporation of ^{32}P-5S RNA into the reconstituted "50S" particles. The reconstitution of "50S" particles was done in the same way as described in the legend to Fig. 1 except that about 0·4 A_{260} units of ^{32}P-labelled 5S (about 800 c.p.m.) were added to the reaction mixture before the incubation. After the incubation at 60° C for 45 min, the reaction mixture was cooled to 0° C. The reconstituted particles were recovered by alcohol precipitation, mixed with a small amount of reference ^{3}H-labelled 50S (about 0·05 A_{260} unit), and then subjected to a sucrose gradient sedimentation analysis (5–20 per cent sucrose in TMAI buffer, 100 min at 45,000 r.p.m.). Fractions were analysed for A_{260}, ^{3}H and ^{32}P.

5S RNA[19]) instead of mature 23S rRNA (and 5S RNA). But we should emphasize that reconstitution of 30S subunits from the same organism is much faster under identical *in vitro* conditions (see Fig. 4) and that this reconstitution is performed using mature 16S rRNA rather than immature precursor RNA. Assuming first order reaction kinetics for both 30S and 50S assembly (see ref. 10), we have calculated the approximate rate constants at 50° C for both 30S and 50S assembly reactions, using experimental data similar to those described in Fig. 4. The calculation showed that the rate of assembly of 30S is about 300 times faster than that of 50S at 50° C. In contrast to this *in vitro* situation, 30S and 50S ribosomal subunits are synthesized coordinately *in vivo*, with about equal numbers of both particles synthesized per unit time per cell in growing bacterial cells under normal conditions. Such considerations suggest that the assembly of 50S subunits is facilitated *in vivo* by some special mechanism which is not involved in the 30S assembly *in vivo*. As will be reported elsewhere, genetic studies in our laboratory have shown that all mutational alterations discovered so far which inhibit 30S ribosome assembly also inhibit 50S assembly, whereas many mutations which abolish 50S assembly do not appear to affect 30S assembly[13,26]. Thus we propose that assembly of 50S subunits somehow depends on simultaneous assembly of 30S subunits *in vivo*, while the assembly of 30S subunits is independent of 50S assembly[26]. The present *in vitro* 50S reconstitution system may offer an experimental system for the investigation of the mechanism for this apparent coupling of 50S assembly with 30S assembly *in vivo*.

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Factor Necessary for Ribosomal RNA Synthesis

by

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A transcription factor, Ψ_r, which preferentially stimulates the synthesis of ribosomal RNA *in vitro* has been identified in crude extracts of *E. coli*. This factor activity is also a component of Qβ replicase.

Ribosomal RNA cistrons comprise only 0·2 to 0·4 per cent of the genome of *Escherichia coli*[1,2] and yet, in a rapidly growing culture, rRNA synthesis can account for up to 40 per cent[2,3] of the instantaneous rate of total RNA synthesis. This high rate of synthesis per cistron is much greater than that of most mRNA species and suggests that there may be some fundamental difference in the transcription processes. Because streptovaricin[4] and rifampicin (unpublished observations of R. F. S.) inhibit rRNA synthesis, and their target is the core RNA

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polymerase[5,6], at least one subunit of the polymerase must be used in the synthesis of rRNA; probably the entire core is used. But when highly purified RNA polymerase with or without the sigma factor is used to transcribe highly purified *E. coli* DNA, no detectable rRNA is synthesized. In such an experiment (Fig. 1) labelled rRNA is hybridized to *E. coli* DNA in the presence of increasing amounts of unlabelled *in vitro* product of the RNA polymerase reaction. This assay can detect rRNA levels in excess of 0·2 per cent of the *in vitro* product. Because, as shown in Fig. 1, this level is not attained, the proportion of rRNA made *in vitro* must be less than the proportion of rDNA in the template. The failure to synthesize appropriate quantities of rRNA suggests that the purified *in vitro* system lacks some effector necessary for the preferential synthesis of rRNA. We report here the identification of such an effector, which stimulates the *in vitro* synthesis of rRNA by RNA polymerase containing sigma. This activity, termed Ψ_r, has been found both in crude extracts of *E. coli* and as a component of phage Qβ RNA replicase.

The effector responsible for the preferential synthesis of rRNA could act in either of two ways. A change in the initiation specificity of RNA polymerase could be mediated by a factor which acted directly on core polymerase. For example, such a factor appears during T4 phage development[7,8]. Alternatively the factor could interact with complete enzyme (core polymerase plus sigma) as suggested for the CAP factor[9,10], which is necessary for the transcription of operons under catabolite repression control.

To search for additional *E. coli* transcription factors, we first fractionated a ribosome-free crude extract by zone sedimentation in a glycerol gradient at high ionic strength ($I = 0{\cdot}5$). The resulting fractions were then assayed for their ability to stimulate transcription of *E. coli* DNA by either core RNA polymerase or RNA polymerase holoenzyme. Assaying with holoenzyme

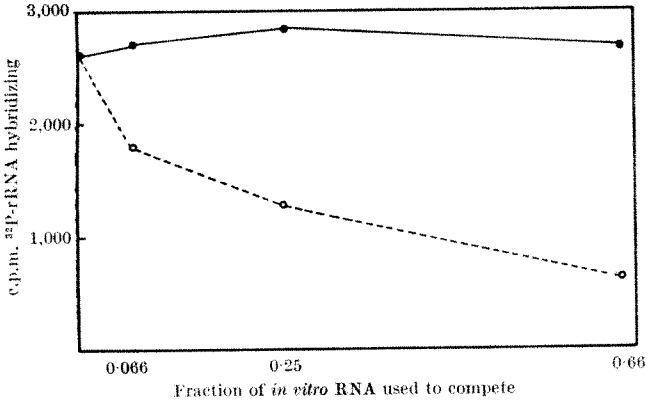


Fig. 1. Competition of radioactive rRNA by non-radioactive *in vitro* RNA. Two 1 ml. reactions containing 25 μg *E. coli* DNA, 10 μg complete enzyme, 0·01 M KCl, 0·04 M Tris-HCl (*p*H 7·9), 0·01 $MgCl_2$, 10^{-4} M EDTA, 10^{-3} M KH_2PO_4, 6×10^{-3} M 2-mercaptoethanol and 0·15 mM triphosphates. One reaction contained in addition 0·2 μg rRNA. A parallel reaction with ^{14}C-UTP showed that 30 μg total of RNA was synthesized per reaction. After incubating for 30 min at 37° C, the reactions were chilled and extracted twice with phenol equilibrated with 2 × SSC (0·3 M sodium chloride–0·03 M sodium citrate). The extracted RNA was precipitated from 2 × SSC at 0·1 M KCl by adding 2 volumes of 95 per cent ethanol and after 2·5 h at −10° C. The precipitate was collected by centrifugation at 10,000*g* for 60 min. The invisible pellet was resuspended in 0·2 ml. 2 × SSC, extracted twice with ether, and bubbled with nitrogen. Each annealing reaction mixture (200 μl.) contained a marked 1/8 sector of a 25 mm 'Millipore' filter, each sector bearing 0·3 μg *E. coli* denatured DNA. Different quantities of the *in vitro* RNA were annealed for 18 h at 67° C in 2 × SSC + 5 per cent dimethylsulphoxide together with 0·02 μg ^{32}P rRNA. After annealing the filter sectors were combined in a test tube and washed by agitation with 5 × 5 ml. 2 × SSC. 2 ml. 2 × SSC and 200 μg ribonuclease A (previously heated to 95° C in dH_2O) were then added and the sectors were kept at room temperature for 1 h. The filter sectors were then rinsed five more times with 2 × SSC, dried and counted. Non-specific binding of rRNA to filters is about 2 per cent of the saturation of 3,000 c.p.m., and at saturation about 5 per cent of the input radioactive RNA hybridizes. ●—●, The amount of ^{32}P-rRNA hybridizing in the presence of increasing quantities of *in vitro* product; ○- - -○, the ^{32}P-rRNA hybridizing when 0·2 μg of rRNA was added to the *in vitro* synthesis reaction.

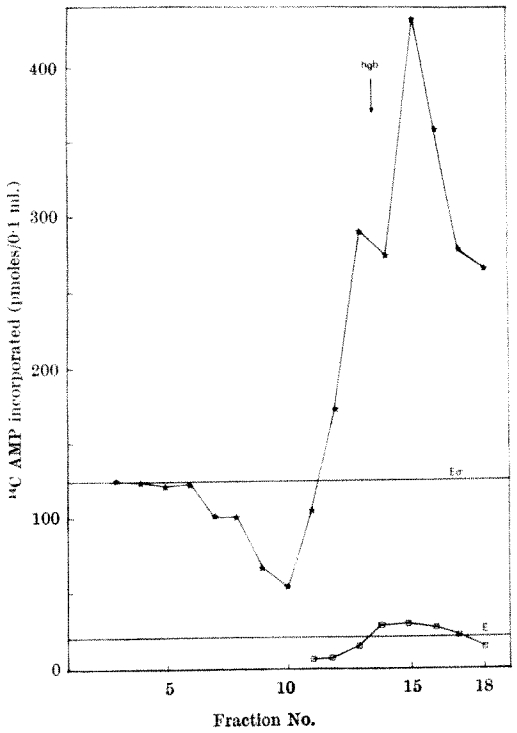


Fig. 2. Zone sedimentation of a ribosome free crude extract. 1 g *E. coli* strain RFS 524 was suspended in 1 ml. 0·01 M Tris-HCl (*p*H 7·9 at 25° C), 0·0001 M dithiothreitol, 0·005 M EDTA, 300 μg/ml. lysozyme and 5 per cent glycerol. The cells were frozen and thawed twice and 2 ml. 0·01 M Tris-HCl (*p*H 7·9), 0·01 M $MgCl_2$, 1·0 M KCl, 0·0001 M dithiothreitol, and 5 per cent glycerol were added to the crude lysate with gentle mixing. Ribosomes together with the bulk of the DNA were removed by centrifugation for 2 h at 100,000*g*. 500 μl. of the clear supernatant was layered on a 12 ml. linear 10–30 per cent glycerol gradient containing 0·01 M Tris-HCl (*p*H 7·9), 0·01 M $MgCl_2$, 0·5 M KCl, 0·0001 M EDTA, and 0·0001 M dithiothreitol. The gradients were centrifuged for 24 h at 41,000 r.p.m. at 7° C in an 'SW 41' rotor. 0·06 ml. fractions were collected and 20 μl. aliquots of the fractions were assayed for factor activity as described in the legend to Table 1. The activities of core polymerase (E) and complete enzyme (Eσ) on *E. coli* DNA without added fractions are shown as base lines. The depression of activity sedimenting at about the 8S region is probably due to the termination factor, ρ[18].

revealed a broad peak of stimulating activity sedimenting at ~3S (Fig. 2). Furthermore, these same fractions possessed no significant RNA polymerase activity of their own and only negligibly stimulated core enzyme. Thus the stimulating activity is not a new polymerase nor a sigma factor. The failure to stimulate core polymerase suggests that it is not a non-specific nuclease which would be expected to stimulate core polymerase by generating nicks in the DNA template[11].

The observed stimulation of RNA synthesis represents the synthesis of a specific class of RNA species rather than merely a general increase in activity. Competition of labelled RNA synthesized *in vitro* in the presence of Ψ_r demonstrated (Fig. 3*a*) that rRNA synthesis was stimulated very strongly. About 30 per cent of the RNA synthesized *in vitro* in the presence of the peak stimulating fraction was competed by rRNA, whereas no detectable rRNA was synthesized by RNA polymerase complete enzyme alone. In this experiment rRNA accounts for about half of the total stimulation of RNA synthesis. We have not yet determined the nature of the other RNA species stimulated. Thus rRNA synthesis requires, in addition to the RNA polymerase complete enzyme, another factor(s) which we have called Ψ_r. These experiments have shown that this factor can stimulate rRNA synthesis at least one hundred and fifty-fold *in vitro*.

Further purification of this crude Ψ_r fraction has not yet been attempted because, as shown below, we discovered that Ψ_r activity is also manifested by a component of Qβ replicase.

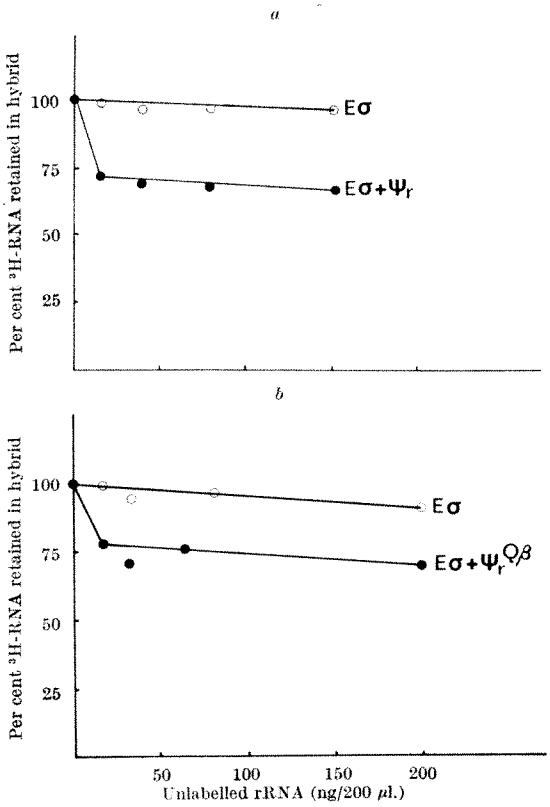


Fig. 3. *a*, Hybridization competition of *in vitro* ψ_r stimulated ³H-RNA by rRNA. *E. coli* ³H-RNA was synthesized *in vitro* in the presence and absence of fraction 15 of the gradient shown in Fig. 2. The reaction mixtures contained in 200 μl. 0·02 M Tris (*p*H 7·9), 0·10 M KCl, 0·01 M $MgCl_2$, 0·006 M 2-mercaptoethanol, 0·15 mM CTP, GTP and UTP, 0·04 mM ³H-ATP at 2·5 Ci/mmole, 75 μg/ml. *E. coli* DNA, 30 μg/ml. RNA polymerase complete enzyme and 200 μl./ml. of fraction 15 as appropriate. The reactions were terminated after 10 min at 37° C by the addition of SDS to 0·05 per cent. Water saturated phenol was then added and, after centrifugation, the resulting aqueous layer was used as the source of ³H-RNA. Aliquots of ³H-RNA were annealed in 2×SSC for 5 h at 66° C with 6 μg/ml. denatured *E. coli* DNA, and the indicated amount of unlabelled rRNA. The reaction mixtures (200 μl.) were then chilled and incubated with 10 μg/ml. heat treated ribonuclease A for 45 min at 25° C. They were filtered on Schleicher and Schuell 'B6' filters, rinsed with 30 ml. 2×SSC, dried and counted in toluene–liquifluor. ○, Competition of RNA synthesized by complete enzyme alone—input 34,200 c.p.m.; RNA hybridized in absence of competitor (mean of duplicate samples), 3,013 c.p.m. ●, Competition of RNA synthesized by complete enzyme and fraction 15—input 62,710 c.p.m.; RNA hybridized in absence of competitor, 6,526 c.p.m. A background of 320 c.p.m. has been subtracted from all points. *b*, Hybridization competition of *in vitro* ³H-RNA synthesized in the presence of Qβ replicase smaller subunit. The reaction mixtures were as described in the legend to Fig. 2, except they contained 0·05 M KCl, 6 μg/ml. termination factor, 0·2 μg/ml. purified small subunits of Qβ replicase where appropriate. They were incubated for 15 min at 37° C. ○, Competiton of RNA synthesized by complete enzyme alone, input 28,320 c.p.m.; RNA hybridized in absence of competitor 2,212 c.p.m. ●, Competition of RNA synthesized by holoenzyme and small replicase subunits, input 43,990 c.p.m.; RNA hybridized in absence of competitor 3,560 c.p.m.

Ψ_r as a Component of Qβ Replicase

There may be a relationship between rRNA synthesis and RNA phage replication, because RNA phages R17 (ref. 12) and Z1K/1 (ref. 13) inhibit rRNA synthesis in a progressive manner between 10 min and 30 min after infection. Furthermore, both RNA phage replication[14] and rRNA synthesis[15] are subject to stringent control when *rel*⁺ cells are starved for an amino acid. One attractive explanation of these phenomena is that RNA phage replicases and DNA dependent RNA polymerase compete for a protein(s) which is necessary for both rRNA synthesis and phage RNA replication.

Kamen[16] and Kondo *et al.*[17] have shown that the RNA replicase induced in infected cells by RNA phage Qβ contains three host specific polypeptides. These specifically copurify with the single phage specific polypeptide, and are thought to be subunits of the replicase[16]. The two smaller host subunits (molecular weights of 45,000 and 35,000) have been separated from the two larger subunits[16]. The subunits were first tested for Ψ_r activity by assaying for stimulation of total RNA synthesis by RNA polymerase complete enzyme on *E. coli* DNA. The pair of smaller subunits but not the pair of larger subunits showed such activity while neither pair was active without complete enzyme (Table 1*a*). The smaller subunits specifically stimulated RNA synthesis on *E. coli* DNA—they had no effect on transcription by core polymerase alone and also failed to stimulate complete enzyme on phage T4 or T7 DNA (Table 1*b*).

The replicase subunits preferentially stimulate rRNA synthesis. In the presence of both of the small subunits and the RNA termination factor, ρ (ref. 18), about 20 per cent of the total RNA synthesized in 15 min at 37° C could be competed by rRNA (Fig. 3*b*). The synthesis of rRNA in the presence of ρ suggests that its transcription is not an artefact of "readthrough" from a contiguous region of the DNA. Interestingly, ρ preferentially decreased RNA synthesis stimulated by the replicase subunits (Fig. 4). This depression was observed both in the initial rate of reaction (measured as early as one min) and in the final extent of synthesis under ionic conditions (0·05 M) which do not allow reinitiation[19]. This result can be accounted for if a fraction of $\Psi_r^{Q\beta}$ directed initiations occur at short transcriptional units, such as those specifying tRNA. The presence of ρ would allow rapid termination and release of short RNA chains, preventing readthrough into adjacent genes (see, for example, ref. 18). Experiments to assess directly the role of $\Psi_r^{Q\beta}$ in tRNA synthesis are now in progress.

To illustrate the purity of the enzyme used in these experiments, SDS–polyacrylamide gels are shown in Fig. 5. The RNA polymerase contained subunits β, β′, σ, and α (ref. 20), while the stimulating fraction contained predominantly replicase subunits III and IV[16], but none of the 65,000 molecular weight phage specific subunit.

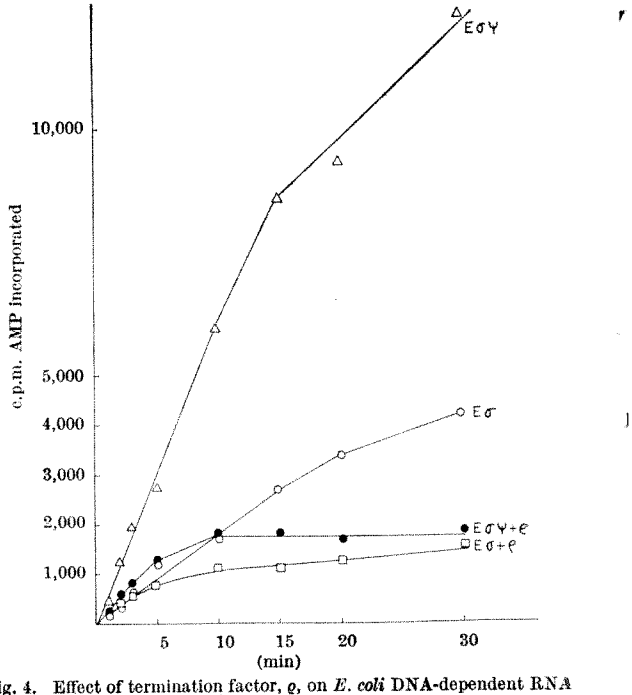


Fig. 4. Effect of termination factor, ρ, on *E. coli* DNA-dependent RNA synthesis with or without $\psi_r^{Q\beta}$. The reaction mixtures were as described in the legend to Fig. 3, except they contained 5 μg/ml. *E. coli* DNA, 15 μg/ml. complete enzyme, 8 μg/ml. $\psi_r^{Q\beta}$ and 6 μg/ml. termination factor, when indicated. The DNA was added to the incubation mixtures (at 0° C) last, the mixtures divided into 50 μl. aliquots, and then incubated at 37° C for the indicated times. Reactions were terminated by the addition of 3 ml. 5 per cent TCA, 0·01 M sodium pyrophosphate, 1 M NaCl, filtered through Millipore 'DA' membrane filters, washed five times with 3 ml. of the same TCA solution, once with 95 per cent ethanol, and then dried and counted in toluene–omnifluor scintillation fluid. A zero-time blank, measured from a sample which was kept at 0° C until precipitated, of 30–60 c.p.m., is subtracted in each incorporation curve.

Table 1. TEMPLATE SPECIFICITY OF Ψ_r STIMULATED RNA SYNTHESIS

(a)

Enzyme	Template	c.p.m. AMP incorporated
Complete enzyme (Eσ)	*E. coli* DNA	1,089
Complete enzyme + replicase subunits III + IV		1,932
Complete enzyme + replicase subunits I + II		1,132
Replicase subunits III + IV		7
Replicase subunits I + II		5

(b)

Enzyme	Template	c.p.m. AMP incorporated minus ψ	plus ψ
Complete enzyme (Eσ)	*E. coli* I	556	1,094
	E. coli II	987	1,830
	T4	5,410	4,784
	T7	7,349	6,385
Core (E)	*E. coli* I	172	171
	E. coli II	260	258

The reaction mixtures were as described in the legend to Fig. 3, except they contained 0·05 M KCl, 0·15 mM ^{14}C-ATP at 35 Cl/mole, 0·2 μg/ml. purified small subunits of Qβ replicase, 15 μg/ml. complete enzyme or 18 μg/ml. core polymerase as appropriate. The DNA templates used are as indicated. Incubation was for 10 min at 37° C. The reaction was stopped by the addition of 3 ml. 5 per cent TCA containing 0·01 M sodium pyrophosphate, and the RNA precipitate resulting was filtered on a 'Millipore' 'DA' filter. The filters were washed, dried and counted in a gas flow counter at a counting efficiency of 35 per cent.

Thus an activity functionally similar to the crude Ψ'_r factor activity was observed in a highly purified defined system.

Both in the specificity of stimulation and in the fraction of rRNA stimulated the activity of the two smaller replicase subunits resembles that of the crude Ψ'_r fraction. We have no evidence whether one or both of the replicase subunits is required for factor activity, nor do we have direct physical evidence that either of the replicase polypeptides is identical to the active entity in the crude preparation.

The method of preparation of Ψ'_r from uninfected cells is similar to that employed by Davison *et al.*[21] for identification of M factor, a transcription factor sedimenting at 4S which stimulated RNA synthesis by both core polymerase and holoenzyme on a variety of DNA templates. Ψ'_r is, however, clearly different from M-factor both on the basis of template specificity and of their relative abilities to stimulate transcription by core polymerase.

Mechanism of Action

What is the molecular mechanism of the action of Ψ'_r? There are two simple possibilities. Ψ'_r could either interact initially with the DNA at or close to the rRNA promoter site, or it could initially bind to the RNA polymerase complete enzyme to form a ternary complex which acted as the recognition unit for promoters under Ψ'_r control. The latter seems more likely because $\Psi'^{\sigma}_{r\beta}$ interacts directly and functionally with the other polypeptide chains of Qβ replicase. Furthermore, a mixture of replicase subunits III and IV will not bind poly C (as detected by retention on nitrocellulose filters), while subunits I + II or intact replicase will bind the polymer (unpublished observations of R. I. K.). This suggests that $\Psi'^{\beta}_{r\beta}$ (subunits III + IV) does not interact with poly C even though it is required for RNA synthesis by Qβ replicase with this template[16]; in a parallel fashion it seems likely that

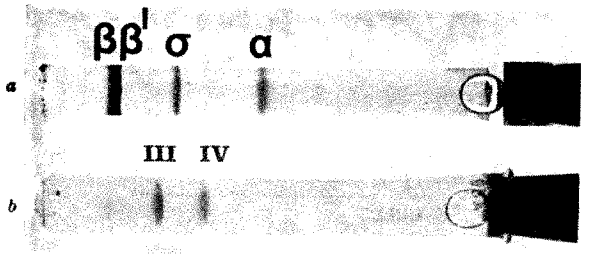


Fig. 5. SDS-polyacrylamide gels of RNA polymerase and Qβ replicase smaller subunits. *a*, Approximately 8 μg RNA polymerase complete enzyme run on a 5 per cent gel as described by Weber and Osborn[26]. The enzyme contained (from left to right in the photograph) subunits β, β', σ and α, with less than 1 per cent of any other polypeptide. *b*, Approximately 3 μg replicase host subunits III and IV, run on a 10 per cent gel. A small amount of replicase host subunit I is barely visible (< 5 per cent) but no other component can be detected (< 1 per cent).

Ψ'_r stimulation of transcription is mediated by interaction with the RNA polymerase rather than with the DNA.

Control of Transcription in Bacteria

The similarities between Ψ'_r and CAP[9] are striking. Both are thought to be involved in the specific initiation of particular classes of RNA. They are approximately the same size and they both require sigma function. Further, they are both functionally involved in transcriptional processes controlled by a nucleotide, CAP activity being activated by cyclic 3′,5′-AMP and Ψ'_r function, as we will show (A. A. T., R. I. K. and Cashel, manuscript in preparation) being inhibited by ppGpp.

The parallels between Ψ'_r and CAP suggest the provocative possibility that these factors are representative of a class of bacterial positive control elements which we propose be generally termed psi (Ψ') factors. The existence of such factors implies that a hierarchy of recognition elements for the initiation of RNA synthesis might operate *in vivo*. Sigma factors are the primary determinant for promoter recognition. The Ψ' factors would act as secondary specificity determinants allowing the RNA polymerase with sigma but not the core polymerase to transcribe general classes of transcription units, each Ψ' factor being specific for a given class. In this context it is worth noting that a mutation of the *lac* promoter apparently relives the requirement for the CAP factor[22]. The regulation of Ψ' factors by low molecular weight effectors provides a system of control that is readily reversible and which would allow the bacterial cell to respond rapidly to environmental changes. Changes of a more irreversible nature, however, such as those associated with sporulation in *B. subtilis* or with lytic phage development involve such phenomena as modification of the core polymerase[23,24], the appearance of a phage induced transcription initiation factor[7,8], or even the *de novo* synthesis of a new RNA polymerase[25].

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Correlated Subpulse Structure in PSR 1237 +25

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PSR 1237+25 has a short-period subpulse system in which subpulses march in a sense opposite to that found in other pulsars and a long-period system which is strongest in the midpulse region where the short-period system disappears.

THE Fourier analysis method of quantifying the class two phenomenon in PSR 1919+21 (ref. 1) has been applied to a sequence of pulses from PSR 1237+25. The results are: (*a*) The two features which appear in a fluctuation power spectrum of a sequence of pulse energies at $P_3 = 0{\cdot}354$ cycles per pulse period (c/P_1) and at $P_4 = 0{\cdot}021$ c/P_1 (ref. 2) are found to arise from variations of different components of the average pulse profile. The latter occurs in the central region of the profile, while the former occurs in the outer regions. (*b*) The variation with position in the pulse profile of the phase of the short-period feature (P_3) in the spectra of energies formed with a narrow time gate shows that the feature is due to correlated subpulse structure in short sequences of pulses. It also indicates that the subpulses evolve in a reverse manner to that found in the five other pulsars in which the detection of the phenomenon has been reported[3-5]; that is, the subpulses are correlated from pulse to pulse at progressively later points within the pulse profile. (*c*) The variation with position in the profile of the phase of the long-period feature (P_4) is difficult to interpret but suggests that this feature is due to an independent system of correlated subpulses. (*d*) The frequent and narrow type II pulse nulls which occur in this object and which are described in ref. 6 are found to arise from a subpulse spacing (P_2) which is broader than the width of those components in the average profile which participate in short-period events. (*e*) The wide and infrequent type I nulls, which are also described in ref. 6, are weakly correlated with the long-period feature (P_4) in the spectra. The data also show that these type I nulls are only occasionally associated with a phase discontinuity of the short-period subpulse evolution as has been shown for a similar null phenomenon in PSR 0808+74 by Huguenin *et al.* (unpublished). The data are insufficient for more definite conclusions about the relations between the long-period feature, the type I nulls and the short-period feature.

The possibility of both forward and backward marching subpulses supports pulsar models where a group of charges which is responsible for the emission is retained from pulse to pulse and is only partially decoupled from the clocking mechanism. The symmetry of the phenomena taking place across the pulse profile supports models with a hollow cone of emission which sweeps across the observer's line of sight. The existence of two systems of correlated subpulses may indicate that both ionic components of a plasma are active in separate regions of the objects.

Observations

The data were obtained as in ref. 1. In this case the receiver parameters were an observing frequency of 607·5 MHz, linear polarization, a bandwidth of 1·0 MHz and a post detection time filter of 1·0 ms. A time filter was used to reduce the correlation between samples which would be present using *RC* time constants with strong pulsed radiation. The radiation intensity was sampled every millisecond over a window of 200 ms centred on the pulse. A new method of synchronizing the sampling window with the pulsar apparent frequency ($1/P_1$) was used. This method uses the radar timing equipment available at the observatory and allows the repetition frequency of the synchronizing trigger pulse to be updated during a run to allow for variations in the pulsar apparent period due to the Doppler effect of the Earth's rotation. By this means the pulsar radiation can be sampled synchronously to within a fraction of a millisecond over a full observing period of 2½ h.

Fluctuation Power Spectra

Fig. 1 presents the digitally recorded data as deflexions from a consecutive pulse number axis which are proportional to the full pulse energies, or the integral of the samples on the pulse less the integral of the same number of samples off the pulse. The first 512 pulses were used in

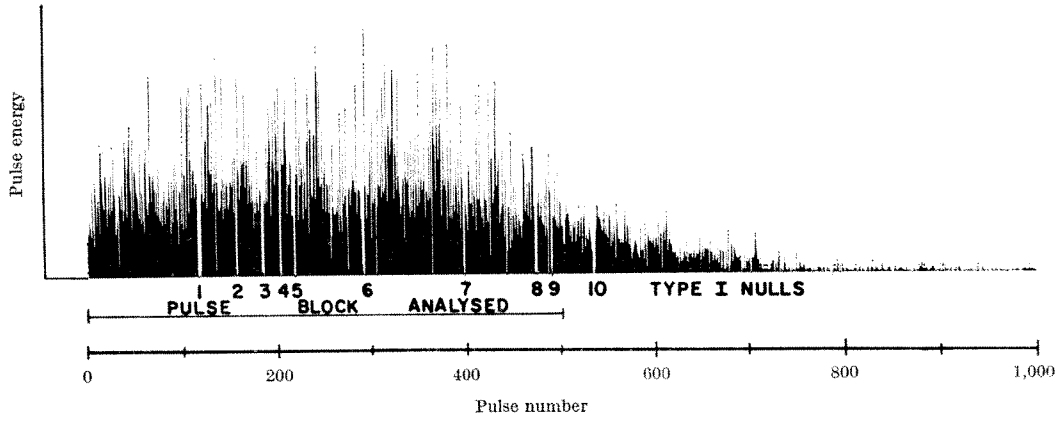


Fig. 1. Pulse energies from observation of PSR 1237+25 on May 17, 1970, presented as deflexions from a pulse number axis (the abscissa). The block of pulses used in the analysis described in the text and the location of type I nulls defined in ref. 6 are noted.

the analysis which will be described below. A fluctuation power spectrum formed from the Fourier transform of this block of full pulse energies confirmed the details of the spectrum which were noted by Lang[2]. His average spectrum is dominated by a very low frequency tail, due presumably to interstellar scintillations, but also contains a short-period feature (SPF) at $P_3 = 0{\cdot}354$ c/P_1, and a long-period feature (LPF) at $P_4 = 0{\cdot}021$ c/P_1.

A power spectrum analysis was then done using the data in a narrow time gate, or longitude box (LB*), for each pulse. Analysis I formed 23 spectra for 23 LBs, each containing three samples (3 ms or 7°·8 of longitude), across the pulse width. Analysis II, which was performed to increase the resolution, used a gate of one sample (1 ms or 2°·6 of longitude) for 61 LBs. The mean level for each LB in analysis II, or the average pulse profile, is shown in Fig. 3*a* with the positions of the 23 LBs of analysis I marked. This average profile as well as those from other observations indicate the presence of five components which have been numbered in Fig. 3*a* from the leading edge to the trailing edge. Components IV and V are unresolved in this average, but are distinct in others.

The fluctuation power spectra from analysis I are presented in Fig. 2. The spectra were formed from the incoherent sum of the power coefficients of two transforms of 256 pulses each and from taking a running mean over five coefficients. The linear scales are dependent on the maximum frequency component in each spectrum. Thus for LBs where the mean level is small, the spectral noise appears large. The zeroes are displaced uniformly. The SPF is very strong, dominating the very low frequency tail, in LBs 3 to 6 and again in LBs 20 to 22. The LPF is equally as strong in LBs 12 to 15. This immediately suggests that the average profile is a composite of independent components. This will be discussed later.

In order to untangle how the average pulse profile is constituted statistically, several quantities were defined and measured. The variation of these quantities with LB is presented in Fig. 3*b* to *e*. For each of the features three quantities were defined as in ref. 1. The "strength" was determined from the integral of the spectral power in the feature above the level of the white component by taking the square root of the integral and dividing by the mean level. The regions of integration for the LPF, SPF, and white component are indicated in Fig. 2. Second, the centre frequency was defined to be the point where half of the power in the feature occurs on either side. Finally, the phase of the spectral component at the centre frequency was determined from the complex coefficients. In this analysis the phases from the two transforms for a given component were added modulo 360°.

The level of the white component in the spectra was defined as the square root of the ratio of the average power in the signal spectra away from the features to the average power in the noise spectra. The modulation index was determined from the square root difference between the integral of the power in the signal spectrum and the integral of the power in the noise spectrum divided by the mean level squared.

Discussion

The variation with LB of the strength of the SPF from the spectra in analysis I (Fig. 3*c*) indicates that only components I, II, IV and V participate in the short period events. The SPF centre frequency (Fig. 3*c*) is constant in components I and V and is offset slightly in components IV and V. The phase function for the frequency 0·363 c/P_1 determined from the spectra in analysis II (Fig. 3*c*) displays a high degree of correlation from one LB to the next in the regions where the SPF is strong. Throughout these two regions the phase evolves slowly.

* The term "longitude" is introduced in place of "pulse phase" which was used in ref. 1 because dual usage of the word phase is confusing and because many authors are referring to the phase in a pulse period as longitude.

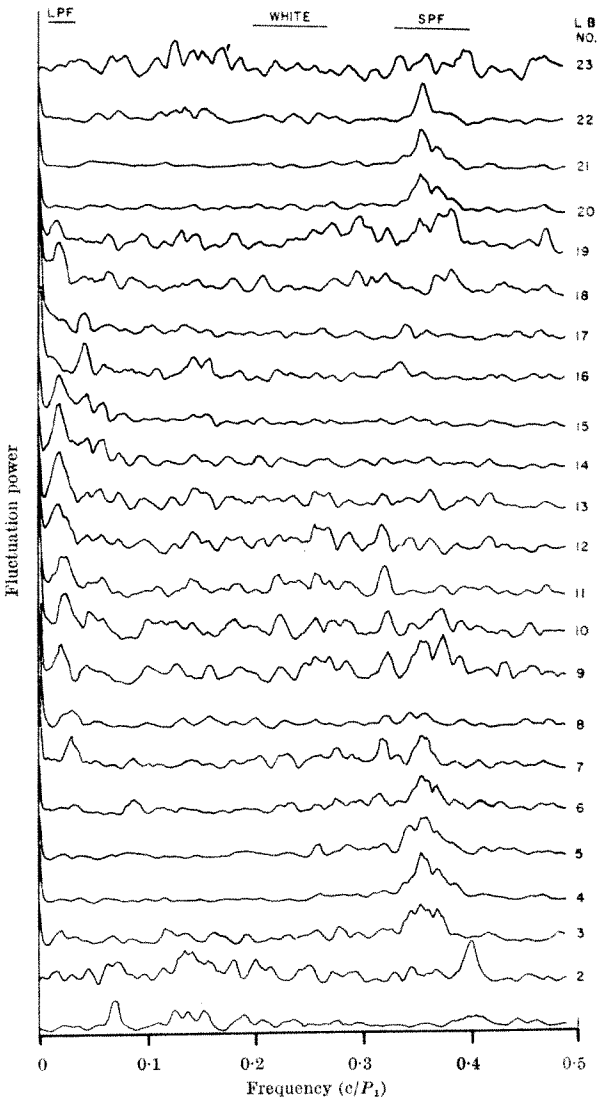


Fig. 2. Fluctuation power spectra of pulse energy for a narrow time gate for 23 longitude boxes (LBs) across the pulse profile. The linear scales for each spectrum vary according to the maximum power coefficient and zeroes for each are displaced. The regions of the long-period feature (LPF) and short-period feature (SPF) are noted as well as a region used to determine the level of the white component in the signal spectra.

It is increasing slowly across component I and decreasing in component II. When the correlation reappears near LB 15, the phase is at first decreasing across component IV and then approaching a constant in component V. As demonstrated in ref. 1 for 1919+21, this correlation of the phase indicates the presence of a class two phenomenon with the gradient of the phase with LB being a measure of the average subpulse spacing P_2. The negative gradient shown in Fig. 3*c* thus implies that the subpulses are correlated from pulse to pulse at progressively later positions within the pulse profile. Qualitatively this phase function then indicates that subpulses arise between components I and II, move rapidly through component I to the leading edge with $P_2 = 34$ ms and slowly through component II towards the trailing edge with $P_2 = -7$ ms (the minus sign denotes a negative gradient in the phase function and therefore the fact that new subpulses appear at earlier points within the pulse profile). In the midpulse region (component III) the subpulses cannot be followed because, for some reason, their strength diminishes to zero. On the trailing edge (components IV and V) an acceleration to the trailing edge occurs with $P_2 = -4$ ms in component IV and P_2 decreasing indefinitely (towards $-\infty$) across component V.

Because the SPF arises in a similar manner in components I, II, IV and V, and because the values of P_2 in components II and IV are similar, it is likely that these components are closely related physically and that some independent effect causes the observed variations in the SPF strength. In fact, the strength and phase functions of the SPF suggest that components IV and V are in some sense mirror images of components I and II with the mirror centred on component III.

It is interesting to compare at this point the SPF strength and phase function for 1237+25 with the to 2·78 lines, or pulses. The phase in the midpulse region was drawn slowly decreasing on the basis of the phase functions for adjacent frequency components which showed more correlation. Individual subpulses are seen to evolve along the average path given by this phase function, especially in the region of component II, although the intricacies of the function and the pulse profile are confusing. The phase of individual subpulse paths relative to the overlay is seen to fluctuate up to 360°, as has been noted for other pulsars[3,4].

Interpretation of the LPF is difficult as the block of

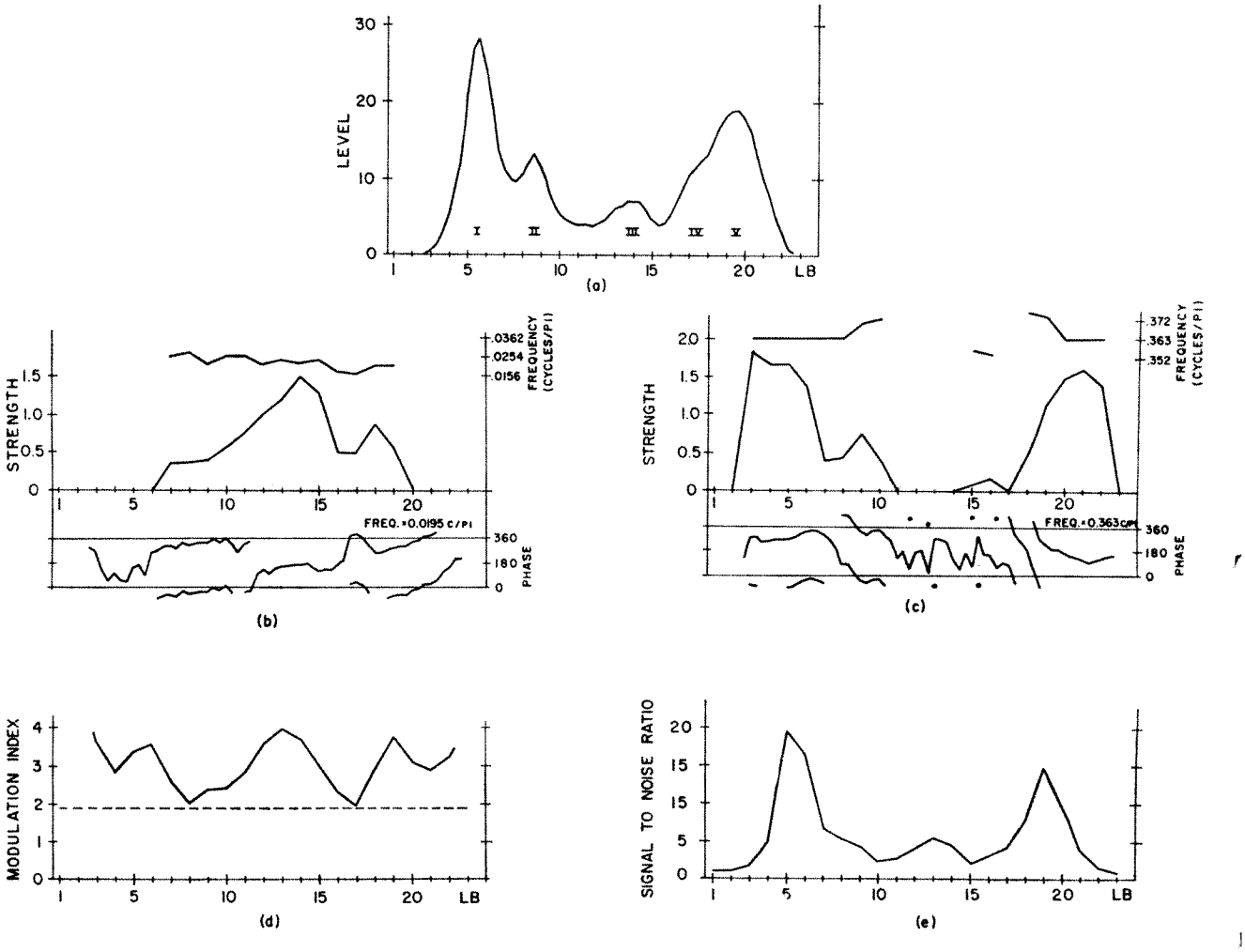


Fig. 3. Summary of the spectra from analyses I and II described in the text. *a*, The mean levels from the spectra in analysis II. Five components of the average profile are noted. *b*, Strength, centre frequency and phase of the long-period feature as defined in the text. The phase function is plotted between −90° and +450°. *c*, Strength, centre frequency and phase of the short-period feature analogous to *b*. *d*, The modulation index for the spectra in analysis I. *e*, The ratio of the level of the white component to the noise (as defined in the text) from analysis I.

corresponding results for 1919+21 (ref. 1). Both have strength functions which are peaked on the leading and trailing edges of the average pulse profile and both have phase functions which flatten out ($|P_2|\to\infty$) at the leading and trailing edges. Although the profiles of the two objects are vastly different, the 1919+21 profile is dominated by a steady component while 1237+25 is not. If this were removed, the profiles would be similar.

To see if the raw data confirm this complex average behaviour an overlay of the phase function smoothed from Fig. 3c and repeated every $P_3=2{\cdot}78P_1$ was placed on a computer printout of the intensity as a function of sample number in the window on a single line and sequential pulse number for successive lines (Fig. 4). The overlay was scaled such that 360° of phase was equal 512 pulses contains only ten full periods of the oscillation. It can be seen in the data that component III is active for only a few pulse periods at infrequent intervals. The large increase in the modulation index in the region of component III (Fig. 3*d*) is a result of this intermittent behaviour. The LPF indicates that the intervals between activity are periodic. The spectrum of a periodic phenomenon with such a low duty rate should contain many harmonics of the fundamental frequency. This indeed is the case as may be seen in Fig. 2. The spectra for LBs 14 and 15 definitely show several harmonics of the LPF, while the spectra for LBs 16 and 17 show the second harmonic as the dominant feature. The latter is related to the dip in the strength and the shift in the centre frequency of the LPF for these LBs (Fig. 3*b*).

The LPF phase function (Fig. 3*b*) shows the phase of the 0·0195 c/P_1 component of the spectra in analysis II increasing slowly across component III where the feature is strongest. This indicates the presence of correlated subpulse structure in long sequences of pulses. The low duty rate of component III mentioned here is thus a result of a second system of subpulses which are particularly strong in component III. The exact path along which they evolve is not well determined from the phase function presented here because the centre frequency of the feature varies by 25 per cent across the pulse. This variation introduces gradients in the phase function not due to evolving subpulses. This is particularly evident between LBs 14 and 18. There the harmonic content in the spectra increases and eventually dominates the LPF fundamental and the phase function of the fundamental wanders. In conclusion, the interpretation is consistent with the data but must be considered tentative.

Finally, the signal-to-noise ratio of the white component in the spectra (Fig. 3*e*) is found to follow the pulse profile level closely. This implies that the amplitude of pulse-to-pulse random variations of the grated energies is proportional to the mean level.

Pulse Nulling Phenomena

Pulse sequences from this pulsar contain two types of pulse nulls[6]. As described in ref. 6, type I nulls last for several pulse periods and occur infrequently while type II nulls are frequent, single pulse nulls. The positions of type I nulls in this block of data are shown in Fig. 1 and examples of both types of nulls are located in Fig. 4. On the basis of the overlay in Fig. 4, the type II nulls can be explained by the occurrence of two successive evolutionary paths of subpulses with a phases of plus and minus 180° referred to the null pulse. When this occurs, components I and V in the preceding and (or) following pulses are active and little energy is detected in the centre pulse.

The type I nulls are often associated with a strong component III following the null, but nulls 5 and 6 in Fig. 4 show that this rule does not occur without exception. Also the type I nulls are occasionally associated with a phase discontinuity of the evolving subpulses. In Fig. 4 at the type I null number 5 the phase was disrupted slightly while at number 6 it remained coherent.

Interpretation

Although this object shows very complex behaviour, the analysis indicates that there is a good chance that it can be unravelled. The independent effect which modulates the SPF subpulse system so that we see two pairs of components may be the geometry of a rotating beam crossing our line of sight. The apparent mirror image effect of these pairs is particularly suggestive of the pulsar models where the radiating region produces a hollow cone of emission[7] at the pole of a strong magnetic field on a rotating neutron star which swings across our line of sight. Manchester[8] finds that components I, II, IV and V show a high degree of linear polarization with a constant angle. His interpretation of the constant angle result is that our line of sight passes through the centre of a cone of emission from a magnetic pole. This is consistent with the results presented here.

The forward marching subpulses in this object remove the asymmetry that up to now has been observed in other objects. The correlation that exists between the subpulse structure in successive pulses suggests, but does not imply, that a group of charges responsible, in some manner, for the emission is retained from pulse to pulse. Either they are radiating themselves, or they are stimulating radiation.

The occurrence of a second subpulse system in the centre of the 1237 + 25 profile with a long repetition period P_4 could be interpreted as an effect of a second component of the plasma that is active in this object. It is suggested that the short period system is due to electrons and the long period system is due to ions. Other interpretations are certainly possible, but it is interesting that Sturrock[9] has proposed a model for short period pulsars such as 0532+21 based on a rotating, magnetic neutron star which has two cones of emission due to an electron polar zone surrounding a proton polar zone. According to his calculations, however, the proton polar zone would not be active in pulsars with 1 s periods due to energy considerations.

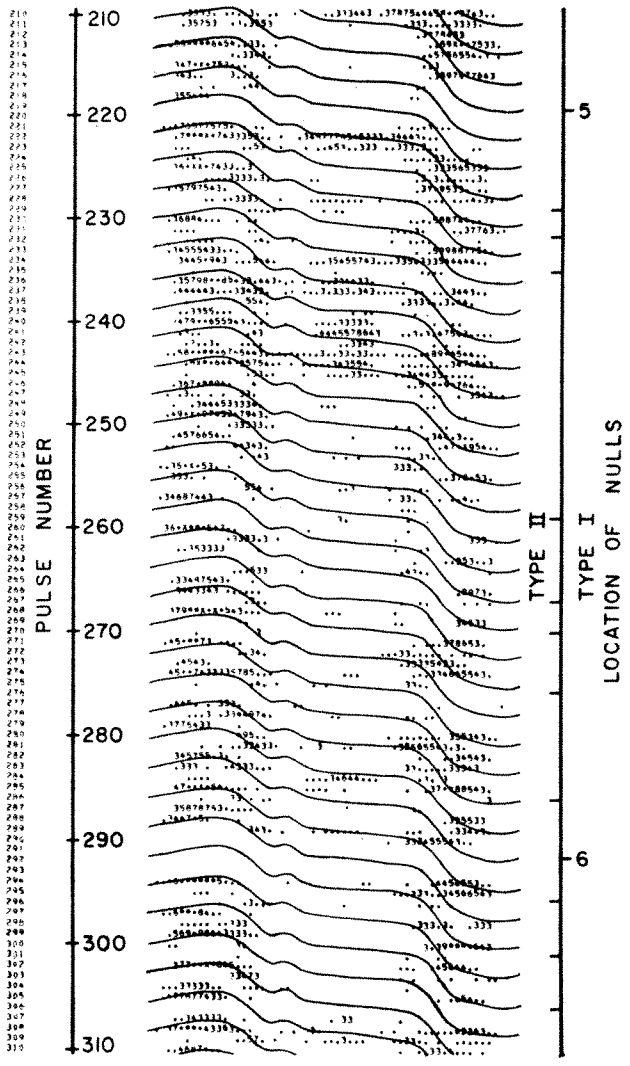


Fig. 4. Computer printout of pulse intensity across the window on a single line for 101 pulses used in the analysis. The phase function of the short-period feature from Fig. 3*c*, smoothed and repeated as described in the text, is drawn over the printout. The locations of type I and type II pulse nulls are noted.

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Lunar Occultation Satellite for High Resolution Studies of Cosmic X-ray Sources

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A proposal for a European X-ray astronomy satellite includes a lunar occultation technique for the more accurate observation of new and existing X-ray sources.

AFTER eight years of research with small rocket and balloon borne payloads, X-ray astronomy currently embraces some fifty discrete sources ranging in intensity from the remarkably bright Sco X-1 source (40 photons cm^{-2} s^{-1} above 1 keV) to the present observational limit near 0·1 photon cm^{-2} s^{-1}. Unfortunately only eight sources have so far been identified with reasonable certainty and the majority of source positions, to one or two degrees, remain much too crude to allow this number to be increased significantly. The variety of the identified sources, comprising three supernova remnants, two peculiar yet visibly faint galactic stars, two strong extra-galactic radio sources and the brightest visible quasar, for each of which the association of a strong X-ray emission has added a new dimension to its apparent nature, emphasizes the primary importance of improved position determination—and hence the possibility of identification—in the future study of cosmic X-ray sources.

More Sources

The total of detected sources is expected to rise to several thousand following the launch later this year of the US X-ray Explorer, the first Earth satellite to devoted to X-ray astronomy and having a sensitivity threshold near 10^{-3} photon cm^{-2} s^{-1} in the kV energy range. This experiment will obtain position measurements accurate to 1′ for the stronger sources and to 10–20′ for those with photon intensity below 10^{-2} photon cm^{-2} s^{-1}, and should result in a considerable number of new optical and X-ray object associations. Yet, as Table 1 indicates, a reliable identification—on positional coincidence alone—will in many cases demand an accuracy of position to several arc seconds, particularly for X-ray sources at low galactic latitude. A similar precision is required for known X-ray sources, such as M87, CenA and the Crab nebula, to locate the region(s) of emission within the structure of the visible and radio counterpart.

Table 1. OPTICAL AND X-RAY OBJECT ASSOCIATIONS

X-ray source intensity above 1 keV (photons cm^{-2} s^{-1})	Number of X-ray sources	Corresponding visible magnitude, M_V	Density of stars of magnitude $\leq M_V$ (arc minute)$^{-2}$ $b^{II}=0$	$b^{II}=90°$
40	1	12·5	0·03	0·004
1	10	16·5	1·2	0·06
10^{-1}	100	19	8	0·2
10^{-2}	1,000 (?)	21·5	42	0·5

The positional accuracy required for an objective source identification is estimated from the density of visible stars having at least the brightness of the optical extension of the X-ray spectrum. This assumes the X-ray source to be optically thin bremsstrahlung of the Sco X-1 type and will underestimate the star brightness if additional cool gas is present and overestimate it in regions of significant dust obscuration.

Against the above background situation, we recently undertook the preliminary study of a European X-ray astronomy satellite with a projected launch in 1974/75. The results are reported briefly here and the proposed payload should shortly be the subject of a technical and cost assessment by the European Space Research Organization.

Occultation Technique

The arc minute accuracy of the X-ray Explorer and other early X-ray astronomy satellites, such as UK5 (to be launched in 1973), is essentially set by the inertial stability of the spacecraft itself. The achievement of a stability in the arc second range is both technically complex and extremely expensive. Thus, in the proposal described here, a lunar occultation technique is suggested, allowing considerable relaxation of the pointing control requirement. Instead, the limit to most source location measurements is set by the determination of the satellite position in space and this can be obtained much more easily and from a ground station. Fig. 1 shows the orbit of the ESRO HEOS A2 satellite which is known to be stable. The high eccentricity and large semimajor axis of this orbit extend the region of sky which is occultable well away from the ecliptic plane, thereby covering a greater number of source positions. A second advantage of the eccentric orbit is the very low angular velocity of the satellite near apogee, where the most precise measurements will be possible. Fig. 2 shows the region of sky occultable with the HEOS A2 orbit and it includes most of the Sco-Sagittarius sources and those in the Ara and Norma constellations, as well as the Crab nebula. It is unlikely that the HEOS A2 orbit is an optimum choice

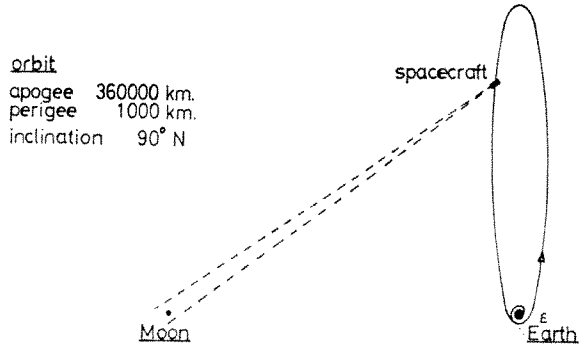


Fig. 1. Schematic of HEOS A2 orbit used to compute occultation area shown in Fig. 2.

for the suggested mission, and further studies could consider an increase in semi-major axis at the cost of payload weight, thereby extending the occulted region to the Centaurus sources. A second, or alternative, launch to the south of the ecliptic plane would then cover the remainder of the galactic centre sources, the Cygnus region and the important cluster of galaxies in Virgo.

Two successive orbits are drawn in Fig. 2 and it may be seen that they are separated by up to 5°. With a lunar diameter at the spacecraft varying from 0·3° to 0·5°, it will clearly be necessary to carry out orbital manoeuvres in order to occult a maximum number of chosen sources, these being known from earlier observations typically to a few arc minutes. An economical method of obtaining small changes in orbit position uses the same gas jet system envisaged for the main altitude control. The high velocity ejection of 0·2 kg of cold gas near orbit perigee can modify the satellite velocity by up to 1 m s^{-1}, causing a shift in the occultation path of several arc minutes in one day and up to several degrees after a month. Clearly it will be necessary to plan the observing programme carefully in advance in order to minimize the use of gas for a chosen number of source occultations. Such planning must also take into account the availability of ground based facilities, necessary for correlative observations.

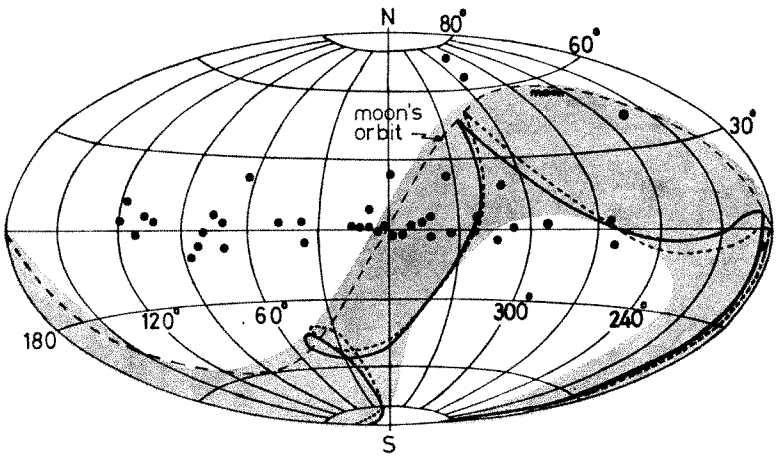


Fig. 2. Illustration in galactic coordinates of the area of sky (shaded) that may be occulted with the HEOS A2 orbit in one year of operation. Also shown are occultation paths of two successive satellite orbits and the distribution of known X-ray sources.

Factors affecting Accuracy

The accuracy of measurement possible with the occultation technique involves two principal factors, namely the determination of the spacecraft–Moon axis in astronomical coordinates and the X-ray counting statistics. For the former, the limit is clearly set by the measurement of spacecraft position and it is estimated that an accuracy of 1 or 2 km should be feasible, corresponding to an angular error subtended at the Moon of the order of 1″. The main X-ray detector in the satellite is envisaged as a conventional gas proportional counter, with effective window area 3,000 cm^2 and a sensitive bandwidth from 1 to 30 keV. A collimator mounted in front of the detector having a field of view of 2·5° (FWHM) will allow for the desired (moderate) lunar pointing accuracy, and will still limit severely the chance of a strong X-ray source other than the object source being viewed simultaneously and consequently adding significantly to the background counting rate. The satellite velocity in the nominal orbit varies from 0·25 km s^{-1} at apogee to 11 km s^{-1} at perigee, with corresponding angular rates of the occultation path from 0·5 to 5″ s^{-1}. It is therefore necessary to observe the disappearance (or reappearance) of an X-ray source within 2 to 0·2 s to achieve a positional accuracy of 1″. An estimate of detector performance, using typical values of efficiency and background counting rates, has shown that a 3σ variation should result from the occultation near apogee of a source of intensity 10^{-2} photon cm^{-2} s^{-1}. Stronger sources observed near apogee would then be limited by the satellite position error, and a similar statistical accuracy is predicted for sources three times stronger when observed near the satellite perigee.

The position measurement discussed above refers, of course, to the coordinate axis parallel to the path of occultation. In the transverse direction the angular distance of the source path from the centre of the Moon will be obtained from the period of occultation and this value will be less accurate than the longitudinal position for rather more than 50 per cent of all possible occultation paths. At worst, that is for a weak source (10^{-2} photon cm^{-2} s^{-1}) passing close to the Moon's centre, the transverse accuracy from a single observation will be of the order of 1′ and for such cases there will also be an inherent ambiguity of transverse position either side of the lunar diameter.

Higher Resolution

The second principal advantage of the occultation experiment lies in its ability to measure the size and structure of X-ray sources with a resolution unattainable by other means. Until now, only the overall extent of the Crab nebula source has been measured (~2′) and the upper limit of 20″ for Sco X-1 represents the best resolution achieved on any source. Many sources are likely to be much smaller than 1″ and extended sources may contain interesting structure on a comparable scale; in the proposed experiment the angular size of a source simply determines the rise or fall time of incident flux as the source crosses the Moon's limb. Table 2 gives the minimum angular dimension reliably measured for both strong and weak sources, near apogee and perigee, and there seems to be no reason why these figures should not be realized in practice. It should perhaps be noted that 10^{-3} arc second represents only 1 m in the lunar profile and thus quite small surface irregularities on the Moon and the finite extent of the X-ray detector may both modify the measured values for the stronger sources.

Table 2. MINIMUM ANGULAR SIZE DETECTED BY THE PROPOSED INSTRUMENT FOR A RANGE OF SOURCE INTENSITIES

X-ray source intensity (photons cm^{-2} s^{-1})	Minimum angular size detected (arc second) At apogee	At perigee
40	5×10^{-4}	5×10^{-3}
1	2×10^{-3}	2×10^{-1}
10^{-1}	3×10^{-1}	3·5
10^{-2}	25	250

In addition to the principal measurements of position, size and structure, the experiment will be capable of several other observations of sources that cross the field of view, whether occulted or not. A given source will remain visible for up to 4 h during a typical transit and the most useful additional study may be the search for short term variability in both intensity and spectrum (including periodic fluctuations). In the special case of X-ray sources associated with a known pulsar, it will be a relatively simple matter to phase analyse the X-ray counting rate using the known radio period and using a satellite clock. An estimated sensitivity of between 1 and 2 per cent pulsed component in a source of intensity 10^{-2} photon cm^{-2} s^{-1} should be obtained in a 3 h observation.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Aggregation of Nitrogen in Diamond

THIS note is an attempt to make quantitative the view[1] that it is erroneous that the impurity nitrogen in diamonds is mostly located in the platelets of diamonds.

The atomic percentage (N) of nitrogen in fifteen diamonds and the absorption coefficient (μ) at 1,282 cm^{-1} have been measured[2,3] by different techniques and are

$$\mu_{1,282} = (300 \pm 20)N \text{ cm}^{-1} \quad (1)$$

for

$$4 \times 10^{-3} < N < 2{\cdot}5 \times 10^{-1} \quad (2)$$

The only exception among the fifteen crystals was slightly inhomogeneous and had a small N. Sobolev *et al.*[1] found that the absorption coefficient at 1,368 cm^{-1} is proportional to the intensity of the X-ray diffraction spike that is caused by condensations, or platelets, in the cube planes[4]. The proportionality constant was not determined. Both these correlations hold over at least the ranges

$$1{\cdot}3 < \mu_{1,282}/\mu_{1,368} < 30 \quad (2, 3) \quad (3a)$$

$$0{\cdot}2 < \mu_{1,282}/\mu_{1,368} < 12 \quad (1) \quad (3b)$$

These data were taken on natural transparent diamonds apparently of types I*a* and II*a*. The rare type I*b* diamond (about 0·1 per cent of clear diamonds[5]) is assumed not to complicate the data.

It is known that there is a negligible concentration of paramagnetic isolated or almost isolated nitrogen in these types[6–8]. The bulk of the nitrogen must therefore exist in other forms such as in the platelets, in smaller aggregates, or in an isolated non paramagnetic form. We assume that the platelets contain nitrogen and, initially, that the nitrogen may exist in only one non platelet form. The correlations above may then be fulfilled subject to one of two conditions: either both the platelet and non platelet forms give absorption at 1,282 cm^{-1} in proportion to the number of atoms in those forms, or, if this is not so, one form of the nitrogen must account for the bulk of the nitrogen.

We consider first the former possibility. The strongest 1,368 cm^{-1} absorption on record[1] is about 25 cm^{-1} in a diamond with $\mu_{1,282} = 5$ cm^{-1}, implying, from (1), a total nitrogen content of 0·06 per cent. Hence the concentration of nitrogen in platelet form is, at most, $N_p = \mu_{1,368}/1{,}500$ per cent, where $\mu_{1,368}$ is measured in cm^{-1}. Then, for diamonds in which $\mu_{1,368} \sim 0{\cdot}5\ \mu_{1,282}$, as is often the case (see Fig. 2 of ref. 3), at most 10 per cent of the nitrogen is in platelet form.

The latter possibility can only satisfy (1) and (3) if the dominant form of the nitrogen is that giving the 1,282 cm^{-1} band. A figure may be put on the degree of domination required by noting that if a specimen has a 1,282 cm^{-1} absorption lower than that predicted by (1) we would ascribe the difference to the presence of nitrogen in platelet form. If the 1,282 cm^{-1} absorption measured is higher than (1), the difference must be due to experimental error. In no specimen has the 1,282 cm^{-1} absorption been measured significantly low (Fig. 4 of refs. 2 and 3) so that at the most the nitrogen in platelet form is 5 to 10 per cent of the total present, for the specimens within the limits of (3*a*) at least.

To distinguish between the two conditions it is necessary to know the absorption at 1,282 cm^{-1} per nitrogen atom for the two forms of nitrogen. Calculations are unreliable because no detailed models for the centres have been confirmed. The temperature independence of most of the impurity induced infrared bands[9] implies a lack of anharmonicity in the vibrational potential and hence uniaxial stress measurements are unlikely to determine the symmetry of the nitrogen forms. As observed in Si[10–13], different defects in diamond may stimulate the same perfect lattice modes. Thus all the neutron induced one photon bands[14] have the same shape and energy (although different relative intensities) as impurity bands in type I*a* diamond, many of which, in turn, may be associated with peaks in the perfect lattice density of phonon states[15]. Some bands in Si[10–13], however, are characteristic resonances of a defect. This characterization is shown in diamond by the failure of irradiation damage to create either the 1,282 cm^{-1} or the localized 1,368 cm^{-1} band[14]. So, although in principle the platelets could vibrate at 1,282 cm^{-1} with an absorption satisfying equation (1), it is unlikely.

We have demonstrated that existing data show that at least 90 to 95 per cent of the nitrogen in diamonds satisfying equation (3*a*) is in an unknown non platelet form if, as seems likely, the platelets cannot give absorption at 1,282 cm^{-1}. If the platelets do absorb at 1,282 cm^{-1} then the fraction of nitrogen in platelet form is at most $0{\cdot}2\ \mu_{1,368}/\mu_{1,282}$ and the non platelet form is again usually dominant. This still leaves sufficient nitrogen to be present in many other forms, including platelets at their known concentration[16]. The arguments given may be readily generalized to allow for several aggregated forms, but the conclusions are unaltered.

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Correlations between Earth's Gravitational and Magnetic Field

IN a recent communication Hide and Malin[1] studied the similarities in the variations of Earth's gravitational and magnetic fields in an effort to determine the undulations on the core-mantle interface. These undulations, if present, can produce significant distortions in the geomagnetic field as it is observed on the Earth's surface[2,3]. These bumps will also distort the gravitational field.

Hide and Malin[1] confined their attention to displacements of magnetic pattern in longitude alone and observed a high degree of correlation between the two fields requiring a displacement of the magnetic pattern 160° to the east. I extend their studies here by considering displacements in latitude. This study will require transforming spherical harmonic series from one polar axis to another.

A rotation can be completely specified by the angle of rotation and the axis about which it takes place. An arbitrary rotation may be represented as a product of rotations. A set of all such rotations forms a group and is completely defined by a matrix. Consider a rotation about ox through an angle $\bar{\theta}$ and rotation about oz through angle $\bar{\varphi}$. Donating the matrices corresponding to each of these rotations by T_φ, T_θ, produces[4]

$$T_g = T_\varphi T_\theta \tag{1}$$

Rotation of spherical harmonic series about the axis ox and oz leads to[4]

$$T_g = \mathrm{e}^{-im\bar{\varphi}}\, u_n^m(\bar{\theta}) \tag{2}$$

where

$$u_n^m(\bar{\theta}) = \frac{(-1)^{n-m}}{2^n(n-m)!}\sqrt{\frac{(n-m)!}{(n+m)!}}\,(1-\sin\theta)^{-m/2}$$
$$(1+\sin\theta)^{-m/2}\frac{\mathrm{d}^n}{\mathrm{d}(\sin\theta)^n}[(1-\sin\bar{\theta})^{n-m}\,(1+\sin\bar{\theta})^{n+m}] \tag{3}$$

where m denotes the order of the harmonic and n the degree, m and n being positive integers. Let a_n^m and b_n^m be spherical harmonic coefficients in the expansion of magnetic potential v. If the magnetic pattern is displaced longitudinally by angle $\bar{\varphi}$ and in latitude by angle $\bar{\theta}$ the coefficients will be modified as

$$a_n^m \rightarrow [a_n^m \cos m\bar{\varphi} - b_n^m \sin m\bar{\varphi}]\, u_n^m(\bar{\theta})$$
$$b_n^m \rightarrow [a_n^m \sin m\bar{\varphi} + b_n^m \cos m\bar{\varphi}]\, u_n^m(\bar{\theta}) \tag{4}$$

If A_n^m and B_n^m be the spherical harmonic coefficients in the spherical harmonic expansion of gravitational potential U, then the correlation coefficient k defined as

$$k = \frac{\sum_{m,n}(a_n^m A_n^m + b_n^m B_n^m)}{[\sum_{m,n}[(a_n^m)^2 + (b_n^m)^2]]^{1/2}\,[\sum_{m,n}[(A_n^m)^2 + (B_n^m)^2]]^{1/2}} \tag{5}$$

will remain unchanged on further displacement of magnetic pattern in latitude. The high degree of correlation observed by Hide and Malin[1] requiring a displacement of the magnetic pattern 160° to the east is not therefore affected by the displacement in latitude. This further supports Hide's suggestion[2] that core motions are probably chiefly zonal.

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Earthquake Lightning

IN some parts of the world, earthquakes are often accompanied by ball lightning, stroke lightning and sheet lightning[1]. The only causal connexion that seems possible is that the seismic strains of the earthquake somehow cause an electric field in the air, which in turn produces ball lightning[2] and stroke and sheet lightning. What is the mechanism of this "seismoelectric effect"?

It is suggested by Terada[3] that the streaming potential of subterranean capillary flow of water causes these electric fields. We estimate, however, that the seismic stresses necessary to produce breakdown fields by this mechanism are several orders of magnitude greater than exist during earthquakes. Electrostatic generation by dust, which is probably important in volcano lightning, is not significant in these earthquakes.

We propose that the piezoelectric effect in the Earth's crust causes the electric field.

The only significant piezoelectric constituent of the crust seems to be quartz. The mere presence of quartz is not sufficient; there must be the right kind of long range crystalline order or texture, for example, $m3:m$ or ∞m. The existence and magnitude of just such order are known from piezoelectric prospecting for quartz-bearing ores[4–6]. The range of order relevant to the seismoelectric effect is the wavelength of seismic waves (~ 2 km). Natural geological structures of this size may exhibit effective piezoelectric coefficients of the order of several per cent that of x cut single crystal quartz.

The long range order implied by these measurements is probably the result of the stress history shared by rocks in one tectonic unit[7–9]. At the relevant temperatures the z axes of quartz crystals tend to line up along the principal direction (eigenvector) of greatest stress. In one rock, for example, 50 per cent of the z axes are within 6·4 degrees of the principal stress direction[10]. There are several processes which can then order the x axes of the z ordered quartz grains. Secondary stresses may order the flats of quartz grains by mechanical action, thus ordering the x axes up to sense. An ordering of their senses occurs in the elimination of Dauphiné twinning by a shift in the direction of principal stress[11].

In rock with a mean piezoelectric coefficient several per cent that of x cut single crystal quartz, and with typical seismic stress changes 30–300 bars, an earthquake makes an average electric field of 500–5,000 V cm^{-1}. For distances of the order of half the seismic wavelength, the generated voltage is 5×10^7 to 5×10^8 V, which is comparable with the voltage responsible for lightning in storms. The impedance presented to this generator by a thin stratum of conductive soil or by conduction through the rock itself does not significantly load it at typical seismic frequencies.

For example, the North Idu peninsula earthquake of November 26, 1930, the best documented instance[3,12] of seismoelectricity (over fifteen hundred sightings), occurred in a region with widespread quartz rich lava flows. The geology and petrology of this area have been extensively treated by Kuno[13–15]; near Mt Hakone, the approximate centre of earthquake lightning, most of the lava flows contain between 15 and 30 per cent by weight of free silica, usually in the form of quartz. Some rocks contain up to 43 per cent free quartz. The rocks are usually crystalline and rarely glassy. In addition to these lava flows there are many regions with exposed dikes and plugs which contain large amounts of quartz and which have crystallized very slowly. One particular quartz diorite plug north of Mt Hakone forms a whole mountain, Yagura-dâke, approximately 1 km across. This plug is only a few km north of the active Hakone fault along which there was extensive slippage during the earthquake of November 26, 1930. The whole North Idu region has been undergoing tectonic processes since the late Tertiary[16] with consequent strong regional shearing stresses. Thus we conclude that extensive long range ordering of quartz rich rocks has probably taken place in this region.

These calculations make certain predictions possible. We expect that field measurements will show ground voltage differences in the North Idu region during earthquakes, differences sometimes large enough to cause atmospheric electric discharges. It surprises us that while minute piezomagnetic fields of seismic origin have

been explored[17], no attention seems to have been paid to such gross piezoelectric fields.

There should also be very low frequency electromagnetic radiation from seismoelectric waves ranging from 10 Hz, the approximate upper frequency of seismic waves, to well below 1 Hz, and from transient stress changes at higher frequencies. The seismic waves provide an effective 1 km³ antenna carrying a current of some 1 to 10 A with a spectral maximum near 1·5 Hz, the approximate maximum for seismic waves. The radiation takes place into the atmospheric cavity whose fundamental frequency is approximately 7 Hz. Electromagnetic radiation from 1·5 Hz seismic waves will be of low power ($\ll$1 W) but radiation from higher frequency transients will be much more intense.

There will also be electrical precursors to earthquakes resulting from changes in stress near earthquake foci. There is a tradition in Japan of predicting earthquakes, sometimes with great saving of life, from unusual clear sky lightning[3]. It may be possible to put this kind of prediction on a more systematic basis using more sensitive and quantitative electric measuring instruments than earthquake lightning.

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Crack Growth under Creep Conditions

In recent years there has been a growing awareness that the techniques of linear elastic fracture mechanics provide a powerful method for quantifying the behaviour of engineering components containing crack-like defects. In the fast fracture of high strength, low toughness materials[1] and in fatigue crack growth[2], it is now established that data from laboratory tests can be confidently applied to predict the integrity of a structure or crack extension in service using sharp crack stress intensity factors. More recently, a study of fatigue crack initiation from sharp notches in mild steel[3] has shown that the number of cycles to initiate a crack can also be calculated using stress intensity factors. In components which operate at elevated temperatures there is a need to develop methods which describe the growth of crack-like defects in creed conditions. This is particularly relevant to welded joints where defects can be evident after stress relief and assessments are required of fitness for use.

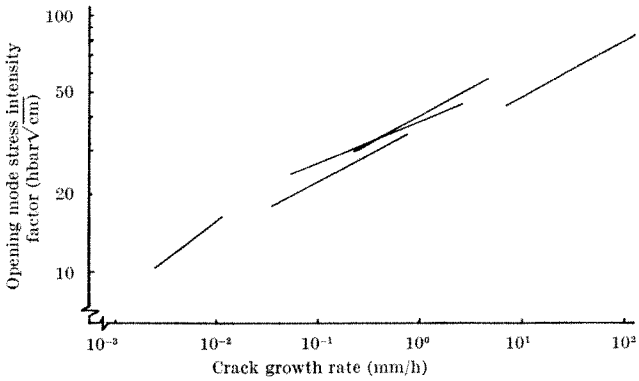


Fig. 1. The relationship between sharp crack stress intensity factor and crack growth rate for 2·25 per cent Cr, 1 per cent Mo steel under creep conditions at 565° C.

The mechanisms of creep fracture in terms of the nucleation and growth of cavities and cracking at grain boundary triple point junctions have been extensively studied[4–7]. Direct studies of crack growth in creep conditions leading to a quantitative description of material behaviour are, however, relatively few[8,9]. This has no doubt been principally because of the lack of a convenient technique for the measurement of crack length at elevated temperature. This difficulty can be overcome by using the electrical resistance method as described by Gilbey and Pearson[10]. The technique has been used in these laboratories during the past three years and has proved reliable; good discrimination has been achieved by the use of high specimen currents, high sensitivity recorders and by careful attention to experimental detail.

Work has been carried out on samples of 2·25 per cent Cr 1 per cent Mo steampipe steel, which had been quenched from 1,350° C to simulate the heat affected zone of a weld. Edge notched rectangular cross-section tensile specimens were tested in air at 565° C using a conventional lever loaded creep machine and the voltage drop across the crack was monitored continuously. The specimen dimensions were: parallel gauge length 75 mm, width 25 mm, thickness 5 mm, and initial notch depth 2·5 mm. Dead loads were applied which produced nominal stresses from 10 to 44 hbar and caused fracture in times up to about 1000 h. Some results are presented in Fig. 1 where each line represents a single test. An equation describing all the tests has the form

$$\dot{a} = \text{constant } K^n$$

where $\dot{a}$ is the crack growth rate, K is the stress intensity factor and where the value of n is about 5·5. This value is similar to the exponent that relates minimum creep rate to stress for materials of this type[11,12]. Correlations of growth rate with nominal applied stress or net section stress produced families of approximately parallel lines. There are obvious objections to applying linear elastic fracture mechanics to a creep situation, but we consider that the present findings represent a useful empirical relationship. Limited tests to verify the generality of the results, by testing at constant stress intensity factor by progressive load reduction with crack growth and by alteration of specimen dimensions, produced crack growth rates in agreement with the equation. The results encourage the view that it may be possible to establish relationships in high temperature crack propagation analogous to these which have already proved so useful in lower temperature applications.

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BIOLOGICAL SCIENCES

Subcellular Distribution of Neutral Protease and Peptidases in Rabbit Polymorphonuclear Leucocytes

SEVERAL peptidases which are active around neutral *p*H have been described in polymorphonuclear (PMN) leucocytes. One of these, which we have found concentrated within the lysosomes of rabbit peritoneal exudate PMN cells, hydrolyses histone but not haemoglobin at neutral *p*H[1,2]. Here we compare the subcellular distribution of this enzyme with that of three peptidases active at near-neutral *p*H in rabbit PMN cells. Hydrolysis of their respective substrates seems to be due to separate activities, and this distinction must precede any attempts to ascribe a function to the enzymes in tissue injury[3–6].

The three peptidases whose subcellular distribution is described are (*a*) leucine 2-naphthylamidase (LNA), representative of several amino-acid naphthylamidases previously described in rat peripheral blood PMN cells[3]; (*b*) acetyl-DL-phenylalanine naphthylesterase (APNE) described in viable rabbit peritoneal exudate PMN cells and implicated in the chemotactic process[4] and; (*c*) benzyloxycarbonyl-L-alanine β-nitrophenylesterase (abbreviated as NBA esterase in ref. 5), which is probably identical to the elastinolytic enzyme[6] in human circulating PMN cells.

In all experiments we used rabbit peritoneal exudate PMN obtained by established procedures[7]. Three subcellular fractions were obtained by differential centrifugation[8]: nuclear debris (400*g* for 10 min); these granule (8,200*g* for 15 min) and a postgranule supernatant were treated with 0·1 per cent 'Triton X-100' to release particulate enzymes. All fractions were assayed for protein content[9], β-glucuronidase activity[10] and neutral protease activity, determined as arginine containing sulphosalicylic acid-soluble material released from calf thymus histone[1]. One or more of the peptidases (LNA, APNE, NBA) were assayed in subcellular fractions of different preparations and their subcellular distribution was compared with that of neutral protease and β-glucuronidase, two enzymes present in the azurophile (lysosomal) fractions of rabbit PMN[1,11]. A recovery of β-glucuronidase in the granule fraction of 77·4 ± 9·8 per cent in five experiments indicated the reproducibility of the fractionation procedure. Because not all of the fractions were assayed for LNA, APNE and NBA, results for each enzyme are given for representative experiments.

LNA activity was measured by diazotization of naphthylamine released with *o*-aminoazotoluene diazonium salt (0–5 mg/ml.) after incubation of equal volumes of subcellular fractions and substrate (0·5 mg/ml. in 0·2 M Tris-maleate buffer *p*H 7·2). Colour development was complete after 10 min when absorbance was measured at 525 nm. Table 1 shows that LNA activity was predominantly in the postgranule supernatant. Further fractionation of the postgranule supernatant by centrifugation at 105,000*g* for 2 h showed that the pellet had a specific activity five times greater than the supernatant. Only a small proportion of LNA activity was present in the granule fraction in which β-glucuronidase and neutral protease were concentrated. The different subcellular distribution of neutral protease and LNA suggests that these are different enzymes. As pointed out by Barrett[12], however, L-leucine naphthylamide may be hydrolysed by several enzymes of varying properties. Indeed, lysosomal and microsomal amino-acid naphthylamidases, LNA among them, are clearly distinguishable on the basis of their activation and inhibition by various reagents[13]. The possibility has been raised that cathepsin B displays LNA activity at neutral *p*H[14,15]. This enzyme, however, was absent from rabbit PMN when benzoyl arginine amide hydrochloride was used as a substrate[16]. Moreover, it was clear that cathepsin B was not responsible for the LNA activity in human rheumatoid synovium[17]. This has been confirmed by the chromatographic separation of LNA from cathepsins B and B′; the latter enzymes were shown to be devoid of LNA-like activity[18]. Only further detailed investigation will reveal whether more than one enzyme is responsible for the LNA activity shown in Table 1.

Table 1. PERCENTAGE DISTRIBUTION OF PROTEIN, NEUTRAL PROTEASE (HISTONE), β-GLUCURONIDASE AND LEUCINE-2-NAPHTHYLAMIDASE IN PMN SUBCELLULAR FRACTIONS

	Nuclear debris	Granule	Postgranule supernatant
Protein	4·0	30·5	65·5
Neutral protease	1·0	99·0	0
β-Glucuronidase	3·5	82·0	14·5
Leucine-2-naphthylamidase	5·0	20·9	74·1

Combined recovered specific activities were: neutral protease, 5·28 μg arginine/mg protein/h; β-glucuronidase, 90·0 μg phenolphthalein/mg protein/h; and leucine-2-naphthylamidase, 6·3 μg naphthylamine/mg protein/h.

Hydrolysis of acetyl-DL-phenylalanine naphthylester was measured in the same manner as described for leucine 2-naphthylamide. The substrate was dissolved in N,N-dimethylformamide and acetone as detailed by Becker and Ward[4]. Table 2 shows that APNE was almost completely absent from the granule fraction; more than 80 per cent of the activity was detected in the postgranule supernatant. Moreover, in an experiment where this fraction was centrifuged at 100,000*g* for 2 h, esterase activity was confined almost entirely to the pellet, the specific activity of which was seven times greater than that of the combined fractions. This enzyme was first found in living PMN cells[4] when it was separated into three components, two of which are irreversibly inhibited by different phosphonate concentrations. One of the phosphonate sensitive enzymes was identified as the esterase of chemotaxis on the basis of its profile of inhibition by a variety of phosphonate compounds. Appearance of esterase activity in microsomal fractions could represent the association of the enzyme with small granules or with relatively intact fragments of the cell membrane itself which sedimented at 100,000*g*. Until it has been located correctly, its role in chemotaxis cannot be fully evaluated.

NBA has been described as a substrate for pancreatic elastase[19] and was subsequently shown to be hydrolysed by crude extracts and semipurified preparations of human PMN lysosomes[6]. These preparations gave similar increases in specific activities for hydrolysis of orcein-elastin and NBA suggesting their identity. NBA esterase activity was also found in rabbit PMN cells (Table 3). Enzyme activity was assayed as previously described[5]

Table 2. PERCENTAGE DISTRIBUTION OF PROTEIN, NEUTRAL PROTEASE (HISTONE), β-GLUCURONIDASE AND ACETYL-DL-PHENYLALANINE NAPHTHYLESTERASE IN PMN SUBCELLULAR FRACTIONS

	Nuclear debris	Granule	Postgranule supernatant
Protein	12·2	29·3	58·5
Neutral protease	0	100	0
β-Glucuronidase	18·1	70·0	11·9
Acetyl-DL-phenylalanine β-naphthylesterase	15·0	4·0	81·0

Combined recovered specific activities were: neutral protease, 2·10 μg arginine/mg protein/h; β-glucuronidase, 164·5 μg phenolphthalein/mg protein/h; and acetyl-DL-phenylalanine β-naphthylesterase, 63·6 μg naphthylamine/mg protein/h.

Table 3. PERCENTAGE DISTRIBUTION OF PROTEIN, NEUTRAL PROTEASE (HISTONE), β-GLUCURONIDASE AND NBA ESTERASE IN PMN SUBCELLULAR FRACTIONS

	Nuclear debris	Granule	Postgranule supernatant
Protein	6·7	30·5	62·8
Neutral protease	1·7	93·9	4·4
β-Glucuronidase	5·7	76·3	18·0
NBA esterase	6·7	59·0	34·3

Combined recovered specific activities were: neutral protease, 6·0 μg arginine/mg protein/h; β-glucuronidase, 110·2 μg phenolphthalein/mg protein/h; and NBA esterase, $\Delta A_{400} = 0{\cdot}462$/mg protein/min.

except that the *p*H was 7·2 and *p*-nitrophenol release was measured at 400 nm. The enzyme (Table 3) was found chiefly in the granule fraction, as was human PMN orcein-elastase activity[6]. The subcellular distribution of NBA esterase activity in human PMN cells has not been reported. NBA esterase differs from neutral protease in that a far higher percentage of its total activity is found in the postgranule supernatant. Other differences observed between the two enzymes are: (*a*) NBA esterase activity of the granule fraction is only partially inhibited (52 per cent) by postgranule supernatant in contrast to the complete inhibition by this fraction of neutral protease[1]; (*b*) total recovery of NBA esterase activity from subcellular fractions approximates that found in whole cells whereas neutral protease recovery amounts to from seven to twenty times that found in whole cells. This finding is explained by the presence of a potent inhibitor of neutral protease in postgranule supernatant fractions[1]. These data support the possibility that NBA esterase and neutral protease are different enzymes, although, because NBA is a more sensitive substrate than histone, smaller enzyme protein concentrations had to be used in assays with NBA as substrate.

However valid the use may be for leucine-2-naphthylamide as a marker substrate for lysosomal enzymes in some cell types[20], our data suggest that the bulk of this activity in PMN is not associated with lysosomes in conditions which permit the localization in these granules of neutral protease and β-glucuronidase. Because the subcellular distribution of hydrolytic activity was distinct for each substrate it would appear reasonable that there exist in PMN at least four separate peptidases active at near-neutral *p*H. The isolation of these enzymes by various procedures will be the subject of a future report.

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Vitamin A Inhibition of Cortisol Induction of HeLa Alkaline Phosphatase

HELA 65 [1], an aneuploid clone derived from a transformed human cervical carcinoma, has a low level of alkaline phosphatase that is elevated 10 to 15-fold by cultivating the cells with cortisol[2–4]. This enzyme "induction" occurs through synthesis of new alkaline phosphatase messenger RNA[3] at a point in the cell cycle just after initiation of DNA synthesis[5], but the result of these alterations is a more active (efficient) enzyme population, not an increase in the steady state level of control enzyme[4]. These findings have been interpreted as cortisol-effected alterations in the translation of recently synthesized alkaline phosphatase messenger RNA[4,5]. The present study shows that vitamin A inhibits this cortisol mediated increase in enzyme activity, suggesting a membrane component as a mutual cellular target for retinol and cortisol.

HeLa 65 cells were grown in Eagle's minimal essential medium (MEM) supplemented with 10 per cent calf serum, 50 units penicillin and 50 μg/ml. each of streptomycin and kanamycin. Kinetic studies of cortisol mediated induction of this phosphatase have shown 72 h as an optimum time for maximum acute effect on the enzyme[3]. Fig. 1 shows a dose response curve of (all-*trans*)-retinol inhibition of cortisol (3 μM) "induction" of alkaline phosphatase after 72 h of cell growth with these agents. The points are an average of triplicate experiments with duplicate biological samples, and standard deviation was less than 10 per cent. The minimum point on the dose curve is 0·2 μg/ml. retinol, for the growth medium contained 10 per cent calf serum, and the serum used for most of these studies contained 2·0 ± 0·3 μg/ml. saponifiable retinol (assay performed by N. Chiba and B. Warriner). Several other compounds of the vitamin A class had this effect, but little or no inhibition was produced by a variety of other fat soluble vitamins (Table 1).

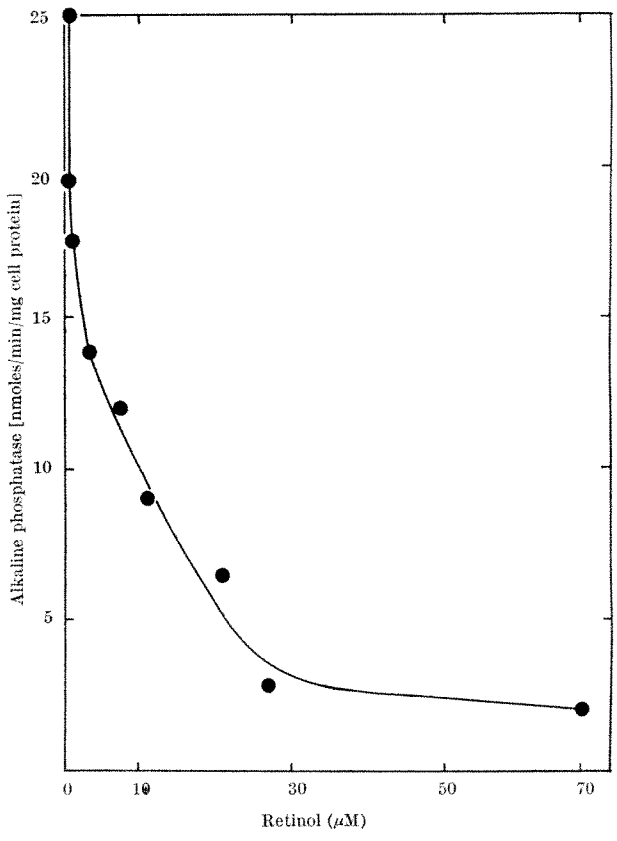


Fig. 1.

Increasing concentrations of cortisol could be counter-effected by increased concentrations of retinol up to about 100 μM, which killed most of the cells. Another HeLa cell clone which has high constitutive levels of the phosphatase (HeLa 71) was not affected by retinol or its derivatives, either in control or hydrocortisone regulated states (continuous growth and subculturing with cortisol). The alkaline phosphatase level of HeLa 65 in the hydrocortisone regulated state could also be reduced to control levels by addition of retinol to the growth medium.

The concentration of retinol in the medium is plotted in Fig. 1, and because HeLa 65 cells concentrate retinol, cellular concentrations are shown in Table 2. In another study[5], radioactive hydrocortisone has been shown not to be concentrated by HeLa 65 cells. Thus the 3 μM cortisol medium concentration amounts to a 3 μM cell concentration, but a cellular retinol concentration resulting in 74 per cent inhibition (10 μM medium concentration) is estimated at about 1,400 μM (140-fold concentration). If each effector interacts reversible at a single type of cellular site, cortisol is about 460 times as effective as retinol (1,400/3). Because HeLa alkaline phosphatase is predominantly a membrane bound enzyme[6], a membrane cellular site is suggested.

Table 1. INHIBITION OF CORTISOL BY FAT-SOLUBLE VITAMINS

Compound	Concentration (μM)	Alkaline phosphatase† (per cent of cortisol treated)
Retinol	10	36
Retinoic acid	1	74
Retinoic acid	6	52
Retinoic acid	10	40
5,6-Epoxy-retinal	1	100
5,6-Epoxy-retinal	10	52
Retinol palmitate	10	50
Cholesterol	3	100
Cholesterol	65	90
Cholecalciferol	35	75
Calciferol	35	100
25-Hydroxy-cholecalciferol*	35	90
α-Tocopherol	17	100
α-Tocopherol	70	80

* From Dr J. A. Campbell, Upjohn Laboratories.
† All values are normalized to the alkaline phosphatase of cells grown with cortisol within any one experiment.

Table 2. CELLULAR CONCENTRATIONS OF RETINOL

Medium	Retinol concentration (μM) Cells*	Fold increase
17	2,400	140
70	8,900	130
175	11,600	67

* Assayed spectrophotometrically by a difference in absorbance at 325 nm between control and retinol treated cells using a 0·5 per cent sodium deoxycholate cell lysate. This value is based on a volume of cell water for HeLa 65 of 3·5±0·4 μl./10^6 cells.

Several studies have reported vitamin A induction of alkaline phosphatase in rat skin fibroblast cells[7,8]. One of these studies[7] showed that the acute elevation of the phosphatase by topical application of vitamin A to wounded rats could be counteracted somewhat by simultaneous topical application of hydrocortisone. This seems to be a sort of mirror image of the HeLa system described above, and suggests some generalized membrane system which interacts with both cell effectors. Preliminary experiments in these laboratories show no effect of either cortisol or vitamin A on human skin fibroblasts cultivated *in vitro*. Cortisol has been shown to antagonize partially the action of vitamin A on both embryonic fowl skin[9] and embryonic cartilage and bone in culture. Again membrane systems have been implicated as site(s) of action.

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Biological Activity of a Polar Metabolite of Vitamin D_3

NEW interest is being shown in the nature of the metabolites of vitamin D, particularly those found in the target tissues, and in the part they play in controlling Ca^{2+} metabolism[1-4]. The first to be identified was 25-hydroxycholecalciferol (25-HCC) which is 1·4 times more potent than cholecalciferol itself[2] and which we have shown to be converted to a more polar metabolite, not yet fully characterized. Experiments with [1-^{3}H,4-^{14}C] cholecalciferol indicated that all the ^{3}H is lost during the formation of this metabolite. This suggests that there is an oxygen function at C-1 as well as at C-3 and C-25 (refs 3 and 4). Contrary to the original reports[5], it is this metabolite (designated compound P) and not 25-HCC that is found in the intestinal nuclei of chicks. The intestine is the richest source of compound P, although it is also found in other tissues, particularly kidney, bone, liver and muscle[3,4].

In the intestine, compound P has never been found at concentrations above 1 ng/g of tissue, although it is the predominant form of the vitamin found in the intestine after a physiological dose of cholecalciferol. Because of the scarcity of material, classical bioassays based on the changes in bone calcium effected by vitamin D could not be done. But because it was of paramount importance to establish whether or not this substance has biological activity we used a modification of the assay procedure of Norman[6], which involves another function of vitamin D, namely the increase in the intestinal absorption of Ca^{2+}, and which requires very small amounts of the vitamin.

Recently Myrtle, Haussler and Norman[7] have reported that their compound IVb, isolated from chick intestine and which has similar properties to compound P, has biological activity although they did not estimate its relative potency. We have attempted to establish the relative potency of compound P.

To prepare sufficient metabolite for the assay, 440 rachitic chicks were dosed with 0·5 μg of [1,2-$^3H_2$,4-^{14}C] cholecalciferol (51 mCi/mmole). The birds were killed 24 h later and the intestines were removed and saponified. The unsaponifiable matter was chromatographed on silicic acid columns to remove cholesterol and other relatively nonpolar material. The fraction containing compound P was further purified by thin layer chromatography with ethyl acetate/heptane (1 : 1) as the developing solvent. In this way 2 μg of [1,2-$^3H_2$,4-^{13}C] compound P in 14 mg of lipid was obtained. The ^{14}C radioactivity was used for measurement of the amount of compound P.

In the bioassay procedure the standard and test substance was administered intracardially to groups of five rachitic chicks. After 24 h a solution of 1 μCi of ^{45}Ca (0·5 μCi/mg) in 0·25 ml. of water was given orally and the blood was collected 1 h later. The ^{40}Ca content of the serum was measured by the procedure of Wales[8] and the ^{45}Ca content was assayed in 0·1 ml. of serum using a Packard liquid scintillation spectrometer.

A preliminary experiment showed that the response of rachitic chicks to 125 ng of compound P, indicated by the serum ^{45}Ca content, was 50 per cent greater than that

obtained with 125 ng (5 IU) of cholecalciferol. Indeed the response was almost equivalent to that obtained with 1500 ng (60 IU) of cholecalciferol. After 24 h, however, the serum ^{40}Ca concentration, (7·5 mg per cent) was unaffected by 125 ng of both compound P and vitamin D, although 1,500 ng of the vitamin induced a concentration of 10·4 mg per cent which is close to the normal level.

The results of a second experiment designed to measure the relative potency of compound P are shown in Table 1. Regression lines were fitted to the data expressing the relationship between $\log_{10}$ of the dose and the response obtained. They fit a common slope. Compound P was 2·9 times as potent as the standard, while 25-HCC was no more potent than cholecalciferol. The fiducial limits were calculated in the usual way to give 95 per cent limits of 1·5–5·4 for compound P and 0·43–1·68 for 25-HCC. Also shown in Table 1 are the serum ^{40}Ca concentrations. The 250 ng (10 IU) and 500 ng (20 IU) of vitamin D_3 and 250 ng of 25-HCC restored normal concentrations but this effect lasted for only 7–14 days. In contrast, the 125 ng (5 IU) of compound P had no effect, again showing that it is no more effective in raising blood calcium levels than vitamin D itself. The blood calcium increasing effect, which one can interpret as the mobilization of bone calcium, might be absent because a single dose of the active compound P has only a short lived effect. The action of a dose of cholecalciferol or 25-HCC, on the other hand, could be more lasting, because these substances generate compound P continuously. This is in keeping with the working hypothesis that vitamin D, in the form of the active compound P, has a direct effect on bone formation, in that it is necessary for the efficient accretion of calcium. It will be of interest, therefore, to test the ability of compound P to maintain bone ash values. Alternatively, it is possible that the polar metabolite of vitamin D does not have the blood calcium increasing action of vitamin D, and that this effect is mediated by 25-HCC as suggested by Trummel *et al.*[9].

Table 1. THE RELATIVE POTENCY OF VITAMIN D_3, 25-HYDROXYCHOLECALCIFEROL AND COMPOUND P ON ^{45}Ca-INTESTINAL TRANSFER AND THEIR EFFECT ON SERUM CALCIUM OF RACHITIC CHICKS

Dose level ng	Vitamin D_3 serum levels ^{45}Ca	Vitamin D_3 serum levels ^{40}Ca	25-HCC serum levels ^{45}Ca	25-HCC serum levels ^{40}Ca	Compound P serum levels ^{45}Ca	Compound P serum levels ^{40}Ca
25	—	—	—	—	2,800	—
62·5	2,200	—	2,600	—	3,100	—
125	2,500	—	3,000	—	4,500	10·2
250	3,700	12·7	4,400	12·3		
500	4,900	12·1				
Relative biological potency (per cent)	100	—	115	—	290	—
95 per cent fiducial limits	—		43 < R.P. < 168		150 < R.P. < 540	

The results are the means of five determinations. Serum ^{45}Ca levels are expressed as c.p.m./ml., the value for the rachitic control group being 1,900 c.p.m./ml. serum. The serum ^{40}Ca levels are expressed as mg per cent with the value for the rachitic control group being 9·6 mg per cent.

In the first experiment the birds dosed with [1,2-$^3H_2$, 4-^{14}C] compound P were killed at 24 h and the ^{14}C and ^3H contents of the tissues were examined. Radioactivity was detected only in the intestinal mucosa, and it amounted to as little as 1·2 per cent of the dose. In subsequent experiments a double labelled compound P (17 ng, 1 nm Ci) was infused into rachitic chicks in 2 ml. Tris buffer (*p*H 7·4) containing 100 mg of bovine plasma albumin. At the end of the 10 h infusion period the radioactivity was distributed over a wide range of tissues that included intestine, liver, kidney, bones and muscles; only 4 per cent of the dose was present both in the intestine and the liver. These infusion experiments, in which we have tried to simulate the apparent circulating concentration, suggest that the injected compound P, derived from the intestine, seems to be rapidly metabolized.

Consideration of the turnover time of compound P and the purity of this particular preparation may well show that the compound is in fact even more potent. The three-fold increase in potency relative to cholecalciferol found in these experiments means that compound P is the most potent substance so far known to have vitamin D activity. It should be emphasized that in these experiments we have tested the biological activity of the polar metabolite of vitamin D formed by reactions involving the metabolic loss of hydrogen from C-1. This compound is distinct from the other compounds of similar polarity which have not lost this hydrogen.

Determination of the mode of action of the functionally active form of vitamin D and its effect on bone metabolism will require further study. 25-Hydroxycholecalciferol, although slightly more potent than cholecalciferol, does not reach a high concentration in the intestine and is not localized in the intestinal cell nuclei[4]. On the other hand, compound P, during the same period, is accumulated in the intestine and is specifically localized in the nuclei[4]. It is at least two and a half times as potent as 25-hydroxycholecalciferol.

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Unique Biosynthesis by Kidney of a Biologically Active Vitamin D Metabolite

BEFORE the molecule of vitamin D acts in the control of calcium metabolism it is converted to a functional form in at least two metabolic steps. The first, a side chain hydroxylation at carbon 25, was shown by the elegant experiments of DeLuca and his co-workers[1–4]. This metabolite is synthesized in the liver from vitamin D[5,6] and is the major form of the circulating vitamin in blood plasma, not only of experimental rats[1] and chickens[7], but also of man[8]. A second more polar metabolite, localized in intestinal cell nuclei[7,9] and having at least three times the biological activity of an equivalent amount of cholecalciferol[10,11], is derived from 25-hydroxycholecalciferol (25-HCC)[12]. When it is obtained from chickens that have been given [4-^{14}C,1-^3H] cholecalciferol, there is a loss of tritium from carbon 1 (refs. 7 and 12). The tritium deficiency and the greater polarity are compatible with oxygen insertion at carbon 1 of 25-HCC.

The site of synthesis of this substance was not obvious. Although the small intestine mucosal cells contain the highest quantities of the tritium deficient metabolite (up to 1 ng/g), it was found in most tissues, with the lowest concentration in blood[12]. The time course of its

formation gave some indication that 25-HCC was converted to the more polar form in the intestinal mucosa and indeed a recent article from the Wisconsin workers suggests this[13].

Attempts have been made in this laboratory to find the anatomical site of the enzyme and to study the mechanism of tritium removal from [4-^{14}C,1-^{3}H] 25-HCC. Conditions for *in vitro* experiments were based on the possible requirements of oxygen, NADPH and Mg^{2+} as in the hydroxylation of steroids by mixed function oxidases[14]. Homogenates, everted sacs, isolated cells and slices of small intestine from vitamin D deficient chickens were incubated with [4-^{14}C,1-^{3}H] 25-HCC in fourteen experiments; all failed to produce any trace of the tritium deficient metabolite. Incubations of 25-HCC with liver, adrenal, parathyroid, thyroid, ultimobranchial body, thymus, rachitic epiphysis, erythrocytes and blood plasma gave similar negative results. It was observed also that surgical removal from the rachitic chicken of the gastrointestinal tract, pancreas, spleen, thyroids, parathyroids, ultimobranchial bodies, adrenals and thymus did not prevent the formation of the metabolite *in vivo*. Kidney, a seemingly unlikely organ, was the tissue which in *in vitro* preparations eventually produced a reduction in the total $^{3}H : ^{14}C$ specific radioactivity ratio with [4-^{14}C, 1-^{3}H] 25-HCC. Chromatography of the lipid extract from such preparations showed that this ratio was reduced by the formation of a polar, tritium deficient metabolite, chromatographically identical to that found *in vivo* (Fig. 1). As kidney was the only tissue to convert 25-HCC *in vitro* in this way, the possibility of its unique role in vitamin D metabolism was investigated in bilaterally nephrectomized rachitic rats. These were each given 125 ng [4-^{14}C, 1-^{3}H] cholecalciferol in 0·1 ml. propylene glycol by intracardial injection immediately after surgery. Mucosa of the small intestine and blood plasma were collected 24 h later and the lipid extracts were chromatographed on silica gel G thin layers. The proportion of the metabolites is shown in Table 1. The nephrectomized rats did not produce any tritium deficient metabolite in contrast to intact controls which in mucosa and plasma contained about 30 per cent and 7 per cent respectively of total ^{14}C as this substance. Nephrectomy did not impair the synthesis of 25-HCC. These results indicate that the kidney is the only site of synthesis of a vitamin D metabolite, postulated to be necessary for vitamin D function.

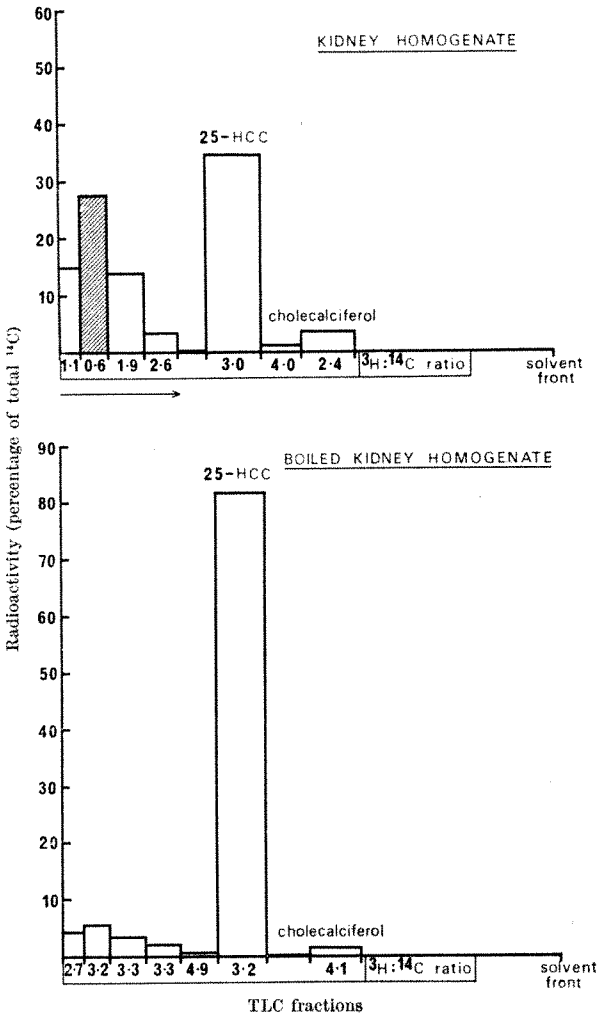


Fig. 1. Thin layer radiochromatogram of $CHCl_3$-methanol extracts from incubation of [4-^{14}C,1-^{3}H] 25-HCC with vitamin D deficient chick kidney homogenate. Silica gel G thin layers were developed with ethyl acetate–heptane (1 : 1) to a 17 cm solvent front. $^{3}H : ^{14}C$ specific radioactivity ratios are given for each fraction. The shaded peak represents the ^{14}C of the tritium deficient metabolite.

Table 1. PERCENTAGE DISTRIBUTION OF VITAMIN D AND ITS METABOLITES IN RACHITIC RATS *in vivo* 24 H AFTER INTRACARDIAL INJECTION OF 125 ng [4-^{14}C,1-^{3}H] CHOLECALCIFEROL

	Nephrectomized rats*		Intact rats*	
	Percentage ^{14}C	Ratio $^{3}H : ^{14}C$	Percentage ^{14}C	Ratio $^{3}H : ^{14}C$
Small intestine mucosa				
Cholecalciferol	39·8	4·2	16·8	4·2
25-HCC	39·7	4·0	37·1	3·3
^{3}H-deficient metabolite	0	—	29·4	0·7
Other polar metabolites	20·5	4·8	16·8	2·4
Blood plasma				
Cholecalciferol ester	0·8	†	0·4	†
Cholecalciferol	31·8	3·2	10·7	3·6
25-HCC	63·6	3·2	78·1	3·1
^{3}H-deficient metabolite	0	—	7·1	1·7
Other polar metabolites	3·7	5·6	3·8	4·2

* $CHCl_3$-methanol lipid extracts of pooled tissues from five bilaterally nephrectomized rats and four intact rats, chromatographed on silica gel G thin layers with ethyl acetate–heptane (1 : 1).
† Radioactivity was too low to calculate reliable ratios.

The mechanism of the metabolic removal of tritium was studied further *in vitro* in kidney from rachitic chicken. Homogenates (12 per cent) of kidney in 0·32 M sucrose, 0·05 M Tris buffer (*p*H 7·4) were incubated in 25 ml. stoppered tubes which contained 2·8 μmoles Mg^{2+}, 30 μmoles nicotinamide, 0·6 μmoles NADP, 6 μmoles glucose-6-phosphate, 0·6 units of glucose-6-phosphate dehydrogenase and 15 μmoles L-malate together with 10–20 mg kidney protein, and 17 ng [4-^{14}C,1-^{3}H] 25-HCC were added in 1 drop of ethanol. The total volume was 1·5 ml. The tubes were gassed with oxygen for 1 min and incubated with continuous gentle agitation at 37° C. The rate of synthesis of the tritium deficient metabolite was linear up to 1 h and had ceased by 2 h. Lipid was extracted with 10 ml. $CHCl_3$-methanol (2 : 1), and the $CHCl_3$ phase, which separated after adding 2 ml. water, was removed. The aqueous methanol residue was further extracted with 10 ml. $CHCl_3$. Recovery of ^{14}C radioactivity in the combined $CHCl_3$ extracts was 85–95 per cent. In boiled enzyme controls recovery was close to 100 per cent. Distribution of enzyme activity in subcellular fractions prepared by differential centrifugation revealed that most of the activity was present in the 700*g* nuclei-debris pellet (Table 2). The major component of this pellet was observed by phase contrast and electron microscopy to be mitochondria which when separated from nuclei, brush borders and erythrocytes by centrifugation on a sucrose gradient contained the enzyme activity. For enzyme induced loss of tritium to occur there was an absolute requirement for molecular oxygen. The heavy mitochondria had little of this activity in the absence of Mg^{2+} or malate, or in the presence of carbon monoxide. These properties are rather similar to those of steroid 11β-hydroxylase found in adrenal cortex heavy mitochondria[15] which requires oxygen, Mg^{2+} (ref. 16) and malate (ref. 17) (the latter being a generator of NADPH via intramitochondrial malic enzyme[18]) and which is inhibited by binding of CO to mitochondrial cytochrome P-450 (ref. 19). Such similarity implies again that the tritium is lost from [4-^{14}C,1-^{3}H]

25-HCC by oxygenation of carbon 1. The fate of the lost tritium was discovered to be in the formation of tritiated water, collected from the total incubation mixture by lyophilization. Recovery of tritium in water exactly matched the loss observed in the lipid extract and in fact proved to be a sensitive assay of the enzyme activity.

What now seems to be the functional intermediary metabolism of vitamin D is illustrated in Fig. 2. The formation of 25-HCC seems to be relatively independent of the supply of environmentally derived vitamin D. Its concentration in plasma of pigs rises as large doses of dietary vitamin are provided[2] and it probably constitutes the circulating reserve of vitamin D activity[13]. On the other hand the synthesis of the kidney metabolite cannot be increased by further administration of vitamin D or 25-HCC (ref. 12). Indeed the administration of 0·5 μg [4-^{14}C,1-^{3}H] cholecalciferol to chickens given 3–5 μg unlabelled cholecalciferol per week for 5 weeks produces very little tritium deficient metabolite and the *in vitro* kidney enzyme activity of such birds was 0–25 per cent of that of vitamin D deficient chickens. We therefore suggest that there is some control over the synthesis of the metabolite by kidney which allows only sufficient quantities for target organ requirements.

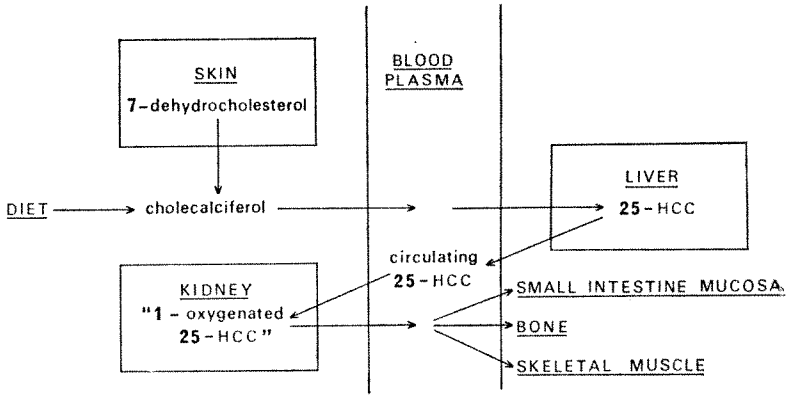


Fig. 2. Functional intermediary metabolism of vitamin D.

Table 2. SUBCELLULAR DISTRIBUTION OF ^{3}H-REMOVING ENZYME ACTIVITY IN HOMOGENATES OF VITAMIN D DEFICIENT CHICK KIDNEY INCUBATED WITH [4-^{14}C,1-^{3}H]25-HCC

	Percentage conversion of 25-HCC to ^{3}H-deficient metabolite	Protein/assay (mg)
(*A*) Subcellular fraction		
Whole homogenate	25·4	19·9
700*g* × 10 min pellet	26·0	18·8
10,000*g* × 10 min pellet	5·6	10·7
100,000*g* × 60 min pellet	0·0	14·9
100,000*g* × 60 min supernatant	0·0	10·0
(*B*) Fractionation of 700*g* pellet		
Whole homogenate	30·8	18·5
700*g* × 10 min pellet	21·2	9·2
Sucrose gradient fractions		
Heavy mitochondria	16·9	16·1
Nuclei	1·3	3·4

The findings reported here could be relevant to the understanding of the pathogenesis of some diseases of calcium metabolism. For instance in chronic renal failure of man there is a variable resistance to the action of vitamin D (refs 20, 21), and an osteodystrophy develops resembling the osteomalacia of vitamin D deficiency[22]. Although previous attempts to identify changes in vitamin D metabolism in renal insufficiency have revealed very little[23,24], the synthesis of the polar biologically active metabolite was not examined and the possibility exists that in renal disease its production from 25-HCC is impaired or abolished. Similarly in familial vitamin D resistant rickets the genetic defect may result in the absence of the kidney enzyme described here. Finally, failure of the kidney vitamin D metabolism to supply sufficient metabolite for intestine and bone to maintain blood calcium levels could explain uncompensated hypocalcaemia such as is seen in dairy cows with parturient paresis.

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Inhibition of Bohr Effect after Removal of C-Terminal Histidines from Haemoglobin β-Chains

THE alkaline Bohr effect is the uptake of protons at a *p*H greater than 6 when oxygen is removed from haemoglobin[1]. In horse haemoglobin the α-amino groups of the α-chain contribute about one-quarter of the alkaline Bohr effect[2]. Recently Perutz *et al.*[3] proposed that histidine 146β contributes about half the alkaline Bohr effect. Their evidence was based on the interpretation of a difference Fourier map of deoxyhaemoglobin after reaction with N-ethylmaleimide[4]. This haemoglobin has lost half its alkaline Bohr effect. We have prepared des-(His 146β) human haemoglobin: it exhibits haem-haem interaction and lacks half the alkaline Bohr effect, and thus fully confirms the ideas of Perutz *et al.*[3].

Carbonmonoxy-β-chains (after removal of *p*-mercuribenzoate[5]) were digested with chromatographically purified carboxypeptidase B[6] in 0·2 M barbitone buffer

(*p*H 8·2) at 37° C for 4 h at an enzyme to substrate ratio of 1 : 1,000. This removes about 60 per cent of histidine 146β, and an equal 10 per cent of both tyrosine 145β and lysine 144β. After digestion the enzyme was removed by passage through a DEAE-cellulose column equilibrated with 0·05 M sodium phosphate buffer (*p*H 6·9). The digested β-chains were then chromatographed on a CM-cellulose column using a salt gradient of 0·01–0·1 M sodium acetate (*p*H 6·0). Three peaks were isolated; the first (presumed to be β-chains from which residues 146, 145 and 144 had been removed) was discarded. A stoichiometric amount of α-chains was added to the second and third peaks and they were digested with carboxypeptidase A[7] to identify them. The results in Table 1 show that peak 2 contains des-(His 146β) β-chains and peak 3 is undigested β-chain. Complete release of arginine 141α after digestion of both haemoglobins with carboxypeptidase B shows that all of this enzyme had been removed after the original digestion of the β-chains.

Table 1. PROPERTIES OF PRODUCTS OF DIGESTION

	Residue	Peak 2 + α-chains	Peak 3 + α-chains	Human haemoglobin
CPA digestion (moles residue/$\alpha\beta$ dimer)	Tyr 145β	0·90	0·90	0·90
	His 146β	0·004	0·95	0·96
CPB digestion (moles residue/$\alpha\beta$ dimer)	Arg 141α	0·93	0·94	0·86
Hill's constant n		2·5	2·8	2·8
Oxygen affinity ($p_{\frac{1}{2}}$), mm Hg, 0·2 M sodium phosphate (*p*H 7·0, 25° C)		2·5	9·5	9·2
Bohr effect $\Delta \log p_{\frac{1}{2}}/p$H		0·23	0·48	0·51

The results of oxygen dissociation curves in 0·2 M phosphate buffer are shown in Table 1. The control haemoglobin (that is, the undigested β-chains recombined with α-chains) has properties identical with normal human haemoglobin. Des-(His 146β)-haemoglobin has a slightly lower Hill's constant n, indicating a slight loss of haem-haem interaction; the alkaline Bohr effect as measured in 0·2 M phosphate buffers between *p*H 7·0 and 7·8 has been halved. The Bohr effect was then measured directly from titration curves and the results are shown in Fig. 1. The control haemoglobin has a normal Bohr effect, while the des-(His 146β) haemoglobin has a halved alkaline Bohr effect. The acid Bohr effect (the release of protons at a *p*H below 6 when oxygen is removed from haemoglobin) in des-(His 146β) human haemoglobin seems to be largely intact. Thus the major part of the acid Bohr effect is not linked to this part of the alkaline Bohr effect. Indeed, the shape of the curve of the dependence of the Bohr effect of des-(His 146β) haemoglobin on *p*H is that calculated from the theoretical model of Wyman[9] assuming that half the alkaline Bohr groups have been removed, but that the acid Bohr groups are intact. It seems that part of the acid Bohr effect is linked to that part of the alkaline Bohr effect coming from the α-chains, for when this is specifically inhibited in des-(Arg 141α)-haemoglobin (which has an n of 1·8), a part of the acid Bohr effect is also inhibited[10]. The rest of the acid Bohr effect is not linked to the alkaline Bohr effect, for des-(His 146β,Arg 141α)-haemoglobin, which has no alkaline Bohr effect nor haem-haem interaction still possesses a significant acid Bohr effect[10].

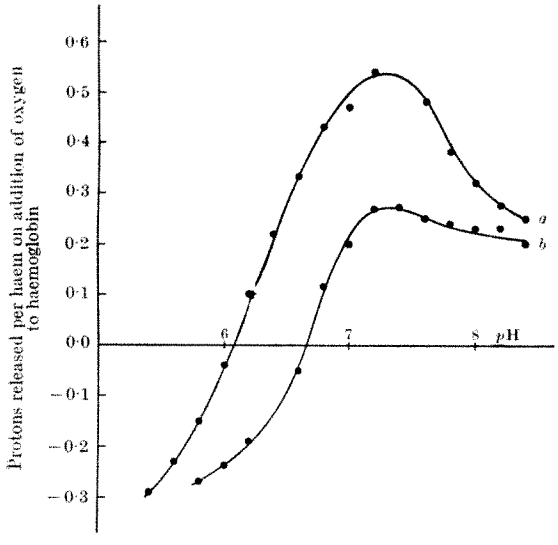


Fig. 1. *a*, Control; *b*, des-(His 146β).

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Glucagon stimulates Brunner's Gland Secretion

GLUCAGON and secretin, chemical homologues having 14 similar amino-acids in identical positions[1], induce choleresis[2], stimulate insulin release[3] and inhibit gastric secretion[4]. Glucagon, however, inhibits, while secretin stimulates, pancreatic fluid and electrolyte secretion[5]. Because three different preparations of secretin—synthetic porcine (Squibb), pure natural porcine (Jorpes and Mutt) and impure porcine (Boots)—stimulate Brunner's gland secretion[6–8], we investigated whether glucagon would affect Brunner's gland secretion in dogs.

In fifteen anaesthetized dogs (18–23 kg) of both sexes, a vagally denervated area of proximal duodenum with an intact blood supply was mounted in a lucite chamber. The main pancreatic and common bile ducts were ligated and divided. The orifice of the accessory pancreatic duct was maintained outside the internal diameter of the chamber. A stable level of anaesthesia was achieved and constant temperature (38° C–39° C) was maintained in both the animal and the duodenal explant. Brunner's gland secretion was studied in the resting state and in response to: (*a*) graded doses of glucagon (Eli Lilly and Co., Indianapolis, Indiana) 0·5 to 25 μg/kg as a single intravenous injection, (*b*) glucagon by continuous infusion (0·50–0·20 μg/kg/min), (*c*) a single dose of glucagon (10 μg/kg) superimposed on a continuous infusion of glucagon (1 μg/min). The order of doses was randomized. Fifteen minute collections of secretion were analysed individually for volume, HCO_3^-, Na^+, Cl^-, K^+ and protein concentration. Blood sugar levels were determined before and after glucagon injection.

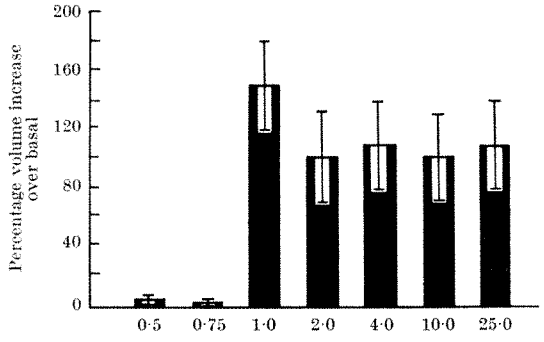


Fig. 1. Effects of graded doses of glucagon by single injection on volume response of Brunner's gland area.

Mean basal Brunner's gland secretion was $0{\cdot}85 \pm 0{\cdot}26$ (s.d.) ml./15 min. Glucagon produced a sharp increase in volume output. The threshold dose for stimulation was 1 μg/kg. Glucagon (1–25 μg/kg) resulted in an increase in secretion of 100 per cent–160 per cent (Fig. 1). The peak response was attained with 1 μg/kg; higher doses did not produce any greater increase in volume. Continuous glucagon infusion alone increased volume output by 80 per cent (Fig. 2); the peak effect was lower than that achieved by a single dose. When a single injection (10 μg/kg) was superimposed on a continuous glucagon infusion, however, further stimulation occurred (40 per cent increase). The peak effect equalled the maximum response to glucagon injection alone. Glucagon did not alter the electrolyte or protein concentration of the secretion but increased their output (Table 1). Blood sugar levels were unaltered by the doses of glucagon used in this study.

Table 1. EFFECTS OF GRADED DOSES OF GLUCAGON ON ELECTROLYTE AND PROTEIN CONCENTRATION OF BRUNNER'S GLAND AREA

Glucagon injection (μg/kg)	Electrolyte concentration (mequiv/l.) Na^+	K^+	Cl^-	HCO_3	Total protein concentration (mg/ml.)
Basal	$146 \pm 1{\cdot}1$	$6{\cdot}2 \pm 0{\cdot}9$	$126 \pm 9{\cdot}4$	$26{\cdot}1 \pm 1{\cdot}5$	$6 \pm 2{\cdot}2$
0·5	$152 \pm 5{\cdot}8$	$5{\cdot}5 \pm 2{\cdot}4$	$123 \pm 4{\cdot}0$	$23{\cdot}8 \pm 3{\cdot}2$	$5 \pm 0{\cdot}0$
1	$150 \pm 4{\cdot}8$	$6{\cdot}1 \pm 0{\cdot}8$	$122 \pm 4{\cdot}9$	$26{\cdot}7 \pm 4{\cdot}4$	$7 \pm 1{\cdot}1$
2	$154 \pm 0{\cdot}7$	$5{\cdot}5 \pm 0{\cdot}7$	$134 \pm 9{\cdot}2$	$27{\cdot}0 \pm 0{\cdot}7$	$6 \pm 2{\cdot}0$
4	$151 \pm 0{\cdot}3$	$6{\cdot}5 \pm 0{\cdot}7$	$136 \pm 19{\cdot}0$	$27{\cdot}0 \pm 3{\cdot}4$	$5 \pm 0{\cdot}5$
10	$151 \pm 4{\cdot}2$	$6{\cdot}0 \pm 0{\cdot}0$	$125 \pm 1{\cdot}4$	$27{\cdot}0 \pm 0{\cdot}0$	$6 \pm 2{\cdot}1$
25	$150 \pm 1{\cdot}0$	$6{\cdot}0 \pm 0{\cdot}0$	$124 \pm 2{\cdot}1$	$26{\cdot}0 \pm 0{\cdot}0$	$6 \pm 1{\cdot}0$

± = Mean s.d.

Glucagon, in doses too small to produce hyperglycaemia, is a potent stimulant of Brunner's gland secretion. Stimulation by single intravenous injection follows an all or none pattern because the response is not dose dependent. A background infusion of glucagon does not potentiate the response to single injections. Thus glucagon and secretin produce similar effects on yet another target organ.

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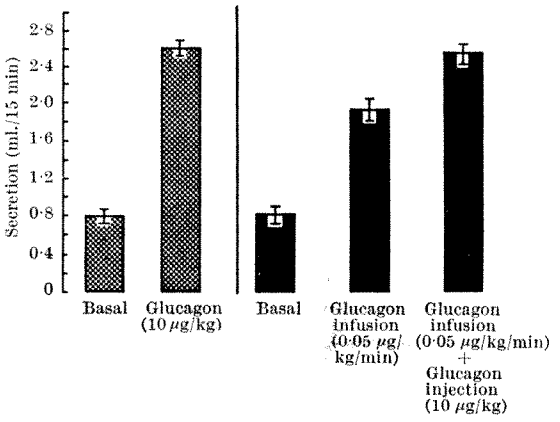


Fig. 2. Volume response to glucagon by single injection, continuous infusion and single injection superimposed on continuous infusion.

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Serum Enzyme Changes during Sleep Deprivation

Substantial increases in serum creatinine phosphokinase (CPK) and aldolase activities occur in many acutely psychotic patients[1–3]. Futher investigation has shown a frequent but not invariant relationship between sleep disturbance and the serum CPK (and aldolase) activity in acutely psychotic patients[4]. In eight acutely psychotic patients, severe sleep disruption, often amounting to total insomnia with particular reductions in rapid eye movement (REM) sleep[5], was associated with high serum CPK activity. We now report that elevated serum CPK activity and aldolase activity occur in normal subjects during and after experimental sleep deprivation, though many characteristics of these increases in enzyme activity are different from those in acutely psychotic patients.

Serum CPK and aldolase activities were studied in seven normal volunteers, each sleep deprived from 40–48 h, on a research ward of the NIH Clinical Center. They included four males and three females with a mean age of 18·7 years (range, 18–21 years) who had all received a brief screening interview by one of us (D. J. K.). A complete battery of blood tests, physical examinations and the Minnesota multiphasic personality inventory showed that they were healthy college students with no remarkable psychopathology. Three pairs of subjects underwent 48 h of sleep deprivation and two subjects (including subject 3 who participated in two separate experiments) underwent 40 h of sleep deprivation. All the subjects were able to complete the experimental protocol without distress or development of psychiatric symptoms.

Blood specimens were obtained at 8–9 a.m. on the morning before the first night of sleep deprivation from all of the subjects for baseline enzyme activities. The samples obtained at 24 and 48 h in the subjects who underwent 48 h of sleep deprivation were called deprivation specimens 1 and 2 (D1, D2 respectively). Three or four specimens during the recovery period (R1–R4) were obtained from these subjects at 24 h intervals thereafter. For the two subjects, 3b and 7, who underwent only 40 h of sleep deprivation, the 48 h specimen was considered the R1 specimen. For these subjects, there was no D2 specimen. Serum from all specimens was obtained by centrifugation, frozen and coded so that all determinations of enzymatic activity were done on a blind basis.

CPK and aldolase activities were determined spectrophotometrically[6,7] and normal serum CPK and aldolase activities for fully active males and females in our labora-

Table 1. SERUM ALDOLASE AND CPK ACTIVITIES IN NORMAL MALES AND FEMALES

	Number of subjects	Mean	s.d.	Range	95 per cent upper confidence limit
		Aldolase (mU/ml.)			
Males	20	1·38	0·67	0·3–2·4	2·7
Females	20	1·73	0·73	0·5–3·2	3·2
		CPK (IU/l.)			
Males	78	39·8	19·9	12–138	80
Females	67	29·2	22·5	7–165	74

tory are given in Table 1. These values are within the same range as those found by other authors using the same methods[8,9]. The activities of serum aldolase and CPK in the subjects who underwent sleep deprivation are presented in Figs. 1 and 2. There were no apparent changes in serum CPK activity in two of the sleep deprived subjects, but most cases showed moderate alterations. In all of the subjects there were marked increases of serum aldolase activity. The serum aldolase activity after the first 24 h of sleep deprivation was significantly greater than that before sleep deprivation ($P < 0.001$). The serum aldolase activity 72 h after the end of sleep deprivation, and the CPK activity following the first 24 h of sleep deprivation and 72 h after the end of sleep deprivation, were also significantly different from baseline enzyme activities ($P < 0.05$). The aldolase activities noted in seven of eight experiments on D1 or R3 or both were quite striking because aldolase activity of greater than 5 mU/ml. is far beyond normal limits and is rarely noted even in acutely psychotic patients[1–3]. The marked increases in aldolase activity on either R3 or R4 in five cases are particularly perplexing in that these changes were occurring 72–96 h after sleep deprivation. The increases in CPK activities on D1 and R3, by contrast, were far smaller than those found in acutely psychotic patients. First, it should be stressed that daily determination of serum CPK and aldolase activity in non-psychotic psychiatric patients for periods of up to 2 months has revealed considerable stability in the activity of both enzymes so that the range of CPK and aldolase activity for a given subject is usually within 15 IU/l. for CPK and 1·0 mU/ml. for aldolase (H. Y. M., unpublished). The increases in aldolase activity during the sleep deprivation and recovery periods, however, were far beyond this range.

A number of factors which could produce increased serum enzyme activity should be considered. Severe and prolonged physical activity can produce increased serum enzyme activity[10–12] but the subjects in this study exercised only moderately in order to keep awake. Further serum CPK increases far more than serum aldolase after exhausting, short-term exercise, and the increases in aldolase after exhausting exercise were much less than those observed in the studies reported here[13].

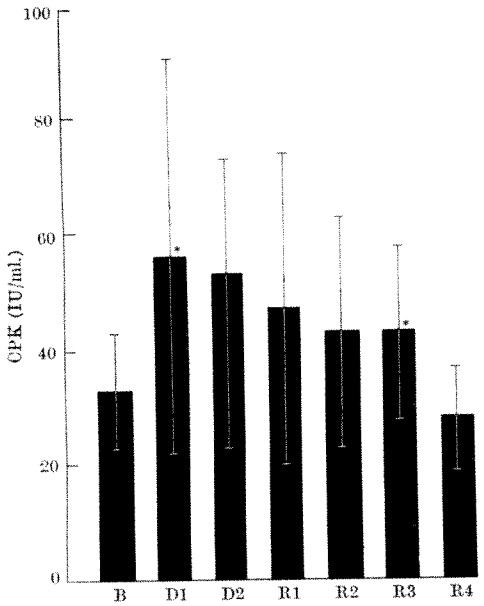


Fig. 1. CPK change during sleep deprivation. B represents the baseline data. D1 and D2 are the two deprivation days. R1–R4 represent the recovery period.

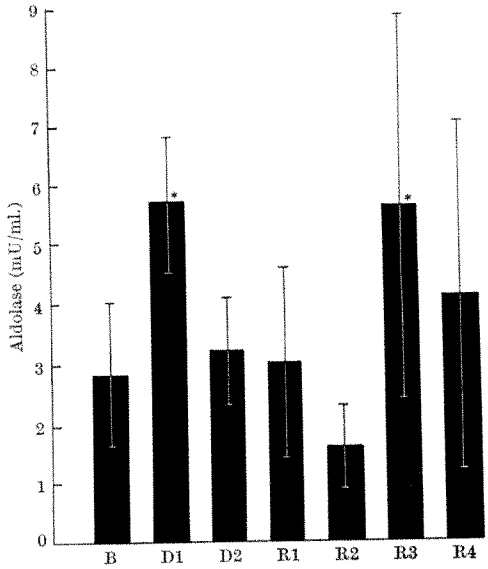


Fig. 2. Aldolase change during sleep deprivation.

Although sleep deprivation much more prolonged than we imposed here seems to produce stress-related biochemical changes, for example, increases in phosphatidyl glycerol and phosphatidic acid, decreases in cardolipin[14], and slight increases in plasma 17OH-corticosteroids[15], moderate psychic stress does not usually increase serum CPK or aldolase activity (refs. 16, 17, and H. Y. M. and S. Hassan, unpublished). Pathologic changes in skeletal and cardiac muscle, liver and the endocrine organs which have been associated with increases in serum CPK or aldolase activity, or both[18–20], would not be likely to be produced in the conditions of these experiments.

It is possible that the large increases in serum aldolase activity found at D1 and R3 are a consequence of the sleep deprivation itself. The transient nature of the increased enzyme activity suggests a temporary increase of cell membrane permeability resulting in greater escape of aldolase into the circulation as the immediate cause of the increased enzyme activity. Isoenzyme studies of the serum aldolase might make possible the localization of the tissue(s) from which the aldolase is being released in increased amounts. It would be interesting to see whether deprivation of selective stages of sleep would produce comparable results.

The relationship between the sleep disturbance and increased serum CPK and aldolase activity in the acutely psychotic patients and the serum enzyme increases in sleep deprived normal subjects is difficult to interpret. In the acutely psychotic patients, the increases in serum CPK activity are much greater relative to normal values than are the increases in serum aldolase activity[1–3]. The converse is true of the sleep deprived normal subjects. The magnitude of the increased aldolase activity after sleep deprivation was much greater than that typically found in acutely psychotic patients, even though many of those patients generally suffered much longer sleep disruption. These differences in enzyme activity between acutely psychotic patients and sleep deprived normal subjects suggest that sleep disturbance in itself may not be the cause of increased enzyme activity in the acutely psychotic patients.

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Decreased Binding of ^{14}C-Homogentisic Acid induced by Ascorbic Acid in Connective Tissues of Rats with Experimental Alcaptonuria

ADULTS with alcaptonuria develop a destructive arthritis secondary to the deposition of a melanin-like pigment in their connective tissues, especially skin, tendon and cartilage (ochronosis). The biochemical steps leading to ochronosis involve the chemical binding of oxidized and/or polymerized derivatives of homogentisic acid (HGA), the metabolite accumulating in the blood in alcaptonuria, to these connective tissues, with subsequent physicochemical alterations in the properties of collagen[1]. HGA seems to be converted to an oxidized polymerized derivative through the formation of a labile, oxidized intermediate, benzoquinone acetic acid (BQA). Both steps in this conversion are catalysed by HGA polyphenol oxidase, a copper-requiring enzyme[2].

No effective therapy or prophylaxis for ochronosis has been reported. Ascorbic acid, which strongly reduces oxidized HGA *in vitro*, has been administered in high doses to patients with alcaptonuria without subsequent reduction in their 24 h urinary HGA excretion level[3–6]. Ascorbic acid, however, can prevent the binding of oxidized derivatives of HGA to collagen and other proteins *in vitro* (T. J. L., J. D. S. and J. E. S., unpublished). D-Penicillamine has recently been seen to form 1,4-addition products with BQA *in vitro* and it has been postulated that this compound might effectively reduce ochronosis through the formation of soluble excretable adducts, as well as by acting as a reducing agent or perhaps by inhibiting HGA polyphenol oxidase[7].

To study more directly the therapeutic potential of these two compounds, we examined the possibility that pretreating rats with experimental alcaptonuria with high doses of ascorbate or D-penicillamine would result in a measurable decrease in the binding of radioactive HGA derivatives to their connective tissues.

Forty male ACI rats, age 10–12 weeks, were randomly divided into four dietary groups: (1) Control—milled rat chow; (2) control diet with 8 per cent L-tyrosine (Nutritional Biochemicals), dry weight; (3) 8 per cent L-tyrosine + ascorbic acid (1·7 g/kg 8 per cent L-tyrosine diet, approximately equivalent to an intake of 8 g/day/70 kg body weight); (4) 8 per cent L-tyrosine + D-penicillamine (3 g with 100 mg pyridoxine/kg 8 per cent L-tyrosine diet). As previously demonstrated[8] quantitative HGA determinations of urine samples from rats on 8 per cent L-tyrosine indicated a prominent homogentisic aciduria. After maintenance on these diets for 92 days, the rats were injected intraperitoneally with ^{14}C-HGA (6·2 μCi, spec. activity 1·08 mCi/mmole) and killed 24 h later. (^{14}C-HGA Lactone (2,5-dihydroxyphenylacetic acid-[1-^{14}C]lactone, New England Nuclear Corp.) was hydrolysed in alkaline conditions with prepumped high purity nitrogen to obtain free HGA. The solution was then adjusted to *p*H 2·5 with 0·2 M HCl and sterilized and packaged by the Pharmaceutical Division, National Institutes of Health.)

Table 1. AMOUNT OF RADIOACTIVITY PRESENT IN DIALYSED CONNECTIVE TISSUE SAMPLES OF RATS WITH EXPERIMENTAL ALCAPTONURIA INJECTED WITH 1-^{14}C-HOMOGENTISIC ACID

Diet	Rat No.	Tail tendon* (c.p.m./100 mg tissue, wet wt.)	Xiphoid process* (c.p.m./100 mg tissue, wet wt.)
Control (without tyrosine)	1	138	—
	2	124	523
	3	117	465
	4	154	418
	5	104	—
	6	301	598
	Mean	156	501
	s.d.	64	69
8 per cent tyrosine	1	507	1,801
	2	512	1,161
	3	604	1,458
	4	519	1,538
	5	595	—
	Mean	547	1,490
	s.d.	43	231
8 per cent tyrosine plus ascorbate	1	233	531
	2	198	430
	3	202	301
	4	112	389
	5	252	556
	6	236	—
	Mean	221	441
	s.d.	48	94
8 per cent tyrosine plus D-penicillamine	1	626	1,349
	2	445	1,393
	3	429	1,358
	4	430	1,351
	5	593	—
	Mean	505	1,363
	s.d.	85	18
Non-injected animals	1	42	74
	2	36	54
	3	32	30
	4	30	59
	Mean	25	54
	s.d.	5	16

* Each value represents the average of two separate samples from each animal and is expressed as net counts per minute over background.

Pieces of tail tendon and xiphoid process were removed from each animal, finely minced, washed with a large volume of distilled water and then dialysed at room temperature against distilled water with three changes over 32 h. After drying for 2 h at 80° C, duplicate 100 mg samples of each specimen were dissolved in 1 ml. of 'Soluene' (Packard) by heating at 55° C for 1·5 h in scintillation vials. 'Toluene-Phosphor' was added to each vial after cooling and the samples were placed in the dark at 4° C

for 30 h. Each sample was counted for 10 min by a Packard Tri-Carb Dual Channel Liquid Scintillation Counter (model 582). Both types of connective tissue samples from rats fed 8 per cent L-tyrosine retained a 2·5 to 3·4-fold greater amount of radioactivity than those on 8 per cent L-tyrosine with supplementary ascorbate ($P < 0.001$ for tail tendon and $P < 0.01$ for xiphoid process). The amount of radioactivity retained by both types of connective tissues of rats fed D-penicillamine was not, however, significantly different from the amount retained for the 8 per cent L-tyrosine diet alone. Small amounts of ^{14}C were also retained in tissues of the control rats (receiving no L-tyrosine) although these values were not directly compared with those of the groups receiving 8 per cent L-tyrosine because the control rats probably had a much smaller body pool of HGA before injection.

This is the first report of direct measurement of the amount of binding of ^{14}C-HGA derivatives to connective tissues *in vivo* both with and without ascorbate treatment and strongly suggests that ascorbic acid reduced the total amount of binding of oxidized and/or polymerized HGA to connective tissue of rats with experimental alcaptonuria. Because the HGA-^{14}C bound to connective tissue constituted a very small fraction of the total HGA-^{14}C administered, the failure to detect differences in the urinary HGA excretion during the earlier experimental treatment of ochronosis with ascorbic acid is understandable.

The failure of D-penicillamine treatment to influence the binding of ^{14}C was unexpected. But D-penicillamine will not react with unoxidized HGA to form addition products and we have been unable to demonstrate the presence of BQA adducts with D-penicillamine in the urine or serum of alcaptonuric patients receiving this drug. Furthermore, the concentration of BQA in alcaptonuric serum is extremely low.

The animal model we used has many similarities to ochronosis[9]. Whether the administration of high doses of ascorbate to patients with alcaptonuria, particularly younger ones in whom pigmentary deposition has not yet produced clinically significant ochronosis, might be an effective method of preventing or inhibiting the deposition of ochronotic pigment in their connective tissues and, hence, delay or prevent the appearance of ochronotic arthritis remains to be demonstrated. We thank Dr George Martin for advice and for providing space in which we kept the animals used in these experiments.

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Determining Body Composition by Radiation Absorption Spectrometry

THE fractional composition of a multi-component absorber can be determined from the attenuation of γ-radiation at several energies[1–6]. This technique has been used to measure the amount of bone *in vivo*[7–11]. We now show that absorption measurements at two energies accurately indicate the relative lean–fat composition of soft tissue, and we have developed a scanning method of determining fat, lean cellular and bone mineral mass *in vivo*.

The attenuation of monoenergetic radiation in a single absorber is given by

$$I = I_0 \exp(-\mu x) \text{ or } \ln I_0 - \ln I = \mu x \qquad (1)$$

where I is the beam intensity after it has passed through the absorber, I_0 is the initial unattenuated beam intensity, μ is the mass absorbance coefficient (cm²/g) of the absorber, and x is the mass per unit area (g/cm²) of the absorber in the beam. For a complex absorber the total absorption coefficient (μ_t) at any single energy is the sum of the fractional absorption contributions of the absorber components

$$\mu_t = \mu_a f_a + \mu_b f_b \ldots + \mu_n f_n \qquad (2)$$

where μ_a is the mass absorption coefficient of component a, and f_a is the fractional contribution of a to the total mass, and so on. For a material composed of n components the fractional composition is obtained from n simultaneous equations of the form of equation (1), each incorporating equation (2). In practice, measurement at more than two energies is difficult because of cumulative uncertainties. This and the scarcity of monoenergetic sources in the useful energy range (20–100 keV) generally limit composition analysis to simple mixtures.

We have used ^{241}Am (59·6 keV) and tin-filtered ^{125}I (27·4 keV) sources with a conventional detector and a single channel analyser[12,13]. Instead of solving simultaneous equations for each set of observations we used the ratio (R) of ^{125}I absorbance to that with ^{241}Am as a direct index by establishing a calibration curve relating R to composition. In soft tissue $R \simeq 2{\cdot}1$ for 100 per cent lean cellular component and $R \simeq 1{\cdot}5$ for 100 per cent fat. Because absorption coefficients of fat and lean are nearly equal with ^{241}Am γ-rays the relationship between the ratio and relative composition of a mixture is linear. Measurements of soft tissue composition by differential absorption spectrometry require high precision and accuracy because of the relatively small differences among the absorption coefficients of low Z materials at these energies.

The accuracy of this new method was high. In soft tissue phantoms (polyethylene and sodium acetate; vegetable oil and water; paraffin and water) absorptiometry indicated fractional composition with a standard error of estimate (s.e.e.) of less than 0·02 (Fig. 1) and with high correlations ($r \simeq 0{\cdot}99$). Other workers using ^{109}Cd reported high accuracy with phantoms[14]. In meat samples (fat content by lipid extraction) the measured R for 100 per cent fat was 1·52 (s.e. = 0·026) while for 100 per cent lean the value was 2·09 (s.e. = 0·016); the s.e.e. in predicting the composition of seven meat samples was 0·03. The measured values of R in the different mixtures tested corresponded closely to values derived from published absorption coefficients[15].

Initially we measured tissue composition *in vivo* at single points on human limbs. Reproducibility over several days was usually high (0·5 per cent uncertainty for R), but composition changed with small position changes. A scanning method was developed which largely eliminated this problem. A photon source (100–200 mCi of ^{125}I, or 125 mCi of ^{241}Am) and detector are mechanically linked and passed several times at uniform speed across an accessible area, usually the middle upperarm[12]. Scanning facilitates both relocation and comparisons among subjects and sites; the total tissue mass varies along the

limb but relative composition of the soft tissue in different segments is fairly uniform. More important, scanning permits measurement of the mass of the components as well as their proportions, and bone mineral can be measured as well.

Theoretically, a third energy is needed for absorption spectrometry of bone mineral together with soft tissue; but the mass absorption coefficient of the mineral is so much greater than that of soft tissue ($\times 2$ at 59·6 keV, and $\times 7$ at 27·4 keV) that the mineral absorption is sharply demarcated in the typical scan (Fig. 2), making it unnecessary in practice. The shaded area in Fig. 2 below the I_0^* baseline shows the bone mineral absorption, and is directly proportional to the mineral mass[12]. The accuracy of measurement is 1–3 per cent from studies on ashed sections of bone[16]. The unshaded area in the scan represents soft tissue absorbance and is approximately proportional to the soft tissue mass in the scan path. The ratio of ^{125}I to ^{241}Am soft tissue scan areas gives an integrated value (ΣR) indicating relative tissue composition in the scan path. The dose from such scans is about 10 mrad and is confined to a narrow (5 mm) area across the limb.

Table 1. FIVE REPEAT MEASUREMENTS IN TWO SUBJECTS OVER 9 MONTHS

	^{241}Am tissue scan		^{125}I tissue scan		ΣR	
Subject	1	2	1	2	1	2
Mean	69·6	104·5	144·2	214·2	2·070	2·045
s.d.	1·95	3·07	3·72	8·32	0·006	0·026
c.v. (per cent)	2·80	2·94	2·58	3·88	0·27	1·28

Immediate reproducibility in replicate upperarm scans ($n = 63$) was within about 0·5 per cent for soft tissue scan area, and 1–2 per cent for bone mineral using either nuclide. Long term precision was examined in replicate scans on nine subjects after a 6 month interval; the uncertainty for scan areas with either nuclide was about 4 per cent while the uncertainty in ΣR was only about 1·6 per cent. Repeated measurements on two subjects over a 9 month period, as well as results on phantoms, showed even higher precision (Table 1). Part of this uncertainty in soft tissue scan areas may reflect actual changes of tissue mass, including those arising from repositioning. In spite of these variations in tissue mass the ΣR was determined with only a 1 per cent error; this typically introduces an uncertainty of about 0·03–0·04 in fractional composition. Such an error is moderately high relative to the usual range in fat fraction (0·10–0·40).

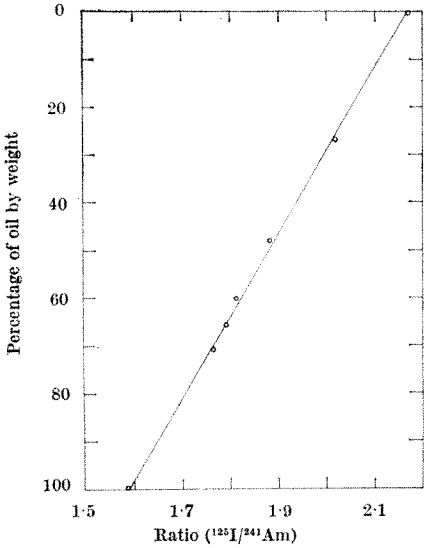


Fig. 1. Relationship between actual composition of a vegetable oil–water mixture and our measure of composition (R); accuracy of composition by absorptiometry is typically 1–2 per cent. $\Gamma = 0{\cdot}999$; s.e.e. = 1·27 per cent.

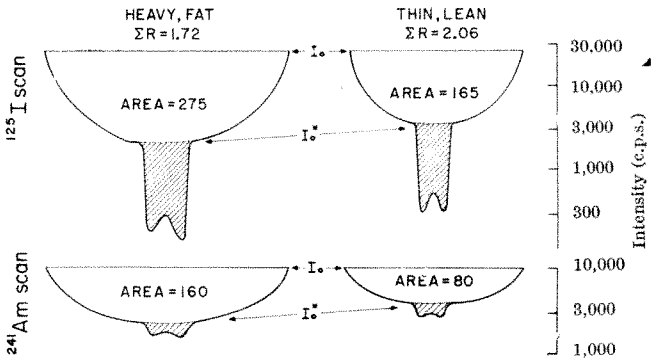


Fig. 2. Absorption scans of the upperarm in a heavy, fat person (left) and a thin, lean person (right). I_0 is the count rate level in air and I_0^* is the level adjacent to the bone. The shaded area represents absorption by bone mineral. The mineral is usually sharply demarcated in the scan and the baseline (I_0^*) is readily determined. The fat person has relatively lower tissue absorption with ^{125}I. The arm of the thin person contained about 7 per cent fat, while that of the heavy person had 65 per cent; these are the extremes of upperarm composition.

Our measurements were highly correlated with some indirect measures of composition. In nineteen young adults the correlation between tissue absorbance and upperarm circumference was 0·98, while the correlation with triceps skinfold (Lange caliper) was −0·91. Upperarm composition in these subjects was about 16 per cent fat, 81 per cent lean tissue and 3 per cent bone mineral.

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Thiaminase I in the Development of Cerebrocortical Necrosis in Sheep and Cattle

MEMBERS of the B group of vitamins are synthesized by ruminal microflora[1], and it has therefore been assumed[2] that ruminants do not require thiamine in their diet. In cerebrocortical necrosis of sheep and cattle[3], however, tissue thiamine levels are low, resulting in cellular anoxia, characteristic damage to the cortical neurones[3], raised

blood keto-acids and lactate, and lowered transketolase[4] activity. Moreover, plasma pyruvate kinase activity is elevated[5], indicating a severe disturbance in carbohydrate metabolism. The decrease in thiamine levels was shown to result from the presence of a thiaminase in the ruminal contents of affected animals[6,7], and we have since found this enzyme in all cases of cerebrocortical necrosis examined in this laboratory. It was present not only in the ruminal contents, but throughout the alimentary tract. We have now examined further the nature of this enzyme.

Thiaminase activity (type I or II) was assayed by extracting labelled 4-methyl-5-hydroxyethylthiazole released by the enzyme into ethyl acetate, in which thiamine itself is insoluble. Materials for thiaminase assay, such as rumen liquor, faeces or bacterial cultures, were suitably diluted or extracted with citrate–phosphate buffer (*p*H 6·4, 0·1 M) and 0·5 ml. of the extract mixed with 0·5 ml. of a solution of substrates in the same buffer. The substrate solution contained 100 μg of [*thiazole*-^{14}C]-thiamine (Radiochemical Centre, Amersham) with an activity of 50 nCi, and 38 μg of nicotinic acid per ml. After incubation for 1 h at 37° C, 2·0 ml. of ethyl acetate was added and the mixture was well shaken. After centrifugation at 2,500 r.p.m. for 5 min an aliquot of 0·5 ml. of the ethyl acetate layer was withdrawn and mixed with 10 ml. of scintillator fluid for determination of radioactivity. A blank without enzyme was always included. The number of counts gave a measure of the thiaminase activity, and was linear with time of incubation and concentration of enzyme.

If the thiaminase was of type I (thiamine : base 2-methyl-4-aminopyrimidine-5-methenyltransferase, EC 2.5.1.2), it would require a co-factor for activation[8–10], for purified thiaminase I itself does not have any thiamine splitting activity[11]. When cerebrocortical necrosis rumen liquor was dialysed against phosphate buffer (*p*H 6·5, 0·01 M) for 36 h at 4° C all thiaminase activity was lost. Restoration of activity by a number of possible co-factors, such as nicotinic acid (and to a lesser degree nicotinamide), aniline, pyridine and 2-methyl-4-amino-5-aminomethylpyrimidine, showed the enzyme to be of type I. Cysteine, cystine, 2-methyl-4-amino-5-hydroxymethylpyrimidine, glutathione, *p*-aminobenzoic acid, hydroxyproline, 1-methylnicotinamide and lysine were without measurable effect.

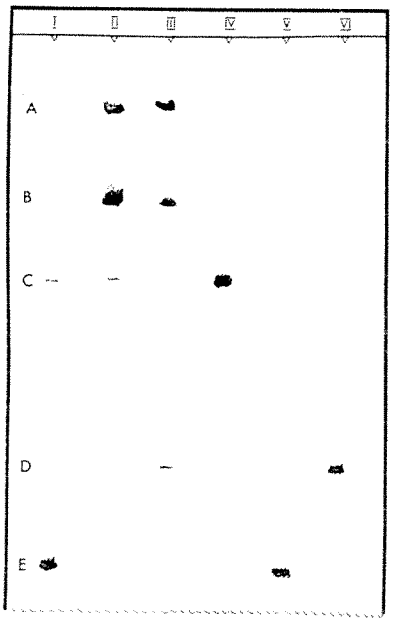


Fig. 1. Idealized autoradiogram of extracts from incubation of cerebrocortical necrosis rumen liquor with various substrates. I, With [*thiazole*-^{14}C]thiamine (ethyl acetate extract); II, [*pyrimidine*-^{14}C]thiamine (defatted alcohol extract); III, 6-^{14}C-nicotinic acid and thiamine (defatted alcohol extract); IV, thiazole or [*pyramidine*-^{14}C]thiamine; V, 4-methyl-5-hydroxy-[2-^{14}C]thiazole; VI, [6-^{14}C] nicotinic acid. Chromatograms (descending) were developed in *n*-butanol–acetic acid–water (4 : 1 : 1) for 16 h. Autoradiograms were obtained by exposure to 'Kodirex' X-ray film for 48 h.

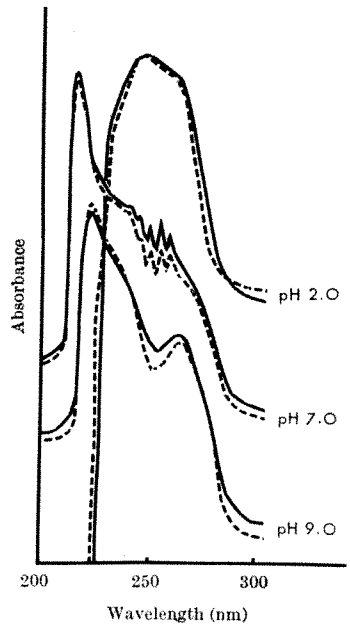


Fig. 2. Ultraviolet absorption spectra wavelength (nm) of pyrimidinyl-nicotinic acid. (*a*) from cerebrocortical necrosis rumen liquor (continuous line) and (*b*) synthetic (broken line) at *p*H 2·0, 7·0 and 9·0.

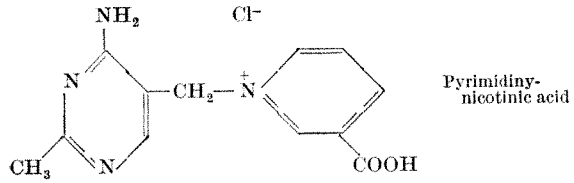
When the ethyl acetate extract from the incubation of cerebrocortical necrosis rumen liquor with [*thiazole*-^{14}C]-thiamine was chromatographed and autoradiographed, a single radioactive spot (apart from a trace of residual thiamine), consistent with the R_f of 4-methyl-5β-hydroxyethyl thiazole (Fig. 1*E*), was seen. When the pyrimidine moiety was labelled instead—by synthesizing [*pyrimidine-2-methyl*-^{14}C]thiamine from [2-^{14}C]acetonitrile—and the defatted alcoholic extract similarly chromatographed and autoradiographed, two radioactive spots *A* and *B* were seen. In separate experiments it was found that spots *A* and *B* were interchangeable, and that their relative concentrations depended on the *p*H of the extract. If the extract was treated with hydrochloric acid, only spot *A* was seen; whereas both spots *A* and *B* occurred when the extract was neutral. When cerebrocortical necrosis rumen liquor was incubated with thiamine and ^{14}C-labelled nicotinic acid (Radiochemical Centre, Amersham), and treated as before, two radioactive spots consistent with spots *A* and *B* were seen (Fig. 1).

Spots *A* and *B* were characterized as pyrimidinyl-nicotinic acid by synthesis of this compound from 2-methyl-4-amino-5-bromomethylpyrimidine and nicotinic acid, or by treatment of a mixture of thiamine and nicotinic acid with sulphurous acid, as described by Matsukawa[12]. Pyrimidinyl-nicotinic acid was further characterized by its positive reaction to Dragendorff reagent, and its greenish blue fluorescence under long wavelength ultraviolet light after being sprayed with alkaline ferricyanide. The ultraviolet absorption spectrum of the compound isolated from cerebrocortical necrosis rumen liquor (spots *A* and *B*) was identical to that of synthetic pyrimidinyl-nicotinic acid (Fig. 2).

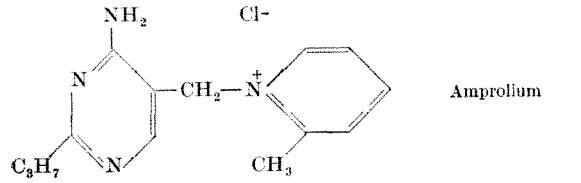
As a model for thiaminase I activity, thiamine and nicotinic acid were incubated with the culture medium from the growth of *Bacillus thiaminolyticus*. Similar patterns of degradation to those described here were seen.

Spots *A* and *B* are taken to be the spots described by Murata[11] as Pm-X and Pm-NiA.

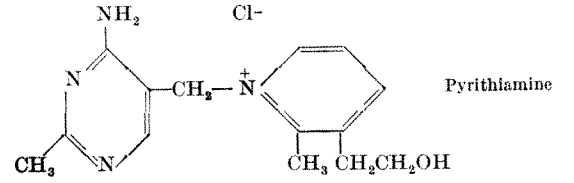
It is possible that this compound has antithiamine properties, for it has elements of similarity to pyrithiamine



Pyrimidiny-nicotinic acid



Amprolium



Pyrithiamine

and amprolium, both known to be thiamine antagonists. If this were so, the presence of thiaminase I in the rumen could bring about rapid thiamine deficiency. Not only would the supply of thiamine be cut off, but active antagonists would be produced which would further deplete tissues of thiamine—a situation in some ways similar to the induction of experimental cerebrocortical necrosis by administration of amprolium to calves and lambs[13].

Cerebrocortical necrosis of sheep and cattle therefore seems to be another example of the growing list of diseases caused by thiaminases: Chastek paralysis of foxes, thiaminase disease of poultry[14] caused by *Bacillus aneurinolyticus*, the nervous disorders of cats and dogs[15] fed canned fish diets, and thiaminase disease in man[16–18]. Further work on assessing the antithiamine index of pyrimidinyl-nicotinic acid and isolation of the causative agent is in hand.

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Placental Transfer and Metabolism of 4-^{14}C-Cortisol in the Pregnant Monkey

The concentration of cortisol in human maternal plasma exceeds that of foetal plasma by as much as two to five-fold[1–3]. This difference has been attributed primarily to the large quantity of non-diffusible cortisol in the maternal circulation—that is, cortisol bound to transcortin (cortisol binding globulin), an α-globulin with selective affinity for cortisol[4]. This concept was challenged by the studies of Gold *et al.*[5], who demonstrated that the concentration of unbound Porter–Silber chromogens in foetal plasma was lower than that found in maternal plasma, suggesting that simple differences in binding globulin concentrations alone cannot account for the distribution of cortisol between maternal and foetal compartments. The more rapid disposition of cortisol by the foetal–placental unit could account, in part, for the differences in cortisol concentration in the mother and foetus.

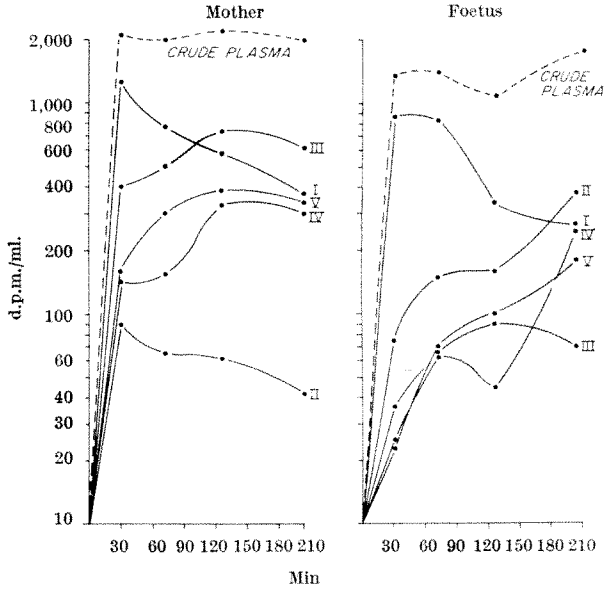


Fig. 1. Radioactivity in sequential maternal and foetal crude plasma samples, and in plasma extracts 1–5 after a single maternal infusion of 4-^{14}C-cortisol at time 0.

Quantitative information concerning this question is at present not obtainable in the human because of the inaccessibility of the foetus and the necessary limitations on human experimentation. As shown by the present study, however, such data can be obtained in a subhuman pregnant primate where it is possible simultaneously to sample maternal and foetal compartments *in situ* after the infusion of tracer amounts of labelled steroid to the mother.

Pregnant rhesus monkeys estimated to be at term (150–160 days) were anaesthetized with intramuscular piperidine hydrochloride and the uterus was exposed by laparotomy. The bidiscoid placentas and the interplacental vessels were identified by transillumination of the uterus. Usually three to four pairs of interplacental vessels are present, each pair consisting of a branch of the umbilical artery and vein. The myometrium overlying a pair of interplacental vessels was incised and a short segment of one vessel was dissected free from its position between the chorion and amnion. The vessel was cannulated with a No. 19 polyethylene catheter, so that we were able to collect foetal blood samples without entering the amniotic cavity. The foetus was then manoeuvred so that the foetal breech was held firmly against an area of

uterine wall free of placenta. An incision was made through the uterine wall and foetal membranes over the area of the foetal perineum. The foetal tail was delivered and, by means of firm traction on the tail, the foetal breech was held tightly against the uterine wall, thus preventing leakage of amniotic fluid. If the foetus was female, an episiotomy was performed and a No. 15 polyethylene tube was inserted through the urethra into the bladder and anchored in place with sutures. In the case of a male foetus, a small polyethylene catheter was inserted into the bladder via the prostatic urethra following a technique described by Chez and Hutchinson[6]. The maternal bladder was catheterized and a branch of the maternal saphenous vein was also cannulated.

Using this experimental model, 5·0 μCi 4-^{14}C-cortisol (specific activity 10 mCi/mmole) was infused into the maternal circulation for 3 min and sequential samples were taken of foetal and maternal blood and foetal and maternal urine. In each experiment foetal viability was monitored by intermittent measurement of the *p*H of blood obtained from the interplacental vessels. At the end of the experiment a living foetus was delivered by Caesarean section.

Table 1. SOLVENT EXTRACTION SEQUENCES FOR PARTITIONING OF PLASMA AND URINE SAMPLES

Extract	Solvent	Volume ratio	*p*H	Presumptive designation
1	Dichloromethane	10 : 1	7·0	Unconjugated "low polar"
2	Ethyl acetate	5 : 1	7·0	Unconjugated "high polar"
3	Dichloromethane	10 : 1	5·0	Glucuronides "low polar"
4	Ethyl acetate	5 : 1	5·0	Glucuronides "high polar"
5	Ethyl acetate	5 : 1	1·0	"Sulphates"

Each plasma and urine sample (0·1 ml.) was counted for crude radioactivity by direct addition to counting vials containing 15 ml. of a water miscible scintillator system (dioxane–cellosolve); vials were counted in a liquid scintillation spectrometer (Nuclear–Chicago, model 720) at a counting precision of ± 2 per cent and an efficiency for ^{14}C of 80 per cent. The samples were corrected for quenching. Each foetal and maternal plasma and urine specimen was then subjected to a serial extraction sequence of solvent partitioning as shown in Table 1. Extract 1 was obtained by extraction with dichloromethane at *p*H 7·0 and the aqueous residue was extracted with ethyl acetate at *p*H 7·0, resulting in extract 2. After hydrolysis with β-glucuronidase 200 U/ml. at *p*H 5·0 for 48 h at 25° C, extraction with dichloromethane and ethyl acetate at *p*H 5·0 gave extracts 3 and 4 and, finally, addition of saturated NaCl and H_2SO_4 at *p*H 1·0 for 48 h at 25° C accompanied by ethyl acetate extraction produced extract 5. After the serial extraction sequence the solvent was removed by evaporation and each extract counted for radioactivity as described previously.

After infusion of the labelled cortisol into the maternal venous system, radioactivity was detectable in foetal plasma at 10 min. A comparison of extractable radioactivity in maternal and foetal plasma (Fig. 1) shows that, following maternal injection, extract 1 radioactivity, presumably cortisol or cortisone, disappears more rapidly from the foetal circulation than from maternal circulation. Extract 2 was the principal metabolite in foetal plasma, whereas it was only a small fraction of the maternal metabolites of radiocortisol. By contrast, extract 3 represented the principal fraction in maternal plasma. Urine specimens collected simultaneously from mother and foetus indicated extract 1 radioactivity as the main excretory product of maternal urine (Fig. 2). In the foetus, however, the principal excretory product was extract 2.

This study is the first to provide data on the simultaneous metabolism and removal rates of radiocortisol from the mother and foetus in any animal species. Although several possibilities have been considered to account for the differences in maternal and foetal plasma concentrations of cortisol, these studies strongly suggest that such differences are partly a consequence of the more rapid rate of removal of cortisol from foetal plasma. This interpretation of the data is based on the assumption that cortisol and cortisone are the major constituents of extract 1. The designation of extracts shown in Table 1 follows that described by Aarskog[7], who used the same solvent extraction sequence. But the activity measured in various extracts may represent several metabolites, the specific nature of which will require further study.

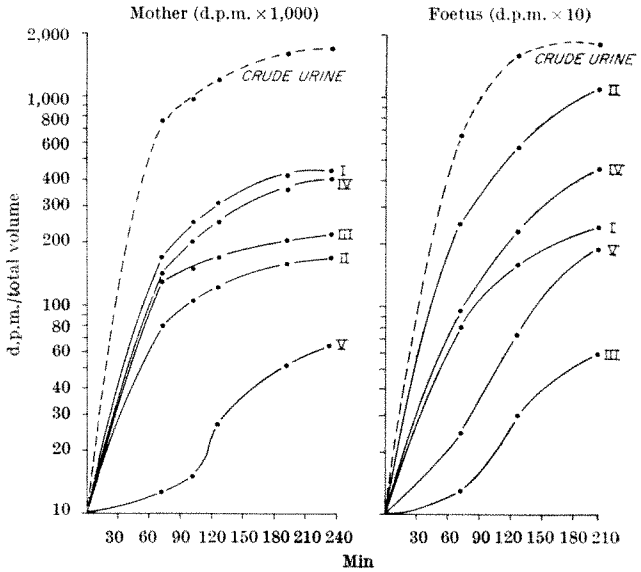


Fig. 2. Radioactivity in sequential maternal and foetal crude urine, and in urine extracts 1–5 after a single maternal infusion of 4-^{14}C-cortisol at time 0.

This study also reveals significantly different metabolic pathways for the removal of radiocortisol from maternal and foetal plasma. Whereas maternal plasma cortisol is metabolized mainly through glucuronide conjugation (extract 3), the principal foetal metabolite of cortisol is not a glucuronide but, rather, a metabolite (extract 2) somewhat more "polar" than cortisol, possibly 6-β-hydrocortisol. While this finding is consistent with earlier studies indicating that the foetus is deficient in enzymes required for glucuronide conjugation[8], it is also clear from the present study that the foetus or the foetal–placental unit has an alternative and apparently efficient mechanism for disposition of cortisol. These differences are further supported by the data concerning the foetal urinary radiometabolites of cortisol where extract 2 accounts for more than 50 per cent of extractable radioactivity.

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In Vitro Support System for the Study of Blastocyst Differentiation in the Mouse

DURING the early implantation stage in the mouse several events in the differentiation of the embryo are initiated. Once the zona pellucida has been lost, attachment to the uterine epithelium occurs, the inner cell mass comes to assume its mesometrial orientation[1], trophoblastic giant cells develop, differentiation of the endoderm begins and the inner cell mass undergoes changes leading to egg cylinder formation. The *in vitro* culture of the attachment stage would allow direct observation and analysis of some of these events and an experimental investigation of the conditions influencing them. Retransfer of eggs to a receptive host animal in order to follow through the effects of experimental manipulations on earlier stages could also, in some cases, be eliminated if *in vitro* development of early implantation stages could be obtained.

Blastocysts cultured in a drop of serum-supplemented medium under liquid paraffin can undergo a form of attachment to the floor of the culture vessel[2]. When this occurs the trophoblast usually migrates out over the glass, pulling the blastocyst down and flattening it, and so disrupting normal organization. A three dimensional culture system has been devised in which development of the attachment stage can occur, with the maintenance of more normal organization, and in which the blastocyst is held in a constant orientation for cinemicrography. This report describes briefly the degree of differentiation occurring in this system and some cinemicrographic observations on the mature blastocyst.

Our principal requirements of a three dimensional culture system for blastocyst development were transparency, a suitable surface for trophoblast attachment and compatibility with culture conditions. One of the materials best fulfilling these requirements was found to be the soft, transparent lens fibre material from the bovine eye. Blastocysts were injected from a mouth controlled pipette into a small fragment of this material placed in a culture chamber suitable for cinemicrography. Earle's BSS and 10 per cent calf serum were added around the lens material and the culture was gassed with 10 per cent CO_2 in air. Control cultures were carried out in small drops of the same medium under the same gas phase. Material for histological examination was fixed in Clarke's fluid and stained with Ehrlich's haematoxylin. For cinemicrography the culture chamber was placed on a microscope enclosed in a warm box at 37° C and time lapse recordings were made at 30 s intervals. To follow relative changes in position of different components of the blastocyst, one of the trophoblast cells was marked as a reference point by the micro-injection of a drop of inert oil using a technique developed in this laboratory[3] (unpublished work of I. B. W., E. Bolton and R. H. Cuttler).



Fig. 1. Formation of endoderm. i, Inner cell mass; p, proximal endoderm; d, distal endoderm; g, trophoblast giant cells.

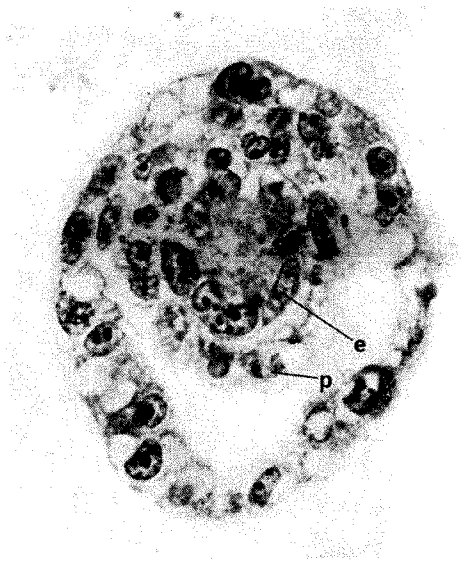


Fig. 2. Formation of egg cylinder. The embryonic ectoderm (e) shows a typical radial arrangement and is surrounded by the characteristic vacuolated cells of the proximal endoderm (p).

Blastocysts were recovered from the uterus 84 h after the estimated time of ovulation and cultured in the eye lens material or as controls for up to 48 h before fixation for histological examination. After loss of the zona, blastocysts in the control cultures either attached and flattened with loss of normal organization[2], or remained unattached and retained their normal shape. In the latter case, no differentiation and very little growth of the inner cell mass occurred and often the inner cell mass was found to have dispersed itself round the inside of the trophoblast shell. Blastocysts in the lens material did undergo differentiation similar in part to that seen when eggs are transferred to ectopic sites[3,4]. Proximal and distal endoderm were found (Fig. 1) and egg cylinder formation was initiated (Figs. 2 and 3). Even if organization failed, growth of inner cell mass derivatives continued (Fig. 4).

These observations suggest that two of the require-

ments for the onset of blastocyst differentiation are attachment and the maintenance of organization. The importance of attachment for the initiation of differentiation has also been suggested before. Tarkowski[5] has shown that rat eggs transferred to the mouse differentiated only in those cases where attachment was successful. When there was no attachment the inner cell mass sometimes spread out and dispersed as in some of the blastocysts in our control cultures, which are comparable with those shown by Tarkowski (ref. 5, Plate 1 and Figs. *A*, *C* and *F*). The difference between flattened blastocysts where no embryonic differentiation is apparent and blastocysts cultured in lens material where organization is maintained suggests that normal cellular relationships are vital in inner cell mass differentiation, as they are in many developing systems.

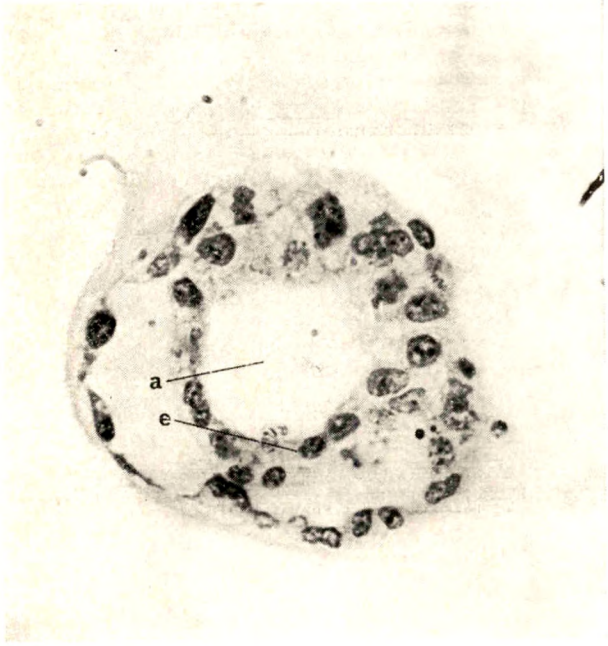


Fig. 3. Pro-amniotic cavity formation. Embryonic ectoderm (e) encloses the pro-amniotic cavity (a).

Cinemicrographic observations on the mature blastocyst in the lens material, after the loss of the zona, revealed several cell activities. During one 12 h recording period the blastocyst underwent three rapid contractions followed by a slower expansion as described by Cole[6]. The cells of the inner cell mass could be seen to be in a constant state of agitation and to push out blunt pseudopods. As a result of this type of activity the inner cell mass, although remaining a discrete group of cells, changed shape several times and actually moved bodily around inside the trophoblast "shell", finally coming to rest some 90° from its original site in a position underlying a region of trophoblast not in close contact with the lens material. The change in position was confirmed by reference to the injected marker drop. The outer membrane of the trophoblast cells pulsated, particularly in those regions where there was no close contact with the lens material; larger hyaline pseudopodia were occasionally pushed out and moved about in an exploratory fashion before being retracted.

The behaviour of the blastocyst as observed *in vitro* corresponds well with histological information on blastocyst structure. Potts and Wilson[7] have shown that the cells of the inner cell mass in the late pre-implantation stages are only loosely associated with each other and with the trophoblast and this is reflected in the way the



Fig. 4. Disorganized growth. Trophoblastic (t) and inner cell mass elements (i) are still visible.

inner cell mass cells seem free to move over each other. At the time of attachment the inner cell mass is often found to be haphazardly positioned in relation to the mesometrial/anti-mesometrial axis. Kirby, Potts and Wilson[1] suggest that mesometrial orientation of the inner cell mass is achieved by migration of the inner cell mass round the inside of the trophoblast "shell"; the results obtained here show that the inner cell mass is capable of such movement.

The gradients, or other factors, which determine that the inner cell mass will come to rest in a mesometrial position are not known. In histological preparations of attaching blastocysts *in utero*, fixation shrinkage frequently causes the blastocyst to pull away from the epithelium. It is often found that it is in the mesometrial region of the trophoblast that separation occurs most readily, suggesting that attachment in this region is weak or not yet complete. It seems from the film recording that the region of trophoblast where there is least contact with the eye lens material is more "attractive" to the inner cell mass than regions which have more contact. Because the mesometrial side of the blastocyst is one where attachment seems to be weakest *in utero* this could explain why the inner cell mass comes to lie mesometrially.

It seems feasible that changes can occur in the trophoblast cells on attachment which could lead to the setting up of gradients in the trophoblast shell to which the inner cell mass would be capable of responding. The nature of such gradients is speculative and could involve several factors such as a change in trophoblast metabolism on attachment, promotion of exchange of materials between the trophoblast and the substratum or a change in surface free energy or surface potential of the trophoblast cells, leading to variations in adhesiveness between those parts of the trophoblast which have attached and those which have not. If inner cell mass orientation was due to differential adhesiveness of the trophoblast shell, it might be expected that the trophoblast of the attached lateral and anti-mesometrial aspects of the blastocyst would be less adhesive to the inner cell mass than the unattached mesometrial region so that, as shown for fibroblasts[8], the cells of the inner cell mass would move up the gradient of adhesion and come to lie mesometrially, at least in the mouse.

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Chromosome Size and Hypodiploidy

IN chromosome preparations from peripheral lymphocytes a certain number of aneuploid metaphases are usually found (about 10–20 per cent hypodiploid and 1–2 per cent hyperdiploid chromosome sets). These aberrations are usually the result of technical artefacts. In cases where a true numerical chromosomal aberration can be expected these unavoidable artefacts can cause erroneous results.

In 1967 Pegoraro *et al.* collected all reported cases of chronic myeloid leukaemia, where the complete chromosomal analysis, based on the Denver classification system was given. They investigated whether the chromosome gain or loss, coincident with the deterioration of the haematological system, showed any distinct pattern. Combining additional and missing chromosomes in the different groups (*A*–*G*), they found no relation to the size of the groups in question. They rather found a close correlation between decreasing chromosome size and the tendency to be gained or lost. The authors therefore assumed that mechanical factors play an important part in chromosomal non-disjunction. Considering only the distribution of the missing chromosomes in the authors' table, however, such a correlation was not seen by us.

Recently Neurath *et al.*[2] tried to establish a correlation between chromosome loss, chromosome size and the age and sex of the individual (7,067 mitoses were studied). Even though they investigated only hypodiploid mitoses of healthy persons, they found the same connexion between decreasing chromosome size and increasing chromosome loss as did Pegoraro *et al.* The only deviation from a nearly linear correlation was the very high loss of *G* group chromosomes exceeding the expected value. Their results were independent of the person's age; the well known tendency towards higher chromosome loss with increasing age[3,4] was verified.

The findings of both groups prompted us to compare our data collected from photographs of 4,884 mitoses. They derived from blood lymphocyte cultures from healthy persons and healthy mothers of mongoloid children (20–37 yr). The mitoses had been collected in a few months and used for chromosome association studies under different preparation techniques; macrocultures in mothers[7], macro and microcultures in controls[5,6]. Because macrocultures and microcultures did not show any different pattern of chromosomal loss, the results could be combined for this study. We found a hypodiploidy rate of 12·9 per cent (Neurath 16·3 per cent) indicating that it stemmed mostly from preparation and not from mitotic non-disjunction.

Our findings are compiled in Table 1, which shows the percentage participation of the different chromosome groups (*A*–*G*) in the diploid chromosome complement and both the raw values of missing chromosomes and the percentage deviation from the expected numbers. The percentage loss difference was calculated by the formula: $L = 100\,(o-e) : e$, where o is the observed number of missing chromosomes for each particular group and e is the number expected on the basis of a random loss. Besides the metaphases with 45 chromosomes, we considered separately the mitoses which lost up to six chromosomes (columns 4–7), so as to investigate the possibility of a combined loss of several chromosomes.

In the female population a group of 33 normal controls and 26 normal mothers of mongoloid children were combined[7] because the pattern of chromosome loss was nearly identical. This means that there seems to be no increased tendency towards mitotic chromosomal non-disjunction in mothers of mongoloid children.

Table 1. DISTRIBUTION OF CHROMOSOME LOSS IN METAPHASE PLATES OF PERSONS WITH A NORMAL KARYOTYPE

Relative group size (per cent)		One missing chromosome: 36 males No.	36 males Per cent deviation	59 females No.	59 females Per cent deviation	Males and females No.	Males and females Per cent deviation	One–six missing chromosomes: 36 males No.	36 males Per cent deviation	59 females No.	59 females Per cent deviation	Males and females No.	Males and females Per cent deviation
A	13·04	4	−74·8	13	−63·0	17	−66·7	17	−61·3	40	−59·6	57	−60·1
B	8·70	8	−24·5	13	−44·4	21	−38·2	16	−45·4	41	−37·9	57	−40·3
C+2*X*	34·78	—	—	118	+26·1	158	+18·4	—	—	343	+29·2	464	+24·1
C+*X*	32·61	40	+0·5	—	—			121	+10·1	—	—		
D	13·04	11	−30·8	23	−34·5	34	−33·3	51	+16·1	62	−37·4	113	−20·9
E	13·04	29	+82·4	57	+62·4	86	+68·6	63	+43·5	148	+49·5	211	+47·7
F	8·70	18	+69·8	24	+2·6	42	+23·5	34	+16·0	73	+10·6	107	+12·2
G	8·70	—	—	21	−10·3	33	−10·1	—	—	52	−21·2	87	−15·2
G+*Y*	10·87	12	−9·8	—	—			35	−4·3	—	—		
Number of:													
Missing chromosomes		122		269		391		337		759		1,096	
Hypoploid metaphases		122		269		391		192		439		631	
Metaphases examined		1,474		3,410		4,884		1,474		3,410		4,884	
Percentage hypodiploidy		8·3		7·9		8·0		13·0		12·9		12·9	

In agreement with the other authors we found a significantly smaller loss of *A* and *B* chromosomes than would be expected by chance. *C*, *E* and *F* chromosomes were more frequently lost than expected. The highest relative loss rate was observed in the *E* group. Unexpectedly, acrocentric chromosomes of the *D* and *G* group were seldom missing but they were not as strongly retained as the large chromosomes of the *A* and *B* groups. There was no significant difference between males and females (*C* and *G* groups have been corrected for the presence of a second *X* or a *Y* chromosome). There were no obvious shifts in the chromosome loss ratios between metaphases with 45 and those with 40–45 chromosomes. A tendency towards a combined chromosome loss could not be found. Our results agree very well with those on the hypodiploid mitoses of Pegoraro *et al.*, but they disagree with the results of Neurath *et al.* We were not able to establish a significant correlation between chromosome loss and size, nor did we see the extremely high loss of *G* group

chromosomes in our material. This seems to be quite significant, for we have found that the common chromosome aberration in human meningeal tumours is the absence of a single *G* chromosome[8]. This finding was confirmed in more than forty tumours and is to our knowledge the first case of a uniform chromosomal aberration in a human solid tumour[9].

It is interesting that the metacentric *E* chromosomes show relatively the highest loss rate, because a loss of *E* chromosomes is reported to be a rather frequent finding in the stemlines of chronic myeloid leukaemia at the beginning of exacerbation[10]. On the other hand, the nucleolus organizing chromosomes, the *D* and *G* chromosomes, are missing relatively seldom. This phenomenon, as well as the close association of these chromosomes in the spread metaphase plate, may arise from the stickiness of adherent nucleolar material.

Our results confirm that the numerical loss of chromosomes shows a certain pattern, but they suggest that this pattern may be a consequence of several different (mechanical) factors and not only dependent on the chromosome size.

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Very Early Replication of Scrapie in Lymphocytic Tissue

THE incubation period of scrapie in susceptible animals is extremely long[1] and its apparently non-viral behaviour is also well known[2]. Biochemical investigations have usually concentrated on brain tissue when histopathological lesions are maximal[3], but it seems important experimentally to locate other sites of active replication. Involvement of lymphocytic tissues has been known for some time[4–6] and agent distribution in organs of the mouse after peripheral subcutaneous inoculation suggested a pathogenesis in which the agent localized and multiplied in lymphocytic tissue before being disseminated to sites of secondary replication such as neural cells[7]. A ten-fold multiplication in the spleen was observed within 8 weeks of inoculation. A genetically controlled delay in the time of an early rise in agent concentration in spleen has been reported after intracerebral or intraperitoneal inoculation[8,9].

Reproducible titres and quickest response are achieved by the intracerebral route[10] which suggests that the pathway of pathogenesis is well defined and amenable to quantitative measurement. Levels of scrapie activity in mouse spleen and brain, two sites of high activity in the infected animal[7], have been determined at intervals from 1–56 days following intracerebral inoculation of groups of inbred C57 mice with inocula prepared from 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions of a standard brain homogenate pool. Seven mice were killed at each interval and whole homogenates of pooled brains or spleens without centrifugation titrated in Swiss White mice[10]. Total levels of activity are expressed in doses of ID_{50}/0·03 ml. inoculum of a 10^{-1} dilution of the standard brain homogenate.

Table 1. TOTAL LEVELS OF SCRAPIE ACTIVITY IN MOUSE BRAIN AND SPLEEN 18 DAYS AFTER INTRACEREBRAL INOCULATION

Inocula	Inoculum dose	Activity in spleen	Multiplication	Activity in brain
10^{-1}†	5·3*	5·2	—	3·9
10^{-2}	4·3	5·2	0·9	3·5
10^{-3}	3·3	4·2	0·9	2·2
10^{-4}	2·3	3·7	1·4	1·6

* All levels expressed as $\log_{10}$ (ID_{50} doses/0·03 ml. inoculum of a 10^{-1} dilution of standard brain homogenate).
† Dilution of brain homogenate.

Spleen activity initially increased for 18 days at a mean rate of 0·16 $\log_{10}$/day, response being identical following the 10^{-1} and 10^{-2} dilutions, with evidence of a ten-fold multiplication (Table 1) quite unrelated to the level of brain activity. Mice receiving a 10^{-1} or 10^{-2} inoculum then maintained a constant level ($10^{5\cdot2}$) in spleen although it continued to rise slowly with the higher dilutions until the same plateau was reached in 42 and 52 days respectively for the 10^{-3} and 10^{-4} inoculum representing even higher multiplication of the original dose. In brain after an initial fall to a residual level, activity began to increase when the spleen attained about $10^{4\cdot5}$ doses. The relative delays in the initial rise in brain activity and its subsequent increase compared with spleen are shown in Table 2. The increase in brain titre, 0·05 and 0·09 $\log_{10}$/day, is lower than the initial change in spleen titre and decreases with time.

Table 2. TIME PERIODS FOR BRAIN OR SPLEEN ACTIVITY TO ATTAIN CERTAIN LEVELS AFTER INTRACEREBRAL INOCULATION

Inoculum	Inoculum dose ($\log_{10}$)	Time for spleen to attain $10^{4\cdot5}$ doses (days)	Time for spleen to attain original inoculum (days)	Time for brain to attain original inoculum (days)
10^{-2}	4·3	14	8	30
10^{-3}	3·3	22	8	38
10^{-4}	2·3	42	7	46

Table 3. TOTAL LEVELS OF SCRAPIE ACTIVITY IN MOUSE SPLEEN AFTER INTRACEREBRAL INOCULATION OF SHEEP OR GOAT SCRAPIE

Inoculum	Time after inoculation (months)	Inoculum dose ($\log_{10}$)	Total activity in spleen ($\log_{10}$)
Suffolk sheep	1	2·2*	3·2*
	2	2·2	4·3
Goat	1	2·9	4·2
	2	2·9	4·2

Expressed as $\log_{10}$ (ID_{50} doses/0·03 ml. inoculum of a 10^{-1} dilution of sheep or goat brain homogenate).

The products of a replication process are therefore present in spleen active in the period 2–3 weeks following injection. No biochemical data exist for actual synthesis in spleen. In its functional behaviour active material could be selectively adsorbed from lymphocytic and gland tissue so that spleen activity is only an indicator of the general level. Comparable levels of activity at similar times in these locations[7] (D. L. M. and A. McL. D., to be published) increase the scale of effective multiplication at an early stage over the whole animal. The identical response following inocula derived from a 10^{-1} or 10^{-2} homogenate indicates a limited number of replication sites. Any replication of residual inoculum in brain following intracerebral inoculation is likely to be extremely slow. After inoculation of a 10^{-1} homogenate an observed slight elevation of levels of activity over that of a 10^{-2} homogenate for the first 40 days can be accounted for by higher levels of residual activity remaining in the brain. It seems necessary for active material to be transported by lymph drainage to lymphocytic tissue where localization and replication take place fairly rapidly and a modified scrapie active material is then gradually returned to the brain in increasing amount. This activated scrapie can now presumably multiply in brain tissue, eventually

causing neuronal damage possibly through metabolic malfunction with the appearance of clinical disease.

If efficiency of localization in lymphocytic tissue is an important factor, the expected time sequence for the critical titre determining rise in brain activity to be reached in spleen following various routes of inoculation would be intracerebral, intraperitoneal and subcutaneous. From experimental data available[7,8] it could take between 28 and 56 days after subcutaneous inoculation while the incubation periods are similar for both the subcutaneous and intraperitoneal routes. Splenectomy of mice before or after intracerebral inoculation has no effect on the incubation period[11] which suggests that the spleen is accumulating agent that has been synthesized elsewhere. Conversely, because the system is saturated with a 10^{-1} or 10^{-2} inoculum, removal of a site of synthesis may have only a marginal effect so that splenectomy performed with higher dilutions of inoculum might be more effective.

We have proposed that during the replication cycle in lymphocytic tissue the scrapie agent is modified. An extreme case is provided by the first passage in mouse of scrapie derived from Suffolk sheep or goats[12] with incubation periods of 13–14 and 11–12 months respectively. Within one month spleens affected by goat scrapie inoculum had reached the plateau levels followed a month later by those receiving sheep scrapie (Table 3). The incubation periods for the recipient mice in the titration groups were longer than expected for mouse passaged material (ME7)[12]. The extent of histopathological lesions in the brains was about midway between the generalized distribution of ME7 and the localized subcortical distribution expected in a first sheep to mouse passage[12]. Applying various criteria a partial adaptation of the species specific scrapie to the mouse and replication is proceeding within 4 to 8 weeks after inoculation.

Another species adapted scrapie inoculation into the host species can be compared with mouse scrapie. Levels of activity found in goat spleen at 10, 14 and 18 weeks after intracerebral inoculation of a 10^{-1} dilution of scrapie goat brain homogenate were equivalent to the activity injected into the animal[13]. These observations were very similar to those obtained with mouse scrapie following a 10^{-1} brain homogenate inoculum.

Whatever scrapie source or route of inoculation is considered it seems that localization in the lymphocytic tissue is essential for initiation of replication and modification. The multiplication process is completed within relatively short periods, comparable with those found with oncogenic viruses, so scrapie replication can no longer be regarded as associated with long time scales. The rate of multiplication is slow, but this agrees with the general low level of scrapie activity compared with virus infections. These low rates of synthesis *in vivo* cast doubt on the possible success of culturing scrapie in cells, or visualization of the agent by electron microscopy. Biological testing is still the only criterion for scrapie potency, but the conceptual change in time scale and tissue type provides a new basis for biochemical investigation for it is conceivable that only a small proportion of the products of synthesis or replication is biologically active. There is undoubtedly a transference of scrapie potent material from localized sites in lymphocytic tissue to the brain[7], and now that the time scale is better defined it should be possible to determine the transfer stage. Tissue biopsy also now seems a possible approach to the diagnosis and progression of the natural disease in sheep. We thank Dr J. T. Stamp, Dr R. M. Barlow, Dr J. M. K. Mackay and Dr J. T. Vantsis for discussion.

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Antigen Binding Cells in Tolerant Animals

The immune response involves the appearance of antibody secreting cells as well as antigen binding cells. Antibody secreting cells can be counted *in vitro* using Jerne and Nordin's local haemolysis in gel (LHG) assay[1], and are then called plaque forming cells (PFC). Antigen binding cells can be recognized by their ability to bind particulate antigens such as sheep red cells (SRB) to their surfaces and have been named rosette forming cells (RFC)[2,3]. It is likely that RFC represent a mixture of antibody secreting cells and non-antibody secreting cells. Howard *et al.*[4] studied the RFC response in the spleens of mice tolerant to pneumococcal polysaccharide. Although serum antibodies could not be seen in these animals a considerable number, larger, in fact, in "tolerant" than in optimally immunized animals, of RFC was found. They explained these findings on the basis that antibody secreting cells existed but that the humoral antibodies produced were neutralized extracellularly by persisting undegraded antigen.

We have studied the cellular basis of tolerance to another polysaccharide antigen. Two to five months old CBA mice were used. Tolerance was induced by repeated injections of 3 mg of alkali detoxified polysaccharide from *E. coli* O55 : B5 (CPS)[5] given 5 times in 3 mg doses during 14 days and maintained by weekly injections of 3 mg of CPS. The animals tolerant to CPS showed a normal PFC response to SRC. The number of PFC in the spleens was tested by the LHG assay of Jerne and Nordin[1] as modified by Mishell and Dutton[6]. Conjugation of the CPS to SRC was performed as described by Möller[7]. CPS stimulates 19S antibody synthesis exclusively for a prolonged period, so only direct PFC were studied[8]. For detection of RFC a slight modification of the centrifugation-resuspension method described by McConnell *et al.*[9] was used. The CPS was absorbed to CBA mouse erythrocytes using the same method as with SRC[7].

Spleen cells from normal, immune and tolerant animals were assayed for the presence of PFC and RFC. In immune animals the assay was done 5 days after one injection of 0·01 mg of CPS at the peak of the PFC response[5]. In one of the tolerant groups, "tolerant mice A", the assay was done 5 days after the last injection of the tolerance maintaining dose of 3 mg of CPS. In the other tolerant group, "tolerant mice B", the animals received an injection of 0·01 mg of CPS 7 days after the last injection of 3 mg and were tested 5 days later.

In non-immune animals the mean number of PFC was $1{\cdot}4/10^6$ spleen cells and of the RFC $115/10^6$ spleen cells. Optimally immunized animals showed a mean number of 154 PFC/10^6 spleen cells and of 382 RFC/10^6 spleen cells (Table 1). In the "tolerant mice A" group the appearance of PFC was completely inhibited except in one animal in which a small response was detected. The RFC, on the other hand, were not abolished; instead

Table 1. CELLULAR IMMUNE RESPONSE IN SPLEENS OF MICE IMMUNIZED WITH *E. coli* POLYSACCHARIDE

	PFC/10^6 spleen cells	RFC/10^6 spleen cells
Normal mice	0·15 ± 0·15 (1·4)	2·06 ± 0·07 (115)
Tolerant mice A	0·13 ± 0·13 (1·3)	2·21 ± 0·14 (164)
Tolerant mice B	0·73 ± 0·19 (5·4)	2·37 ± 0·04 (235)
Immune mice	2·19 ± 0·08 (154)	2·58 ± 0·06 (382)

The figures are given in $\log_{10}$ values for the mean ± standard error. The corresponding antilogs for the mean are given in parentheses. Tolerant mice A were tested 5 days after the last tolerance maintaining dose of 3 mg of CPS. Tolerant mice B were given 0·01 mg of CPS 7 days after the last injection of 3 mg of CPS and assayed 5 days later. Immune animals were tested 5 days after the injection of 0·01 mg of CPS.

there was a tendency for an increase in number compared with the unimmunized mice, with a mean of 164 RFC/10^6 spleen cells. In the "tolerant mice B" group a mean number of 5,4 PFC/10^6 spleen cells and of 235 RFC/10^6 spleen cells was demonstrated. The number of RFC in this group is significantly increased over background values but is not as high as in optimally immunized animals (Table 2).

Table 2. *P* VALUES FOR DIFFERENCES IN MEAN NUMBER OF RFC BETWEEN VARIOUS GROUPS OF MICE

	P value
Normal—tolerant A	Not significant
Normal—tolerant B	$P < 0.005$
Normal—immune	$P < 0.001$
Tolerant A—tolerant B	Not significant
Tolerant A—immune	$P < 0.05$
Tolerant B—immune	$P < 0.02$

For designation of the various groups see Table 1.

These findings cannot be explained on the basis suggested by Howard *et al.*[4] that the tolerant state is brought about by neutralization of antibodies by excess antigen. Thus tolerance to endotoxin appears to inhibit selectively the steps leading to the appearance of antibody secreting cells, without affecting those resulting in RFC to a similar extent. In fact, Howard has recently reinvestigated the immune status in mice tolerant to pneumococcal polysaccharide and has also found antigen binding cells in situations where the antibody secreting cells are paralysed by very high antigen doses (personal communication).

There are several alternative explanations for our findings. The receptors on the RFC in tolerant animals may be directed to other antigenic determinants than the antibodies secreted by the PFC in immune animals. These determinants may occur in such a low density on the sensitized SRC that antibodies against them do not cause plaques. Tolerant animals may contain cells actually secreting antibodies directed against the same determinants as antibodies producing plaques in immune animals but of such a low affinity[10] that they are detected as RFC but not as PFC. Or tolerance induction could lead to blockage of antibody secretion without affecting division and differentiation of precursor cells for antibody production. These three possibilities are unlikely for reasons which will be fully discussed elsewhere (O. S., unpublished).

A further possibility is that tolerance is caused by the inhibition of division and differentiation of precursor cells for antibody production. The RFC in tolerant animals may in this case be formed by thymus derived lymphocytes or by bone marrow derived lymphocytes which do not give rise to PFC. Recent findings have shown that thymus derived and bone marrow derived lymphocytes cooperate in the antibody response to certain antigens[11]. The lipopolysaccharide antigen of *E. coli* is not dependent on thymus derived lymphocytes to induce a humoral antibody response (Andersson and Möller, personal communication), but this does not necessarily imply that there are no receptors for the antigen among the thymus derived lymphocytes. The independence of thymus derived lymphocytes for an adequate immune response to CPS may be related to the fact that the structure of this antigen is characterized by repeating units of identical determinants. Such an antigen may bind well to bone marrow precursor cells with multivalent receptors and stimulate them to antibody production in the absence of thymus derived lymphocytes. If thymus derived lymphocytes were thus stimulated to respond in tolerant animals, they would appear as RFC but not as PFC, which may explain the discovery of RFC in tolerant animals. Recently, experiments involving isoantigenic markers have revealed that a high proportion of the RFC that appear in mice immune to SRBC are made up of thymus derived lymphocytes[12]. But the percentage inhibition of RFC against CPS after treatment with anti-theta serum were −2 to +8 per cent and −16 to +18 per cent for tolerant and immune animals respectively compared with 20–32 per cent for RFC against SRC (O.S., unpublished). The RFC against CPS are therefore not likely to be thymus derived lymphocytes but rather lymphocytes from bone marrow.

It is possible that a high dose of antigen will only stop division of bone marrow derived lymphocytes with receptors for the antigen above a certain level of affinity. Such cells would give rise to PFC after an optimal immunization dose. Bone marrow derived lymphocytes with receptors of lower affinity would be stimulated to divide but not to produce antibodies after optimal stimulation. In tolerant animals these cells are not prevented from dividing because they escape tolerance induction in consequence of the low affinity of their receptors. Such cells could explain the RFC in tolerant animals. If this alternative is correct it would also imply that a level of affinity, sufficiently high to bind the antigen to the cell surface, is not high enough to induce antibody production.

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Opsonin Depletion after Surgery

PREVIOUS studies have demonstrated a state of decreased host resistance after surgery[1–3]. While the precise pathophysiological causes of this phenomenon are unknown, recent findings suggest that depression of the reticuloendothelial system (RES) may be involved[4,5]. Intravascular phagocytic clearance of foreign colloidal and particulate matter by the RES is highly dependent on the presence of circulating serum or plasma factors called "opsonins"[6–8], so RE depression after surgery may be related to depression of opsonic activity. Indeed, the role of opsonins in resistance

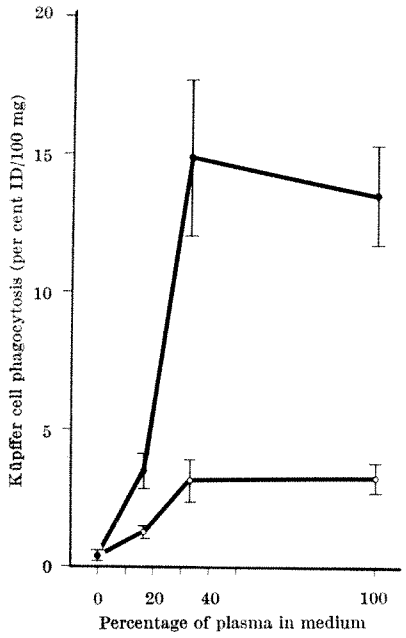


Fig. 1. Hepatic Küpffer cell phagocytosis of test lipid emulsion in normal (●) and 30 min post-surgery plasma (○) as a function of the incubation plasma concentration. Uptake is expressed as the percentage of the injected dose phagocytosed per 100 mg liver (per cent ID/100 mg). Each point represents the mean ± standard error of six to twelve samples.

to infection[9], and the findings of marked RE depression buring periods of plasma opsonin depletion[6,7], suggest that ooth the reduced resistance and the depressed RE activity dbserved after surgery may be related to a functional depression of the opsonic system.

To test this hypothesis, plasma opsonin activity was evaluated in normal rats and in rats at a post-surgery interval (30 min) known to be associated with significant RE depression[4]. Furthermore, because a depression in opsonin activity could reflect either the absence of plasma opsonin or the presence of an inhibitory substance in the blood, the potential inhibitory effect of post-surgery serum on *in vivo* RE activity was evaluated. Non-fasted male Holtzman (Holtzman Co., Madison) rats weighing 250–350 g were used. Serum and plasma (heparinized) were obtained by vena cava puncture with the use of light ether anaesthesia. Plasma opsonic activity was evaluated by an *in vitro* tissue slice technique, in which the comparative ability of normal and post-surgery plasma to stimulate Küpffer cell phagocytosis was determined[6,7,10]. Slices of normal liver were prepared with a Stadie–Riggs tissue slicer and incubated with a ^{131}I-labelled lipid emulsion in 3 ml. of heparinized (50 USP/ml.) incubation medium containing 0, 16·7, 33·3 or 100 per cent plasma. The plasma was diluted with Krebs–Ringer phosphate (*p*H 7·4). All samples were gassed with 95 per cent O_2 and 5 per cent CO_2 and incubated with oscillation at 37° C for 30 min. At the end of the experiment, the liver slices were removed, washed in isotonic saline and analysed for particle uptake as previously described[6,7,10]. Plasma opsonic activity was assessed in terms of the degree of phagocytosis, and Küpffer cell phagocytosis of the colloid was expressed as the percentage of the injected dose phagocytosed per 100 mg tissue (per cent ID/100 mg). The radio-iodinated test emulsion used *in vitro* was a gelatinized ^{131}I "RE test lipid emulsion"[6,7,10]. It was prepared as an anhydrous base with glycerol, ^{131}I-triolein (Mallinckrodt Nuclear, St Louis), and lecithin mixed in a ratio of 10:10:1 by weight, respectively[10,11]. Before use, the lipid base was diluted with a 0·1 per cent gelatine containing 5 per cent dextrose and water solution previously adjusted to *p*H 7·4. In this study, 2,000 μg of the lipid emulsion was added to each incubation flask. Liver slice uptake of the ^{131}I lipid emulsion was determined with an 'Auto-Gamma' crystal scintillation counter (Nuclear–Chicago, DesPlaines).

The effect of normal and post-surgery serum on RE activity was evaluated with a slight modification of the carbon clearance technique[12], with which the rate of colloid clearance from the circulation before and after the intravenous injection of serum was determined. Rats were injected with colloidal carbon (Gunther–Wagner, Hanover, Germany) at a dose of 16 mg/100 g body weight, and the rate of disappearance of carbon from the circulation was determined by spectrophotometric analyses of serial 0·1 ml. aliquots of whole blood[13]. Carbon concentration was plotted semi-logarithmically against time and the $t/2$ for the vascular clearance of the foreign colloid by the RES was determined. From the clearance curve, the "phagocytic index" K was calculated according to the expression

$$K \text{ (phagocytic index)} = \frac{\log C_1 - \log C_2}{T_2 - T_1}$$

where C_1 and C_2 represent the circulating colloid concentration at times T_1 and T_2, respectively. K can also be calculated from the $t/2$ value, according to the expression:

$$K \text{ (phagocytic index)} = \frac{0{\cdot}301}{t/2}$$

where 0·301 is the $\log_{10}$ of 2, and the half-time is in minutes. In the present study, the K for the vascular clearance of a single dose of carbon was determined before (0–7 min post-carbon injection) and after (8–14 min post-carbon injection) the intravenous injection of serum or saline. Saline or serum injection was performed 7 min after carbon injection at a dose of 0·5 ml./100 g body weight, and comparisons between the pre and post-injection K values were made.

The surgery consisted of mid-line laparotomy (5 cm) with brief intestinal manipulation under ether anaesthesia. The incision was closed with silk suture. Control rats were exposed to a comparable ether anaesthesia stress.

Küpffer cell phagocytosis of the lipid emulsion was significantly ($P < 0{\cdot}001$) enhanced by the presence of normal plasma compared with Krebs–Ringer phosphate (Fig. 1). Phagocytosis in a 33·3 per cent normal plasma concentration was 42-fold greater than in the absence of plasma. In contrast to the marked opsonic stimulation of phagocytosis by normal plasma, minimal stimulation of phagocytosis was induced by plasma obtained at 30 min post-surgery. Uptake in 33·3 per cent post-surgery plasma was only 21 per cent of that observed in comparable concentrations of normal plasma.

Table 1. EFFECT OF NORMAL AND POST-SURGERY SERUM ON RATE OF COLLOIDAL CARBON CLEARANCE FROM THE CIRCULATION

Group evaluated	Material injected (intravenous)	Vascular clearance rate Pre-injection (K_1)	Post-injection (K_2)
Control	Saline	0·011 ± 0·008	0·012 ± 0·002
Normal	Serum	0·017 ± 0·002	0·029 ± 0·003
Post-surgery	Serum	0·011 ± 0·002	0·013 ± 0·002

K_1 reflects clearance between 0–7 min after carbon injection, and K_2 reflects clearance between 8–14 min after carbon injection. Saline, normal serum, or 30 min post-surgery serum was injected at 7 min post-carbon injection. Each group represents 3–9 animals. Means ± standard error of the means are presented.

Injection of saline or 30 min post-surgery serum in the course of carbon clearance did not significantly alter the rate of carbon clearance as indicated by the respective pre-injection (K_1) and post-injection (K_2) values for the phagocytic index K (Table 1). By contrast, the injection of normal serum in the course of carbon clearance significantly enhanced ($P < 0{\cdot}05$) the subsequent rate of carbon clearance from the circulation.

These results confirm and extend previous findings on the importance of plasma or serum opsonic activity for Küpffer cell phagocytosis[6,7,10], and show that 30 min after surgery in the rat there is a marked depression in circulating opsonin activity. The failure to alter the rate of carbon clearance by the injection of post-surgery serum in the course of colloid clearance suggests a minor role

for a serum inhibitory substance in the altered phagocytic activity after surgery.

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Metabolic Effects of Polychlorinated Biphenyls in the American Kestrel

POLYCHLORINATED biphenyls (PCB) have been widely found in wildlife samples[1,2]. PCB are used as plasticizers in plastics, resin and chlorinated rubber[3]. They have been recommended for improving lindane residues[4] and have been shown to increase the insecticidal properties of DDT and dieldrin[5]. PCB are highly stable, insoluble in water and soluble in lipids; so that they can persist and undergo biological magnification in the environment.

Sax[6] reported the toxicity of chlorinated biphenyls as moderate to high. One PCB has been shown to produce chick hydropericardial oedema and growth depression proportional to dietary levels (200 and 400 p.p.m.) of the toxicant[7]. These effects were accompanied by such pathological changes as: cream-coloured pancreas, enlarged haemorrhagic kidney, enlarged adrenal gland, small spleen, dermatitis, defeathering and transient changes in blood glucose, haemoglobin and haematocrit levels. Intramuscular injection of PCB has been shown to increase the rate of steroid metabolism in bird liver homogenates[1] as has been shown for organochlorine pesticides[8]. Although a cause and effect relationship has not been demonstrated, PCB residues have been associated with decreasing populations of fish-eating birds and birds of prey[1].

American kestrels were trapped, allowed to acclimatize for a week, paired (one male and one female per cage), acclimatized for another week and then placed on *ad libitum* experimental diets. The kestrels' diet consisted of one day old dead cockerels. The dead cockerels were injected in the breast region at either 0·5 p.p.m. or 5·0 p.p.m. (w/w) of either 'Aroclor 1254' or 'Aroclor 1262' (Monsanto trade names for PCB containing 54 per cent chlorine and 62 per cent chlorine respectively) dissolved in sesame oil. The higher dose is roughly equivalent to 2 mg/kg PCB to each kestrel. Cockerels for control kestrels were injected with sesame oil only. It is assumed that the kestrels consumed essentially all of the dietary 'Aroclor', for the only parts of the cockerel not eaten were the beaks and feet, and the pellets regurgitated by the kestrels consisted of cockerel down. Each pair was kept in a burlap-covered $6 \times 6 \times 8$ foot wire cage and fed experimental diet once a day for 5 months. At the end of the test period, the birds were killed, livers removed, and the microsomal fraction was prepared and incubated as described by Conney and Klutch[9]. Radioactive oestradiol and its metabolites were extracted with dichloromethane and separated by paper chromatography[10] with changes in procedure as reported previously[1,8].

Histological examination was carried out on kestrels fed 5 p.p.m. 'Aroclor 1254' for 14 days. A central portion of the liver was removed immediately after death and fixed in Carnoy's fluid for 45 min. Sections were cut at 5 μm. All sections were treated with deoxyribonuclease (0·1 mg/ml. in 0·1 M phosphate buffer (*p*H 7·5)–0·1 per cent gelatine–0·03 M $MgSO_4$) for 3 h at room temperature to remove DNA which would otherwise stain with azure B. All sections were then stained in azure B (0·1 per cent in 0·1 M citrate buffer, *p*H 4·0) for 2 h at 40° C, washed briefly with water and differentiated overnight with *t*-butyl alcohol[11]. The density of the staining with azure B was measured cytophotometrically at 545 nm (ref. 12). Ten readings of the per cent transmission of a standard area of cytoplasm were made for each of five control and five experimental livers. The mean for control birds was 39·7 ± 3·3 whereas for the experimental birds it was 26·8 ± 3·6. An analysis of variance of both the individual values and the means gave calculated F values which exceeded the 0·005 tabular F; we therefore conclude that the decrease in percentage transmission is a consequence of the presence of more cytoplasmic RNA in the 'Aroclor'-treated kestrels.

Table 1. *In vitro* MICROSOMAL BREAKDOWN OF OESTRADIOL
Amount of polar metabolites formed in nmoles/g microsomal fraction (sample size, 4 for each group)

Diet	Mean	s.d.
Control	0·00	0·00
0·5 p.p.m. 'Aroclor 1254'	13·65	7·35
5·0 p.p.m. 'Aroclor 1254'	60·50	31·20
0·5 p.p.m. 'Aroclor 1262'	19·05	11·50
5·0 p.p.m. 'Aroclor 1262'	47·95	25·45

A dose-dependent *in vitro* breakdown of oestradiol to a more polar metabolite occurred in livers from kestrels fed either 'Aroclor 1254' or 'Aroclor 1262' (Table 1). There was no apparent difference between the effects of the two 'Aroclors' nor was there any difference between sexes. No such conversion took place in the livers from the control birds.

The increase of hepatic enzyme activity can thus be correlated with an increase of cytoplasmic RNA. Proliferation of the endoplasmic reticulum of rat liver cells following the administration of chlordane[13] and DDT[14] has been noted although these changes were not quantified. Our findings suggest the physiological actions of PCB are similar to those of DDT and its metabolites. This industrial contaminant should be considered as an additional burden to the ecosystem comparable with that imposed by the organochlorine pesticides.

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Connectance of Large Dynamic (Cybernetic) Systems: Critical Values for Stability

MANY systems being studied today are dynamic, large and complex: traffic at an airport with 100 planes, slum areas with 10^4 persons or the human brain with 10^{10} neurones. In such systems, stability is of central importance, for instability usually appears as a self-generating catastrophe. Unfortunately, present theoretical knowledge of stability in large systems is meagre: the work described here was intended to add to it.

Most of these large systems, often biological or social, are grossly non-linear, which increases the difficulties associated with them. Here we consider linear systems merely as a first step towards a more general treatment.

We have attempted to answer: What is the chance that a large system will be stable? If a large system is assembled (connected) at random, or has grown haphazardly, should we expect it to be stable or unstable? And how does the expectation change as n, the number of variables, tends to infinity?

Monte Carlo-type evidence[1,2] had suggested that the probability of stability decreased rapidly as n was increased, in some cases perhaps as fast as 2^{-n}, an exponentially-fast vanishing of the chance that the system will be stable. This result, however, was for systems that were fully connected, where every variable had an immediate effect on every other variable. While this case is obviously important in theory, it is not the case in most large systems in real life: not every person in a slum has an immediate effect on every other person, and not every cell in the brain directly affects every other cell. The amount of connectedness ("connectance") is often far below 100 per cent. We have studied how such incomplete connectance affects the probability of a system's stability.

Let the linear system's state be represented by the vector $\mathbf{x}$ ($= <\mathbf{x}_1, \ldots, \mathbf{x}_n>$, where each $\mathbf{x}_i$ is a variable, a function of time), and its changes in time by the matrix equation

$$\dot{\mathbf{x}} = A\mathbf{x}$$

To "join the variables at random" is to give the elements in A values taken from some specified distribution. "Non-connexion from $\mathbf{x}_i$ to $\mathbf{x}_j$" corresponds to giving the element a_{ji} the value zero. Thus, if the specified distribution has a peak at zero, sampling from it will give the equivalent of a dynamic system with many non-connexions. The connectance, C, of the system can then be conveniently defined as the percentage of non-zero values in the distribution. Thus, if the coefficients are drawn from a distribution with 99 per cent zeros, and if $n = 1{,}000$, then each line of the equation would contain about ten non-zero coefficients, corresponding to a system in which each variable is directly affected by about ten other variables.

Because our work was essentially exploratory, we used the distribution in which the non-zero elements were distributed evenly between $-1{\cdot}0$ and $+1{\cdot}0$. The elements in the main diagonal, corresponding to the intrinsic stabilities of the parts, were all negative, distributed evenly between $-1{\cdot}0$ and $-0{\cdot}1$. Thus each sampled value of A corresponded to a system of individually stable parts, connected so that each part was affected directly by about C per cent of the other parts.

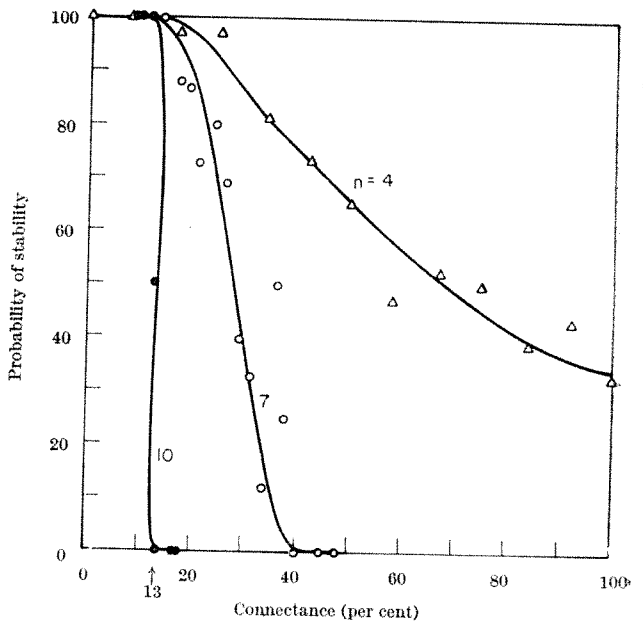


Fig. 1. Variation of stability with connectance.

On a digital computer, a value for n was given and a value for C. Random numbers appropriately distributed were then sampled to provide a matrix A. Hurwitz's criterion was applied to test whether the real parts of A's latent roots were all negative (the stable case) and the result recorded. Further samples, giving further As, allowed the probability of stability (P) to be estimated. The probability was then re-estimated for another value of C, and so on, until the variation of P with C became clear.

The results showed the feature that we wish to report here. As the system was made larger, a new simplicity appeared. Fig. 1 shows a selection of the results, enough to illustrate the principal fact.

When $n = 4$, the probability that the system would be stable depended on C in a somewhat complex curve (which could perhaps be predicted exactly). But as n increases, the curve changes shape rapidly towards a step-function, so that even when n is only 10, the shape might be so regarded, at least for some practical purposes. Thus, even at $n = 10$, questions of stability can be answered simply by asking whether the connectance is above or below 13 per cent: 2 per cent deviation either way being sufficient to convert the answer from "almost certainly stable" to "almost certainly unstable".

The matter is being investigated further, but it may be of general interest to notice that this work suggests that all large complex dynamic systems may be expected to show the property of being stable up to a critical level of connectance, and then, as the connectance increases, to go suddenly unstable.

This work was supported in part by the US Office of Scientific Research.

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[1] Ashby, W. R., *Design for a Brain* (Chapman and Hall, London, 1952).
[2] Gardner, M. R., *Critical Degenerateness in Linear Systems*, Tech. Rep. No. 5.8 (Biological Computer Laboratory, University of Illinois, Urbana, Illinois 61801, 1968).

Book Reviews

PERSPECTIVES OF POPULATION

Population, Resources, Environment

Issues in Human Ecology. By Paul R. Ehrlich and Anne H. Ehrlich. (Series of Books in Biology.) Pp. 383. (Freeman: San Francisco and Reading, September 1970.) 84*s*.

Life Without Birth

A Journey Through the Third World in Search of the Population Explosion. By Stanley Johnson. Pp. xxii + 364 + 24 photographs. (Heinemann: London, September 1970.) 50*s*.

In spite of the objective evidence of an increase in the expectation of life from about twenty-five years in 1750 to about sixty years in 1970, the controversy over the possibility of improving the human condition continues to rage. *Population, Resources, Environment* is a notable contribution in the tradition of Malthusian pessimism. This beautifully produced book, appropriately swathed in a jet-black dust-cover, is a catalogue of apparently insuperable obstacles strewing the paths to further progress in the directions in which man's endeavours are leading. Although each individual extrapolation is suitably qualified by reference to ways in which the obstacles might be circumnavigated, the pall of gloom gathers deeper over the reader the further he advances.

There is a great deal of well arranged basic information on demography, ecology and food technology which is neutrally reported and attractively presented. This part of the work is useful textbook material at the sixth-form level. It is in assessing the significance of current trends and projecting these into the future that the authors' judgment becomes most obtrusive and at the same time conjectural.

The total available resources of the Earth do not suffice to develop duplicates of industrial societies all over the globe, even for the current level of population. Fuel, dozens of metals, productive land, marine resources are all inadequate and the authors document their case with care and ingenuity, interleaving the text with "boxes" containing capsulated case-histories and elementary lessons in fundamentals such as the law of natural selection, thermodynamics and the chemistry of herbicides. Many pollution hazards are detailed. Some of these hazards such as residual insecticides are grave threats to the ecological balance of life on Earth; other hazards described are speculative, of local significance only or are highly fanciful. In their anxiety to document the case against "growth mania", the Ehrlichs fail to make appropriate distinctions of this kind.

The section on family planning and population control suffers most severely from lack of balance. A table (p. 242), purporting to show the progress achieved by population programmes by 1969 measured against the targets set, conveys an impression of total failure, but all the figures quoted for 1969 are in fact from earlier years—in some cases for years before the programme being assessed started operation. Instead of a careful appraisal of the problems and principles involved in the task of providing family planning services to total populations of agricultural societies and of inducing them to make use of them early in the process of family formation, the Ehrlichs devote the bulk of the space available to a consideration of dramatic and quite impractical strategies, some of which belong in the pages of the more lurid science fiction magazines. Indeed, valuable and comprehensive as this book is, it consistently fails to achieve a detached and balanced view of hazards and remedies because of the over-developed sense of doom and drama which pervades it. As if the limitations of the Earth and the byproducts of man's struggle to master it were not alarming enough, the authors throw in for good measure the possibilities of political and natural catastrophe which the future may hold—nuclear and biological warfare on the one hand, world inundation when Antarctica slumps into the sea or the Arctic ice-cap melts on the other. At the end of the book, the Ehrlichs call for de-development of the industrial countries. They may be right that this is the only course that will avert world disaster, but it seems the least feasible of all.

I approached Stanley Johnson's book with antipathy. After flicking through the pages I concluded that this was a dilettante travelogue loosely linked with human interest anecdotes of the hardships of population growth and as meandering as the author's journey. I left it with the conviction that it is by far the best non-technical book on the current population crisis yet available. Mr Johnson is not a specialist in any aspect of population study, but he is a specialist in observation, in insight and in communication.

Life Without Birth is indeed a travelogue, documenting the population scene in twelve countries (including Red China whose Tibetan profile the author glimpsed against the sky from Nepal). Each short chapter extracts what is relevant from the history and political alignment of the country and knits it together with population problems policies and programmes through conversations with officials and academics and the kaleidoscope of impressions that impinge on the traveller and which Stanley Johnson so unerringly dissects into its components.

The book has an air of innocence about it which is quite unusual. The author seems to have approached his task with no preconceptions and, if his repeated modest disclaimers are to be accepted, no relevant technical knowledge. If this approach has led to a somewhat uncritical acceptance of much that he was told by officials and advisers with their own preconceptions to advance or interests to guard, it has nonetheless resulted in a series of remarkably clear and comprehensive pictures of the past, present and future of population growth and policy in the countries visited. Although it is packed with factual information this never becomes overwhelming because it is placed in its logical setting and becomes part of an absorbing story. Whether it is a device or the very truth, the author's technical innocence enables him to take the reader's hand and lead him through the task of understanding the elementary demographic theory without which no population story can be told.

The contents of the book defy summary, for it has no didactic purpose and no capsulated messages. It is for those who like to form opinions on the basis of fact rather than slogans. It is thus an old-fashioned book and it is a surprise to find its author is only 29 years old. It is also a well written book, rising to the level of the genuinely epigrammatic time and again, and it is a courageous book, mincing no words in castigating Pope Paul VI for Humanea Vitae, or Westminster (perhaps a little unfairly) for the lack of official support for Hong Kong's family planning work.

Although written for a lay readership, *Life Without Birth* should be read by every "population expert" and "multidisciplinary family planning worker" in the world, for it has the three-dimensional quality which comes from the correct use of perspective.

D. Wolfers

NEW SCHOOL CHEMISTRY

Nuffield Advanced Science

Chemistry. (Penguin: Harmondsworth, Middlesex, 1970. Published for the Nuffield Foundation.)

Students' Book 1: Topics 1 to 12. Pp. 410. 30*s*.

Teachers' Guide 1: Topics 1 to 12. Pp. 342. 30*s*.

Ion Exchange: A Special Study. Pp. ix+109. 13*s*.

THESE books are the first to be published of a complete set which will cover the requirements for Nuffield A-level chemistry. The remainder will appear in the near future: a second *Students' Book* and *Teachers' Guide*, a *Book of Data*, four more *Special Studies* (biochemistry, chemical engineering, food science, and metallurgy), experiment sheets, and some programmed texts.

The content of the books is excellent. I regret that I cannot praise them without some not-so-faint damns. The first criticism is the perennial one of cost. The explanatory notes for the new London A-level chemistry syllabus, for example, contain frequent reference to the Nuffield course; and there is no doubt that the *Students' Books* would serve very well as texts for students coping with the new courses. But to equip my present sixth-form chemists, as I would like to, with the two *Students' Books* alone would (assuming that the *Students' Book 2* will be the same price as *Students' Book 1*) cost about £250; which is two-thirds of my normal annual departmental allocation for all books, chemicals, and everyday apparatus. And the binding and appearance of these books do not make me confident of their ability to resist constant usage even for just two years.

Only two of the project team responsible for this course remain as full-time teachers; and rather too many of the teachers and former teachers in the team came from well-favoured independent and direct-grant schools. The team's collective view of what is at present possible for the average teacher in the average school with average facilities and technical help and on an average budget may be too rosy. One eventual result, however, could be the establishment of a norm for the provision of resources for chemistry teaching which determined teachers can quote when arguing with unhelpful local education authorities.

All teachers will recognize among their pupils the existence of two extreme types: the clever idler and the apparently slow-witted but conscientious slogger. The one redeeming feature of the old syllabuses was that their factual and descriptive content gave scope for the hard worker to pass the eventual exam, and sometimes to pass well. From the objective point of view I am not suggesting that this feature was necessarily a good thing; but, speaking subjectively, I am all for rewarding hard work and character, and I fear that the slow but steady worker will have little luck with the more conceptually intense Nuffield-type courses. The possibility must be faced that there may be fewer A-level entrants in chemistry although these should have a better understanding of the subject.

These criticisms—if criticisms they are—are no fault of the project team. But the team can, of course, be held responsible for the uniformly high quality of the material in these books. The *Students' Book 1* is clearly written and easy to read. Each topic contains instructions for experimental work, discussion of underlying theory, passages of background reading incorporated into the text, and problems. Nuffield courses undergo exhaustive testing before publication; nonetheless, I have tried to carry out my own validation. Since the beginning of term, I have been building up ideas about ionization energies, energy levels, and so forth with my first-year sixth form. I have deliberately stuck to the Nuffield text on these topics. Impressions on both sides are excellent. The *Teachers' Guide* is a necessity, particularly for those teachers to whom much of the material (and some of the chemistry!) will be new. Each section of each topic is set out thus: objective, timing (somewhat optimistic in places), suggested treatment, supporting material and suggestions for homework, and answers to problems in the *Students' Book*. There are lists of materials and apparatus required for the experimental work. Much extra theory is included for the benefit of the teacher. *Ion Exchange* is one of the five *Special Studies*, one of which has to be selected by each student as part of his course. The book makes an excellent introduction to the subject, with particular and welcome emphasis on the applications of ion exchange in industry, medicine, and agriculture.

In his foreword to each book Brian Young writes that "the need is for courses which are satisfying and intellectually exciting in themselves—not for courses which are simply passports to further study". In the hands of capable teachers and reasonably well-motivated pupils, these books should certainly increase the volume of satisfaction and excitement associated with any A-level course in chemistry.

MARTYN BERRY

INTEGRATED SOCIAL SCIENCE

Understanding Society

Readings in the Social Sciences. Edited by the Social Science Foundation Course Team, the Open University. Pp. xx+712. (Macmillan: London, October 1970. Published for the Open University.) 50*s* boards; 20*s* paper.

IT has generally been an American rather than a British tradition to produce volumes of readings in the social sciences for undergraduates. The British tradition has been for the undergraduates to look up the relevant articles for themselves in the library and to be guided by their tutors, but in the American system of mass lecturing, with comparatively heavy reading loads for the undergraduates, the practice of issuing prepared volumes of readings has been usual. The American practice has also been useful to people who teach in English universities and colleges, because, in fact, it seems that only a minority of students actually do the looking up for themselves which they are expected and intended to do.

The Open University, dealing with part time students scattered over the whole of the country, usually only with access to the local public libraries, manifestly cannot expect people to browse among the back runs of learned journals, and they have produced a volume of readings for the Social Sciences Foundation Course. This volume of readings is based on two principles. The first is that it contains, on the whole, the results of original thought or research by fairly distinguished people in a number of fields, including social anthropology, geography, economics and sociology. The second is that this is intended to be seen by the student and by his tutors as an integrated approach to the study of the problems of society.

The individual articles in this volume are sometimes excerpts from books, sometimes articles complete in themselves and obviously some disciplines are better represented than others. Nevertheless, it is a well chosen and interesting series of essays which suggests to the reader and especially, one hopes, to the careful student, how specialists in a particular subject set about their work. Integrated courses in the social sciences have become popular in recent years, principally because of the holistic philosophy that some teachers have had, that, because man and mankind are one, the study of mankind also needs to be unified. There is an obvious fallacy here, for any disciplined approach to the study of mankind or of nature must entail dealing with small pieces of things in detail as well as larger and more embracing general philosophies. The result of a great many integrated courses seems to

be a smattering of knowledge in a great many fields and a tendency to dismiss with obloquy those disciplines which are particularly difficult to study. It is, I think, not an accident that the natural sciences have always been extremely cautious in choosing this approach to their own disciplines.

The great lack in this volume is of any mathematical and statistical approach to the study of society. The great advances in economics have come about largely through the applications of mathematical techniques, and this is equally true of other disciplines. There is in this volume a lack of model building illustrated as a technique in the social sciences, and an implicit dismissal of the understanding of statistical procedures. In this sense the bias of the volume is probably understandable but may be unfortunate. Nevertheless, with this one grave lack, the volume may be commended as an interesting guide for the interested and ignorant student who nevertheless wishes to undertake some work which is both stimulating and fairly rigorous. It resembles the great works of H. G. Wells and his collaborators on popularizing the social and physical sciences and mathematics, and for that reason it has a splendidly nostalgic flavour.

JOHN VAIZEY

SYMMETRY AND PARTICLES

Unitary Symmetry and Elementary Particles

By D. B. Lichtenberg. Pp. xiii + 239. (Academic: New York and London, May 1970.) 121*s*.

THIS book is definitely mathematical rather than physical, and possesses both the strengths and weaknesses of such an approach. On the one hand, the mathematics is fully (and relatively simply) explained, starting from the fundamental axioms concerning groups. Group representations in vector spaces are considered, first for Lie groups in general, then for SU(n) and finally for SU(3). The eight-fold way itself first appears on page 152, and departs on page 183. (SU(6) and quarks occupy the rest of the book.)

On the other hand, there is a kind of mismatch between this mathematics and the physics it is supposed to describe. For example, the machinery for reducing an arbitrary product representation in SU(3) is irrelevant, because only singlets, octets and decuplets actually exist. Again, SU(6) does not actually apply to any physical object (except for SU(6)w which is not treated). And, of course, quarks, whether or not they exist, can hardly have the simple dynamics required in many applications of the quark model.

This mismatch is, of course, partly a feature of the subject, for which the author is not entirely responsible. The book is well written and may be useful as a reference book; but at the price it is hardly a good buy for the individual.

D. H. LYTH

PHYSICS OF COMMUNICATION

The Physics of Transmission Lines at High and Very High Frequencies

Vol. 1: Primary and Secondary Parameters, Travelling Waves, Pulses. By P. Grivet. Pp. xiii + 451. (Academic: London and New York, June 1970.) 150*s*.

A COMMUNICATION transmission line is often regarded as an important element of a communication system but a rather dull subject for a 450-page book. Professor Grivet destroys this view by providing an excellent treatment of the physical principles of the subject within a frequency band which embraces telephone lines and microstrip. The text is based on lectures delivered by the author in Paris and Orsay and, as such, is unlikely to fit the structure of a physics or electrical engineering degree course in Britain. Nevertheless, any lecturer planning a course in this field would do well to consult this book.

A successful attempt has been made to harmonize the field theory and circuit theory approaches to the subject. The calculation of capacitance, inductance and resistance is considered in some detail and 60 pages are devoted to methods involving the complex plane. The chapter on the quasi monochromatic wave includes a clear explanation of group velocity and the adequate discussion of delay equalization is typical of the extent to which the author has used practical systems to illustrate the theory. In this respect the text will also appeal to those telephone engineers who seek a perspective view of their subject, for the author has included an adequate historical background to the development of current practice. Pulsed operation of lines is discussed in some detail but the chief omission is an extensive treatment of matching techniques. This in part reflects a cut-off in the treatment at VHF.

The translation by Dr P. W. Hawkes seems uniformly excellent and the author's clear style of expression has been captured. The text can be unreservedly recommended and I await with interest further volumes in the series.

P. J. B. CLARRICOATS

AURORAL SUBSTORMS

Polar and Magnetospheric Substorms

By Suyn-Ichi Akasofu. (Astrophysics and Space Science Library.) Pp. xviii + 280. (Reidel: Dordrecht, 1968.) 55 florins.

THIS is an excellent reference book for research workers in auroral and magnetospheric physics, and good general reading for others interested in knowing of recent progress in the study of that fascinating and beautiful natural phenomenon, the aurora.

Over a hundred years ago it was realized that there was a connexion between strong auroral displays observed occasionally from well inhabited parts of the globe, "magnetic storms" (which occur on a world-wide scale on such occasions) and large sunspot groups and flares on the Sun's surface. It is now known that around the auroral oval (a narrow belt a few degrees of latitude wide near 70° magnetic latitude, with its centre displaced about 3° from the Earth's dipole pole toward the dark hemisphere) auroral displays occur practically every night, but the magnetic disturbances accompanying them are localized (hence the term polar substorm). Polar substorms occur with greatest frequency and intensity during world-wide magnetic disturbances.

Professor Akasofu pioneered the analysis of hundreds of all sky camera photographs of the aurora taken from many different places during the International Geophysical Year, and built up a large-scale picture of the sequence of events (as observed in the visible light portion of the electromagnetic spectrum) which occur during a polar substorm around the auroral oval. He showed that auroral substorms usually last an hour or so and occur every few hours, with maximum activity centred at those stations close to local midnight. He showed that different types of activity, and the direction of motion of the auroral forms observed at any one station, depend both on the local time of observation and on the phase of the substorm. It is now recognized that many other ionospheric and magnetospheric phenomena exhibit characteristic behaviour dependent on local time in and near the auroral oval or on magnetic field lines leading into it during polar substorms.

In his book Professor Akasofu summarizes and gives detailed references to observations both from the ground and from rockets and satellites of the sequence of events

which occurs during polar substorms in various wavelength ranges of the electromagnetic spectrum, and with trapped and precipitating charged particles, taking a chapter to describe each different group of experimental observations. In his last chapter he gives an account of several different theories which have been advanced to explain the nature and origin of polar substorms. It is generally accepted that the substorm energy derives from the solar wind, but no theory advanced so far has produced an explanation for the way in which solar wind energy is introduced into the magnetosphere. He concludes by pointing out that the large, and as yet unsolved, problem in this field is to determine the nature and causes of the magnetospheric electric fields responsible for the polar substorms.

PAMELA ROTHWELL

UPPER ATMOSPHERES

Chemistry of the Ionosphere

By A. D. Danilov. Translated from the Russian. (Monographs in Geoscience.) Pp. xv+296. (Plenum: New York and London, 1970.) $22.50; 210*s*.

IN the past decade the development of space research techniques, coupled with the organization of world-wide cooperative studies in solar-terrestrial physics, have resulted in a vast accumulation of new detailed experimental data on the Earth's upper atmosphere. The interpretation of these data in terms of basic physical and chemical processes is a major task of current interest. This volume, in the words of the author, "is an effort to present an integrated review of information on the most important aspects of the chemistry of the upper atmosphere, covering both laboratory investigations of the pertinent chemical processes and studies for clarifying the role and rate of these processes in the atmosphere". The term "upper atmosphere" is here used to denote the height range 60 to approximately 800 km and throughout the book the emphasis is on the reactions involving charged particles. The principal topics covered include a summary of current data on atmospheric density, temperature, composition, electron and ion concentrations; elementary photochemical processes in the ionosphere; dissociative recombination, ion-molecular processes, the role of negative ions, the dissociation of oxygen and of nitrogen, reactions involving oxygen and nitrogen, reactions involving neutral and ionized hydrogen and helium atoms.

No consideration is given to the airglow, nor to certain other related aspects of the chemistry of the ionosphere such as rocket experiments on the high altitude release of chemical reagents. The justification for such omissions is that in the case of the airglow, the subject is large enough to merit a separate volume and the results of chemical releases are not discussed, because it is not considered that they have as yet added much "of importance for the study of basic problems in ionospheric chemistry".

The original Russian text was prepared in 1965, and it is recognized that in the past few years certain new theoretical and experimental studies have been published which bear on some of the problems discussed in the book. In a brief appendix the author summarizes some of the more important relevant papers published recently, and, in particular, some attention is given to the complex pattern of chemical reactions now emerging from rocket studies of the D-region.

The book is eminently readable and is provided with a very useful selected bibliography covering a total of nearly 600 references. The translation and production are both excellent and the volume will be invaluable for ionospheric workers requiring a concise, authoritative text.

W. J. G. BEYNON

Film Review

KALEIDOSCOPE OF MAN

The Body

Produced by Tony Garnett. Directed by Roy Battersby. (112 min.) At the Classic, Piccadilly, London.

IN its way, the film *The Body*, produced by Tony Garnett and directed by Roy Battersby, should be a courageous and major undertaking. In recent years very few producers have been able to make for the commercial cinema feature-length, semi-documentary films which do not use name stars. Unfortunately, compared with our human frames whose working it depicts, *The Body* is slightly less than the sum of its parts, although it does make a compelling, if contentious, 1 hour 40 minutes of cinema.

The film, at least those parts of it dealing with the body corporeal, is loosely based with credit on Mr Anthony Smith's recently published and well received book. However, parts of the film deal with the body politic and are merely tangentially polemic comments devoted to the thesis that we are all brothers under the skin. These parts of the film are loosely based without credit on books by Marcuse, Fanon, Reich, Laing, Marx, Engels and Trotsky. In these sections the film is tediously obvious and unsatisfying.

The hero of the film is indeed the human body in all its workings. Mr Tony Imi's outstanding and magnificent photography illustrates and explores brilliantly and sensuously the nature of our physical world. Under his lens, nipples look like volcanic peaks; the curves of the abdomen take on the appearance of a countryside; the hairs of the hand become like a forest of trees. The body beautiful, the body loving, the body eating, the body excreting, the body moving and the body working. To support all these functions stunning X-ray, endoscopy and schlieren techniques are triumphantly employed.

As such there is no story, only a collection of sequences whose internal order is difficult to divine. These excursions into the corpus mysterium are generated on a wave of neo-paganism, which in turn is carefully nurtured and based in a neo-Eden. Here bodies beautiful, leavened with bodies decrepit, discard clothing in carefully contrived and lengthily considered innocence. The attitudes in this new Eden of these noble (strictly working-class, jam butty) savages can be described as a cross between the Esalen institutes of California and a progressive Workers' Educational Association gymnastics class. Within this studio paradise our lads and lasses indulge in the innocent and not so innocent tactile exploration of each other, leading from sequence to sequence and situation to situation.

Mens sana in corpore sano. Don't you believe it! Every Eden has its serpent! In this case it is the exploitation of our wonderful bodies as wage slaves or the smothering of this magnificent, moving, loving, laughing (not to mention breathing, copulating, energy balancing) human apparatus, in the pollution engendered by our ravishing of our environment.

Thus the opportunity is provided for Miss Vanessa Redgrave and Mr Adrian Mitchell to bare their souls on the soundtrack while others bare their breasts for the picture. They glumly remind us of the doom about to overtake our fragile remains or the dreariness of our production line existence.

Within this framework are some outstanding sequences:

- The clever graphic demonstration of the tons of food eaten by the average Western man in his lifetime, contrasted with the average Indian peasant's consumption.
- A moving and wonderful birth sequence. The midwife, like Juliette's nurse, fussing over mother and baby with a humanity that shares the event with the world, trans-

cending the voyeurism implicit in eavesdropping on such an intimate moment in a married couple's life.

- A funny, brilliant pastiche of a baby crawling over glass. Every hand print and foot print lights up as it walks—it sounds simple, but the camera work and lighting make it a magical moment.
- A magnificent introductory tracking shot of bodies (some 98 of them I believe) ranging from the very young to the aged and infirm.
- Richard Gregory's optical illusions are beautifully demonstrated, as well as some of J. Tanner's film on growth.

There are also some boring sequences. A demonstration of foreplay (some in schlieren no less!) and sexual intercourse is as lengthily tedious as it is unnecessary. Whilst scrupulously satisfying edicts of the British Board of Film Censors, it seems designed more to titillate than reveal. It seems to be included, along with other sequences, to meet some film distributor's estimate of the audience's taste rather than being concerned with the needs and integrity of the subject.

This is, alas, the essence of the film. Too often while pretending to be "vulgar" in the original and honourable sense of the word, the element of voyeurism implicit in the producing of today's "flesh" movies makes the film "vulgar" with all that the modern meaning implies. It thus mars what otherwise might have been an outstanding film.

"Birth, copulation and death,
I should be bored,
I should be bored."

So far as this film is concerned, T. S. Eliot just misses hitting the nail on the head, but he is not far off the mark.

AUBREY SINGER

Correspondence

Soviet Restrictions

SIR,—I am glad to see from Jukes's letter (*Nature*, **228**, 589; 1970) that I am not the only one who thinks the letter from Bikerman (*Nature*, **228**, 297; 1970) extraordinary. To compare his own failure to obtain a travel grant with the trials of Medvedev is an insult to a very courageous man. No one is presumably preventing Bikerman from leaving the USA, whereas, to judge from Medvedev's testimony, the attitude of the Soviet authorities borders on the psychotic. On balance, it is Bikerman's letter, not Medvedev's book, which is "subtle communist propaganda".

Cahn's subsequent point (*Nature*, **228**, 485; 1970) about the non-arrival of Russian speakers at meetings is unfortunately only too common. He might have added that, when they do turn up, they have often been allocated barely two pieces of hard currency to rub together. The consequences of this can be extremely embarrassing.

Yours faithfully,

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Science Citation Index

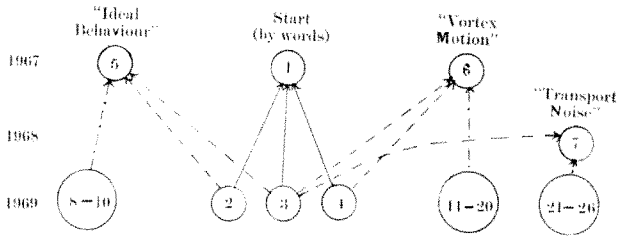
SIR,—Following E. Garfield's article[1] about heavily cited authors, several apparent shortcomings of the *Science Citation Index* (*SCI*) have been pointed out[2–4]. I could list many other reasons why the *SCI* apparently should not work, and why the "most cited author" list may be misleading.

The reason why the *SCI* does work and the list is valuable becomes clear if we talk about probabilities rather than certainties. Thorough investigations[5] of citation practices show that various apparent shortcomings do not normally affect the system; they are low probability events or possibilities—in contrast to the high probability that a well cited article reflects a beneficial progressive impact.

Hopefully, the system will be used with discrimination, unlike the usage described by Croom[4]. Hopefully also, the assessment of an author by citation counts will be qualified by a consideration of the activity in his subject area, nature of the research front, and so forth—in conjunction with any other facts or opinions that may assist in the assessment. I think we may assume that Garfield's remark: ". . . it is up to the scientific community to prevent abuse of the *SCI* . . ." means that the *SCI*, author lists and such like, should be applied with reasonable intelligence.

A reader of the top of the league table will doubtless reflect on the nature of the author's impact—whether it be inventive, original, interpretive or methodological. Moreover, if our author is cited consistently, year after year, perhaps long after he published, it may be concluded that his impact endures.

The set of subjects symbolized in the references of an article is usually associated with the subjects, concepts, methods, apparatus and so on, described in the text (there are also occasional excesses of undeserved self-citing and bibliographic mutual back-scratching).



A, Locate starting article by words in the *Permuterm Index*: 1.
B, Enter *Citation Index* to locate citing articles. Examine references keyed to specific aspects of the subject in the text: →, 2, 3, 4.
C, Select references symbolizing those aspects: — — →, 5, 6, 7.
D, Enter the *Citation Index* to locate articles, — · → 8–10, 11–20, 21–26, which cite the "subjects" 5, 6, 7 respectively.

Fig. 1. Identification of subject aspects—"The physics of superconductors".

Fig. 1 illustrates the citation connexions between articles about "The physics of super-conductors". Articles 5, 6 and 7 were rather easily identified with certain aspects of the subject when using the *SCI* without subject expertise. Articles citing them relate to similar aspects.

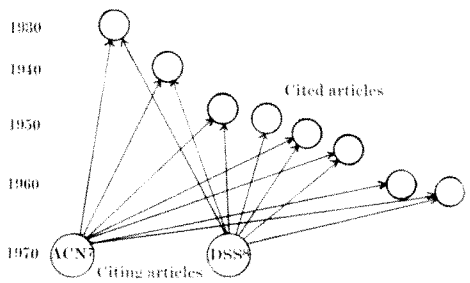
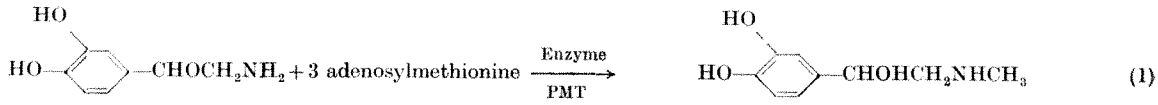


Fig. 2. Similarity of articles indicated by "bibliographic coupling". Two articles, each containing eight references, have six of them in common.



(1)

Norepinephrine (noradrenaline) Epinephrine (adrenaline)

An artefact called "bibliographic coupling"[6] was used when considering this subject. An extreme example of it recently occurred in *Nature*, when two articles[7,8] (published independently) contained virtually the same set of references (Fig. 2). A study of such diagrams helps to identify subject similarity, particularly when titles are inexplicit, and the articles appear in journals with apparently quite different disciplinary coverage (quite a common occurrence).

Quite complex concepts may be conveniently symbolized by a reference. Thus the enzyme assay shown in equation (1) is symbolized by:

Saelens, J. K., *et al.*, *Biochem. Pharm.*, **16**, 1043 (1967)

which leads to:

Nikodije, B., *et al.*, *Biochem. Pharm.*, **18**, 1577 (1969). "Catechol-o-methyltransferase 1. An enzymatic assay for cardiac norepinephrine" (a 1969 article which cites Saelens).

Because only 0·005 per cent of authors are in the exalted category mentioned by Garfield, and because the outcome of the average *SCI* search is usually based on the works of the remaining 99·995 per cent, one look-up in the *SCI* usually locates a small number of relevant articles, together with an even smaller amount of "noise"[9]. This is one of the main virtues of the system.

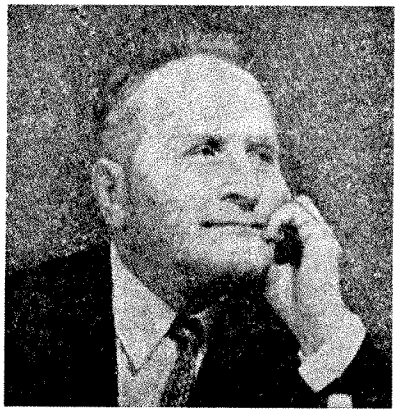
Yours faithfully,

A. E. CAWKELL

ISI European Branch,
132 High Street,
Uxbridge,
Middlesex.

[1] Garfield, E., *Nature*, **227**, 669 (1970).
[2] Comfort, A., *Nature*, **227**, 1069 (1970).
[3] Oliver, P. T. P., *Nature*, **227**, 870 (1970).
[4] Croom, D. L., *Nature*, **227**, 1173 (1970).
[5] Cawkell, A. E., *J. Doc.*, **24**, 299 (1968).
[6] Kessler, M. M., *Inform. Stor. Retr.*, **1**, 769 (1963).
[7] Neville, A. C., *Nature*, **225**, 199 (1970).
[8] Smith, D. S., *Nature*, **225**, 199 (1970).
[9] Barlup, J., *Bull. Med. Libr. Ass.*, **57**, 260 (1969).

Obituaries



Professor David Al'bertovich Frank-Kamenetskii

ON July 2, 1970, Professor David Al'bertovich Frank-Kamenetskii, one of the leading scientists and science journalists of the Soviet Union, died suddenly in his fifty-ninth year.

David Frank-Kamenetskii was born in Vil'nyus in 1910, the son of a chemical engineer who later became a professor at the University of Irkutsk. He graduated in the department of non-ferrous metallurgy of the Tomsk Technological Institute and after working for a period in a factory and a period as a teacher in a technical college he was invited by Academician N. N. Semenov to go to the Institute of Chemical Physics of the Academy of Sciences of the USSR in Leningrad, where in 1938 he presented his thesis for the degree of candidate of chemical sciences. In 1943 he became a doctor of physical and mathematical sciences, and became head of the department of technical chemistry in Gor'kii State University. From 1956 onwards, he worked in the Kurchatov Institute of Atomic Energy and at the same time founded and directed the Department of Plasma Physics in the Moscow Institute of Physics and Technology.

Frank-Kamenetskii was known for his basic research in a number of fields, including the theory of combustion and chemical kinetics, exothermic reactions, astrophysics and plasma physics; in the last field he introduced the concept of magnetosonic resonance. His works include *Diffusion and Heat-Transfer in Chemical Kinetics*, *Oxidation of Nitrogen in Combustion and Explosions*, *Physical Processes within Stars* and also a number of popular works on physics and astrophysics. During recent years he was deputy chief editor of the popular science magazine *Priroda*.

For his own research work, and for his achievements in the popularization of science, he was awarded a number of prizes and honours, including the Order of Lenin, the Order of the Red Banner of Labour and three State Prizes of the Soviet Union.

Academician S. S. Medvedev

THE death occurred on August 13, 1970, of Academician Sergei Sergeevich Medvedev, one of the most renowned Russian specialists in the physics and chemistry of high molecular compounds. He was in his eightieth year.

Sergei Medvedev was born in Moscow in 1891. After graduating from Moscow University in 1918, he played an active part in the organization of the Central Asian University of Tashkent and became head of the Central Chemical Laboratory of the Uzbek Council of Agriculture. In 1922 he founded the laboratory of oxidation and polymerization processes in the L. Ya. Karpov Physical Chemistry Institute in Moscow, holding the post of head of the laboratory until the end of his life. From 1938 onwards, he was an instructor at the Moscow Institute of Fine Chemical Technology. He became a corresponding member of the Academy of Sciences of the USSR in 1938, and was elected to full membership of the Academy in 1958.

Medvedev's research dealt with many pure and applied aspects of polymerization, including the production of synthetic rubber, the effect of nuclear radiation on polymerization and the production of synthetic textiles.

He was the first to give an experimental demonstration of the presence and role of free radicals in the polymerization process, and discovered a number of laws governing the course of polymerization.

His publications include *Research on the Laws of Emulsion Polymerization* (1963) and *Obtaining Syndiotactic Polymethylmethacrylate in Water Emulsions* (1966). He was awarded a number of orders and prizes for his work, including the Stalin Prize (1946), the Order of Lenin and the Order of the Red Banner of Labour.

Announcements

University News

Dr Douglas Bond has been appointed to the chair of civil engineering in the **Queen's University of Belfast** and **Dr Adrian Roberts** has been appointed to the chair of engineering mathematics.

Professor J. H. Kellgren, director of the Rheumatism Research Centre and professor of rheumatology, has been appointed dean (part-time) of the Medical School, **University of Manchester.**

Dr H. O. Foulkes, Department of Pure Mathematics, and **Dr C. Grey Morgan,** Department of Physics, have been appointed to personal chairs in the **University College of Swansea.**

ERRATUM. In the article "Variation of the Luminescence Lifetime of a Molecule near an Interface between Differently Polarizable Dielectrics" by K.-H. Tews, O. Inacker and H. Kuhn (*Nature*, **228**, 276; 1970), on p. 277, column 1, line 11 from bottom, ref. 5 should be replaced by ref. 6; on line 2 from bottom, ref. 2 should be replaced by refs. 2 and 3. Equation (5) should read

$$f(x) = \frac{4\sin x}{x^3} + \cos x - \frac{4\cos x}{x^2} + x\left(\mathrm{Si}(x) - \frac{\pi}{2}\right)$$

ERRATUM. In the article entitled "Influence of Hydrostatic Pressure on the Reversible Polymerization of Fibrin Monomers" by D. Collen, G. Vandereycken and L. De Maeyer (*Nature*, **228**, 669; 1970), all figures referring to pressure should be read as thousands.

ERRATUM. In the article by M. Paecht-Horowitz, J. Berger and A. Katchalsky (*Nature*, **228**, 636; 1970), a sentence was unfortunately left out in the opening paragraph. The last five lines should be substituted by: ". . . starting material for primaeval biosynthesis. So far, however, only low molecular weight peptides have been obtained, both with the phosphates of amino-acids and with dicyandiamide, and such peptides would have a doubtful claim to be the precursors of contemporary biopolymers. By thermal polymerization in anhydrous conditions we have obtained larger polymers[8,9], but it is unlikely that such conditions prevailed in the prebiotic state." In addition, the title of Table 1 should be "DATA ON PEPTIDES (1)".

CORRIGENDUM. The article "Variability, Structural Glycoproteins, and Classification of Herpes Simplex Viruses" (*Nature*, **227**, 1253; 1970) was published without a final correction. Although the authors were in general agreement with the statement regarding the unsuitability of either the name herpesvirus hominis or herpes simplex, the manuscript should have noted that they were not in complete agreement with the designation of subtypes. A. J. Nahmias and W. R. Dowdle preferred to retain the type 1 and 2 designations, reasoning that the biological, clinical and epidemiological differences are sufficient to justify considering them as distinct serotypes.

British Diary

Monday, November 23

Building Linear Filters from Logic Circuits (7.30 p.m.) Mr M. H. Ackroyd, Institution of Electrical Engineers, at the University of Leicester.

Engineers in General Management Posts—the Army's Experience (5.30 p.m.) Major General R. L. Clutterbuck, Institution of Civil Engineers, at Great George Street, London SW1.

Fire Precautions in Locomotives and Rolling Stock (5.30 p.m.) Mr J. M. Jarvis, Institution of Mechanical Engineers, Railway Division, at 1 Birdcage Walk, London SW1.

Founders of Radio-Analytical Chemistry (6 p.m.) Professor R. Spence, Society for Analytical Chemistry, Radiochemical Methods Group, at the Polytechnic of the South Bank, Borough Road, London SE1.

Microwave Ultrasonic Devices (6 p.m.) Mr R. F. Humphreys, Institution of Electronic and Radio Engineers, Aerospace, Maritime and Military Systems Group, at 8–9 Bedford Square, London WC1.

The Rejection of Laplacian Physics: a Turning-Point in the History of the Physical Sciences in France (5.30 p.m.) Mr Robert Fox, British Society for the History of Science, in the Lecture Theatre of the Science Museum, Exhibition Road, London SW7.

Tuesday, November 24

Biogenesis and Chemogenesis of Recent Marine Sediments (2 p.m.) Geological Society, Marine Studies Group, at Burlington House, Piccadilly, London W1.

Distribution Research at the Electricity Council Research Centre at Capenhurst (5.45 p.m.) Mr G. Ratcliff, Institution of Electrical Engineers, at the Renold Building, UMIST, Manchester.

High Cereal Yields (5.15 p.m.) Sir Joseph Hutchinson, FRS, Royal Society of Arts, Commonwealth Section, at John Adam Street, Adelphi, London WC2.

Offshore Drilling Rigs (two-day symposium) Royal Institution of Naval Architects, in the Weir Lecture Hall, 10 Upper Belgrave Street, London SW1.

Particle Size Analysis of Unusual Materials (6.30 p.m.) Mr J. E. C. Harris, Mr H. J. Scullion and Professor H. Heywood, Society for Analytical Chemistry, Particle Size Analysis Group, in the Physics Lecture Theatre, Imperial College, Prince Consort Road, London SW7.

Porphyria and Photosensitivity (5.30 p.m.) Dr I. A. Magnus, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Tenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

The Design and Construction of Butterworth Wharves, Malaysia (5.30 p.m.) Mr J. W. T. Tapp, Mr A. J. B. Dixon and Mr A. C. Carpenter, Institution of Civil Engineers, at Great George Street, London SW1.

The Identification of Rogue Materials in Reactor Construction (4.30 p.m., discussion) Institution of Mechanical Engineers, Nuclear Energy and Steam Plant Groups, at 1 Birdcage Walk, London SW1.

The Relationship of Styles of Management to Productivity (5.30 p.m.) Professor Richard Beckhard, Institution of Electrical Engineers, at Savoy Place, London WC2.

Wednesday, November 25

Chromatographic Techniques in Toxicology (2.30 p.m.) Society for Analytical Chemistry, Chromatography and Electrophoresis Group, at Chelsea College of Science and Technology, Manresa Road, London SW3.

Carbon Fibres for Engineering Applications (6 p.m.) Mr L. N. Phillips, Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1. (Thomas Hawksley Lecture.)

Continuing Education for Electronic Engineers (7.30 p.m.) Mr K. G. Stephens, Institution of Electrical Engineers, at the Star and Garter Hotel, Dorking, Surrey.

Cyclic Sedimentation and Faunal Response in the Cretaceous Basin of Western Interior North America (5 p.m.) Dr Erle G. Kauffman, Palaeontological Association, at the Geological Society of London, Burlington House, Piccadilly, London W1.

Early Timber Bell Towers (5.30 p.m.) Mr K. W. E. Gravett, Royal Institution, Library Circle, at 21 Albemarle Street, London W1.

Electronic Techniques in Archaeology (7.30 p.m.) Mr M. J. Aitken, Institution of Electrical Engineers, at Sumpner Building, University of Aston, Birmingham. (Silvanus P. Thompson Lecture.)

Famous Experiments in the Royal Institution (5.30 p.m.) Sir Lawrence Bragg, CH, FRS, Royal Institution, at 21 Albemarle Street, London W1. (Lecture for Fourth Form pupils from Schools in London and the Home Counties. To be repeated on November 26, December 1 and 2.)

Laminar Flow Devices and HEPA Filters (2.30 p.m., symposium), in the Mechanical Engineering Department, Imperial College, London SW7.

Progress in Taint-Free Packaging (6.15 p.m., symposium) Society of Chemical Industry, Food Group, jointly with PIRA, at the Zoological Society, Regents Park, London NW1.

Psychiatry, Science and Non-Science (5.30 p.m.) Dr E. T. O. Slater, University of London, at the Institute of Psychiatry, De Crespigny Park, London SE5.

Recent Advances in Isoenzymes (2 p.m.) Professor J. H. Wilkinson, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

Sir George Cayley "On Aerial Navigation", 1809-1810 (1 p.m.) Mr Charles H. Ginns-Smith, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Sleep and Dreams (7.15 p.m.) Mr C. Evand, Institution of Electrical Engineers, at the College of Technology and Art, High Wycombe, Bucks.

Thomas Huxley Manuscripts (6 p.m.) Mrs Jeanne Pingree, Society for the Bibliography of Natural History, in Conference Suite 3, College Block Imperial College, London SW7.

Thursday, November 26

Applications of Lasers (2.15 p.m.) Professor E. D. R. Shearman, Institution of Electrical Engineers, at Mander College, Bedford.

Applications of On-line Analytical Techniques to Process Control (10 a.m. colloquium) Institution of Electrical Engineers, in association with the Institute of Measurement and Control and the Institution of Mechanical Engineers, at the IEE, Savoy Place, London WC2.

Diophantine Equations (5.30 p.m.) Dr G. L. Watson, University of London, in the Engineering Theatre, University College London, Malet Place, London WC1. (Inaugural Lecture.)

Dynamic Characteristics of Silicon Controlled Rectifiers (7 p.m.) Mr R. G. Dancy, Institution of Electronic and Radio Engineers, at Medway College of Technology, Chatham, Kent.

Flow Measurement in Fan Testing (6 p.m. discussion) Institution of Mechanical Engineers, Fluid Plant and Machinery Group, at 1 Birdcage Walk, London SW1.

IgE and Hypersensitivity in Man (5.30 p.m.) Dr D. R. Stanworth, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Eleventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Modern Trends in Pollution Control (4 p.m.) Dr G. Mattock, Society for Analytical Chemistry and the Andersonian Chemical Society, in the Thomas Graham Building, University of Strathclyde, Cathedral Street, Glasgow C1.

Press Coverage of Pharmacy's History (7 p.m.) Mr O. Waller, Pharmaceutical Society of Great Britain, and the British Society for the History of Pharmacy, at 17 Bloomsbury Square, London WC1.

Putting Roland Into the Computer (1.20 p.m.) Professor W. Woledge, University of London, at University College London, Gower Street, London WC1.

New Horizons in Meteorological Instrumentation (6.30 p.m.) Dr H. T. Ball, Institution of Electronic and Radio Engineers, jointly with IEE, at the University Engineering Laboratories, Trumpington Street, Cambridge.

The Control of Fertilizer Granulation Plants (2.30 p.m.) Dr J. A. Bland, Dr J. L. Hawksley and Mr W. J. Perkins, Fertilizer Society, in the Lecture Hall of the Geological Society, Burlington House, Piccadilly, London W1.

The Social Impact of Modern Biology (three-day meeting) British Society for Social Responsibility in Science, at Friends House, Euston Road, London NW1.

Friday, November 27

Industrial Training (7.15 p.m.) Mr H. R. Peel, Oil and Colour Chemists' Association, at the Royal Hotel, Bristol.

Laser-Flash Photolysis (1 p.m.) Dr M. West, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Perspectives on Pesticides (2.15 p.m.) British Pest Control Association, at the National Liberal Club, Whitehall Place, London SW1.

Some Diseases of the Rat (symposium) Laboratory Animal Science Association, at the Zoological Society of London, Zoological Gardens, Regent's Park, London NW1.

The Life of the Common Porpoise (9 p.m.) Professor R. J. Harrison, Royal Institution, at 21 Albemarle Street, London W1.

Vitamin D Metabolism in Man (4 p.m.) Professor S. W. Stanbury, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

Monday, November 30

Organization of Pollution Studies in Hydrology (4.30 p.m., informal discussion) Institution of Civil Engineers, Hydrological Group, at Great George Street, London SW1.

The Exploration, Protection and Management of the World Ocean—a Challenge for the 1970's (5.30 p.m.) Dr S. J. Holt, University of London, at University College London, Gower Street, London WC1.

The Possibility of Predicting the Life of Hydraulic Equipment Under Operating Conditions (4.15 p.m., discussion) Institution of Mechanical Engineers, Fluid Plant and Machinery Group, at 1 Birdcage Walk, London SW1.

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Building Research Station. Current Paper 22/70: Foundations of Buildings in Clay. By J. B. Burland. Pp. 4. (Garston, Watford: Building Research Station, 1970.) *gratis*. [710

Freshwater Biological Association. Thirty-eighth Annual Report for the year ended 31 March 1970. Pp. 160. (Ambleside, Westmorland: Freshwater Biological Association, The Ferry House, 1970.) 5*s*. [810

Ministry of Transport: Road Research Laboratory. RRL Report LR 348: Road Accident and Casualty Rates in 1968. By H. D. Johnson. Pp. 20. (Crowthorne, Berkshire: Accident Analysis Section, Road Research Laboratory, 1970.) *gratis*. [810

Scottish Marine Biological Association. Annual Report 1969/1970. Pp. 77. (Oban: Scottish Marine Biological Association, 1970.) [810

London Under Stress: a Study of the Planning Policies Proposed for London and Its Region. Pp. 80. (London: Town and Country Planning Association, 1970.) 10*s* (50*p*). [910

University of London. University College Calendar 1970/1971. Pp. civ+290. (London: University College, 1970.) [1210

The British Glass Industry Research Association. Fifteenth Annual Report 1970. Pp. 55. (Sheffield: The British Glass Industry Research Association, 1970.) [1210

Social Responsibility and the Scientist in Modern Western Society. By Professor Sir Ernst Chain. (Robert Waley Cohen Memorial Lecture 1970). Pp. 26. (London: The Council of Christians and Jews, 1970.) 5*s*. [1210

Ministry of Transport: Road Research Laboratory. RRL Report LR 338: A Post Driving Technique for the Erection of Tensioned-Beam Crash Barriers. By I. B. Laker. Pp. 8+4 plates. RRL Report LR 342: Seat Belts—The Proportion of Cars Fitted and of Occupants Using Them. By B. N. Farr. Pp. 10. (Crowthorne, Berkshire: Road Research Laboratory, 1970.) *Gratis*. [1210

Other Countries

Republic of Botswana: Ministry of Commerce, Industry and Water Affairs. Annual Report of the Geological Survey Department for the year ended 31st December 1968. Pp. 2$. (Lobatse, Botswana: Geological Survey, 1970.) [510

OECD. Centre for Educational Research and Innovation: Purpose, Programmes, Progress. Pp. 19. (Paris: OECD, 1970.) [610

New South Wales State Cancer Council: Special Unit for Investigation and Treatment. Publication No. 16: Human Mammary Cancer—Analysis of Haematological, Biochemical and Anthropometric Observations. By Stanley H. Chorlton, N. R. Hughes and Maureen Larkin. Pp. 33. (Sydney: Australasian Medical Publishing Co., Ltd., 1969.) [610

Institutt for Atomenergi, Kjeller Research Establishment. Kjeller Report No. 137: Studies of the Effects of Nitrogen in an Austenitic Stainless Steel with 18 per cent Chromium and 9 per cent Nickel. By Paul Storvik. Pp. iii+52. (Kjeller, Norway: Institutt for Atomenergi, Kjeller Research Establishment, 1969.) [610

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 138: Gaschromatographie der Pflanzenschutzmittel—Tabellarische Literaturreferate I. Von Dr Winfried Ebing. Pp. 43+tabular literature abstracts, Ser. 1. (Berlin-Dahlem: Biologische Bundesanstalt für Land-und Förstwirtschaft, 1970.) 21 DM. [610

Records of the Australian Museum, Vol. 28, No. 2 (27th August, 1970): Results of an Archaeological Survey of the Hunter River Valley, New South Wales, Australia, Part 1: The Bondaian Industry of the Upper Hunter and Goulburn River Valleys. By David R. Moore. Pp. 25–64+plates 4–14. (Sydney: The Australian Museum, 1970.) $2. [110

Geological Survey of Botswana. Quarter Degree Sheets 2026D and 2126B: Moestse—Matsitama. (Lobatse, Botswana: Geological Survey, 1970.) [110

Republic of South Africa: Department of Fisheries. Division of Sea Fisheries Investigational Reports. No. 80: Exploitation and Recovery of Right Whales *Eubalaena australis* off the Cape Province. By P. B. Best. Pp. 20. No. 87: Exploratory Fishing for Tuna Off the South African West Coast. By C. S. De V. Nepgen. Pp. 26. (Sea Point, Cape Town: Director of Sea Fisheries, 1970.) [810

Hydrological Map of Cyprus. (Nicosia: Geological Survey Department, 1970. Obtainable from Lands and Surveys Department.) £0.600 mils. [910

OECD. Uranium: Resources, Production and Demand. (A Joint Report by the European Nuclear Energy Agency and the International Atomic Energy Agency.) Pp. 54. (Paris: OECD, European Nuclear Energy Agency; London: HMSO, 1970.) 13 francs; 20*s*; $3; 11.50 Sw. francs; 9.10 DM. [910

US Department of Commerce: National Bureau of Standards. NBS Special Publication 260: Catalog of Standard Reference Materials. Pp. vi+77. (Washington, DC: Government Printing Office, 1970.) $0.75 [910

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DECEMBER 5, 1970

Communications Without Talk

THE precipitate dismissal of Lord Hall, the chairman of the Post Office Corporation, just a week ago is probably one of the best things to have happened to the British telecommunications industry since the Post Office was made a public corporation just over a year ago. Lord Hall, to be sure, has been unlucky in having had to take the brunt of the inevitable strains that there would be in turning the British Post Office from a government department into a public corporation. To expect that somebody qualified in the comparatively abstract concepts of medicine, politics and the law should turn out to be well suited as the head of a commerical enterprise seeking to find its feet in unfamiliar terrain was probably over-optimistic of Mr Harold Wilson and the other members of the Labour Government. In the circumstances, it is hardly a surprise that Mr Christopher Chataway, the new Minister of Posts and Telecommunications, was able to beat off the censure motion by the Opposition in the House of Commons earlier this week. Unfortunately, nothing said in this predictably acrimonious debate has helped to clarify the problems with which the Post Office Corporation must contend, most of which are painfully apparent in the first report to be published under the new arrangements (HMSO, £1 10*s*).

The Post Office is one of the largest enterprises in Britain. Its income is something like three per cent of the Gross National Product and is growing more quickly than that of any barely comparable enterprises at more than ten per cent a year. It is in the ordinary sense a conglomerate, with a responsibility not merely for delivering the mails and operating the telephone service but also for a bank (the giro system) and for a computer bureau (the National Data Processing Service). The new report says that the objective of the reorganization carried out a year ago was to allow it the more efficiently to meet the objectives of providing an efficient service to potential users and an economic return on capital employed, but the complacent way in which the report says that the corporation was pointing in the right direction—"the influences of change and sharpened commercial awareness had been gathering momentum", for example—is at least one reason why confidence in Lord Hall's capacity to continue as chairman must have been shaken. Even if an annual report should avoid washing dirty linen in public, it might be expected not to affirm the opposite of the truth. And the plain fact, clearly revealed by the reports of the auditors accompanying the first report, is that the Post Office Corporation is still in a thorough muddle by commercial standards. Briefly, the auditors say that the Post Office accounts are kept in such a way that assertions about the value of assets cannot be verified, that stocks of equipment cannot be checked against the records, that debtors' accounts are unverifiable and that the method of recording balances of the giro customers does not adequately distinguish between the transfers of money from one post office to another and from particular post offices to customers. Plainly there is a long way to go before Lord Hall's establishment can look its commercial competitors, let alone its customers, in the face.

Good accounting or the lack of it is, however, not the most serious of the issues raised by the publication of the new report. The most serious charge against the British Post Office in the past ten years, still largely valid, is that the organization as a whole has done much less than it should have done to exploit the technology of the seventies. In the latest report, there is some cheerful talk about the rate at which would-be telephone subscribers are being supplied with telephones, but there is very little in this over-glossy document to suggest that the Post Office Corporation is really based on "a highly innovating, business-oriented technology". For example, the report has nothing to say about the potential involvement of the Post Office Corporation in cable television, but its defenders in the House of Commons and particularly the ex-Minister of Posts and Telecommunications, Mr John Stonehouse, are anxious that the right to deal with matters like this should not pass to other organizations. In a breathless chapter on "technology", the new report talks of an experiment planned for 1973 in which millimetre waves will carry 300,000 telephone conversations along a copper tube spanning 20 miles of East Anglia without acknowledging that similar experiments are already well under way elsewhere. And there is no sense that the new corporation, stripped of what were supposed to be the inhibitions of the civil service, would feel free to keep itself out of financial trouble with the government by devising and then selling things which potential customers would enjoy using. The Post Office may have ceased to be a government department, but it is a long way from pretending to be even a non-profitmaking commercial enterprise.

What is to be done? On the face of things, even the present Post Office Corporation is in need of splitting up, partly because of the internal inconsistencies in its structure and partly because the monopoly with which it thinks it is blessed is frequently an inhibition and not an incentive. Thus, in the present circumstances, when it is increasingly difficult to make a profit on the collection and delivery of mail, it is only understandable that the Post Office should find it increasingly hard to decide what must be done. Should all postal charges be increased by close on 50 per cent? Or has the time come to make some more subtle variation of the pricing

structure ? In particular, now that the Post Office's own interest in telephones has diminished the force of the old Victorian argument in favour of a uniform postage rate that rural citizens should be dealt with as those in towns, there is, for example, room for considering the basis on which uniform postal charges are imposed. This certainly is what any commercial organization would do. The chances are, moreover, that such a radical reappraisal of the principles of the Post Office's operations would lead not to greater costs but to greater efficiency. In exactly the same way, without crabbing the corporation's freedom to engage in banking or data processing, there is a case for thinking that enterprises like these could be and should be established gradually, by competing first for the cream lying free in the market and only afterwards hoping to set up the nationally integrated system on which political ideologists (and a good many civil servants) dote. All this follows from what is called, in the business schools, management science.

The Post Office Corporation is also a technological anachronism. One of the puzzles of the new annual report is that research and development expenditure, at just over £13 million in the year finishing on March 31, can be as small a proportion of the total capital expenditure. To be sure, there has been a welcome increase (of 43 per cent) over the year, chiefly on account of exchange equipment, where the Post Office is plainly seeking to make up for some of its misfortunes of the past few years. It is also true that a good deal of the research carried out in telecommunications is placed with commerical suppliers and charged for in the equipment eventually supplied. At the same time, however, it seems likely that the money being spent on research and development is too little to meet the needs of the late seventies and the eighties.

New Ways with the Moon

THE success with which the Soviet Union last week dispatched a vehicle on wheels to the surface of the Moon should be an occasion for reconsidering the ways in which the Soviet Union, the United States and several other nations might usefully collaborate in the exploration of the Solar System. At no time since President Kennedy pinned the reputation of the United States to a successful landing on the Moon within a decade have circumstances been so appropriate for serious exploration of ways in which some form of international collaboration might be brought about. For if there has ever been a race to the Moon, by now it must be clear that the race is over. The United States, to be sure, has achieved its expressed objective and has already turned its attention from the further exploration of the Moon to more ambitious schemes such as that for building a launching rocket which can be used time and time again for servicing laboratories. But who knows whether the Soviet Union had ever intended to participate in the supposed race to the Moon ? It would be entirely consistent with what the Russians have always said about the importance of sending instruments and not people on these journeys to regard their latest exploit as the attainment of their objective. There is certainly a good chance that the technique that has succeeded on the Moon may now be applied elsewhere more easily than can be the American Apollo system, so that what the Russians may have lost in glamour they may have gained in long-term benefit. Certainly their method has been cheaper. But in the United States as well as in the Soviet Union, this is evidently a time for asking what comes next.

In the United States, this question is inextricably bound up with doubts about the immediate future of the National Aeronautics and Space Administration. Dr Thomas Paine resigned two months ago but his successor is still not appointed. Worse still, the passage of close on a year since NASA committed itself to the space shuttle as the next objective of importance has not engendered confidence in the value of this proposal. There is a serious danger that the shuttle, like many earlier aircraft projects, will turn out to be disappointing in performance but much more expensive even than its supporters now confess. Certainly the decision to develop the shuttle, if confirmed by the Nixon Administration's next budget proposals due in February, will dangerously concentrate NASA's new development in space on a single project that must inevitably mean that resources cannot easily be spared for other ventures. Most probably, this simple truth is now as painfully evident inside the Administration as it is to outsiders. May it not then be that the time has come for a radical reappraisal of the programme as a whole ? And may not international collaboration be a valuable alternative to present plans ?

Dr Thomas Paine, the retired administrator, has a creditable record in this field. Especially in the past two years, he has consistently argued that expensive schemes for space research and development may be most valuable as stimulants of international collaboration. From time to time, he has chided the Soviet Union for dilatoriness in carrying out the Russian part in minor agreements for collaboration. In the past few months, there has been welcome progress in the setting up of talks on matters such as the design of universal couplings for spacecraft manufactured in different countries—this may not be an important problem but it is at least a useful one to start with. And both the Soviet Union and the United States have been ready in the past few months to collaborate with other nations—both the American and the Russian experiments on the surface of the Moon have, for example, provided opportunities for people elsewhere (and the French seem to have been the quickest off the mark) to obtain laser reflexions from the Moon.

By now, enough is known of the pitfalls of international collaboration to know that the simplest kinds of collaboration are also often the least useful. The European Launcher Development Organization (ELDO) is a telling example of how not to let the wish to collaborate become the father of half-baked thoughts. Where the prestige of two great powers is involved, there are evidently risks that collaboration may become simply a means of attempting tasks that seem unnecessary to the two nations separately. Everybody knows, however, that the past few years have seen both the United States and the Soviet Union attempting exploits which are hard to justify except in terms of prestige or bravado. By comparison, the international collaboration which may now grow up should be designed so as to avoid earlier and current excesses. In particular, whatever some European governments may think, the development of the space shuttle in the United States is an unsuitable subject for collaboration because it is unnecessary. And to begin with, the need for mounting experiments with diverse national origins in the same spacecraft is much less urgent than the need somehow to coordinate forward plans in such a way as to ensure that the fullest use is made of the available resources.

Geophysics Teaching in Disrepute

SUCCESS in research is as much a matter of asking the right questions as getting the right answers. It is a pity, then, that the authors of the Royal Society report on British postgraduate training in the solid earth sciences (*Postgraduate Training in the United Kingdom 5. Solid Earth Sciences*. The Royal Society, November 1970) should have failed to apply this familiar principle to the investigations which have taken them all of four years to complete. For the kindest thing that can be said about the report is that its existence at least acknowledges the subject to be worth study. And, to be sure, it recognizes in a hand-waving way a few of the problems facing the education of earth scientists—most important, the thorny question of the proliferation and continued existence of small ineffective geology departments scattered throughout the British university system. But otherwise it is a missed opportunity.

The basis of the report is the replies to a questionnaire sent to all university departments of geology and geophysics, only thirty of which responded in full. The limited scope of the investigation can be gauged from the selection of the questions asked: what qualifications do you require prospective PhD students to possess? Are there regular colloquia or seminars for PhD students? Should supervisors act as internal examiners or not? Would you favour the award of classes in the PhD degree? How frequently do candidates fail? *Et hoc genus omne*. It is perhaps only fair to acknowledge that there is a little merit in this concentration on the purely operational aspects of postgraduate earth science training if only because it has not been dealt with before. But if it is a useful exercise to ask geology departments to give information on, for example, the duration of their PhD courses, it may be presumed to be even more useful to quote the results in full rather than to present eight selected replies. For where even the total number of geology and geophysics departments is statistically small, data of this kind only make sense if they are more or less complete. The best that can be said about the technique of quoting a few choice responses is that it can be gloriously entertaining, especially as it is pretty easy to match the replies with their authors.

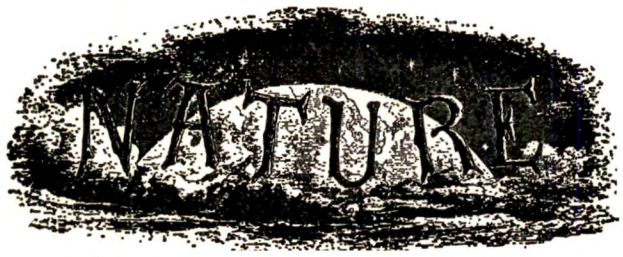
So what should the report have concerned itself with? The truth is, of course, that the authors have put the cart before the horse. It would have been more profitable to consider first just what present and future postgraduate training in earth sciences should achieve. A little research here would not have come amiss. What do students actually do when they have their PhDs? For that matter, just how many PhD students are there in the earth science departments? And to what extent do they match up to demand in both number and discipline? If it is true, as the report suggests, that the exploration companies "regard the years of training for PhD as a reduction in the working life of the men in question", is the PhD only of use as an entrance fee to a university teaching post or the Institute of Geological Science?

All this leads to even more fundamental questions. For one thing, it is hard to see how a rational evaluation of postgraduate training can be carried on separately from a consideration of undergraduate (and even school) education, if only because of the purely mechanical point that teaching and research are usually carried out under one roof. The fact is that the whole question of earth science education in Britain needs a thorough airing, but even a limited investigation of postgraduate training makes no sense in the absence of any clear thought about what Britain can do with limited resources. There is also the problem of too many earth science departments. The one truth that shines through the report is that many departments are quite simply too small to sustain viable research groups.

This said, the report does have some useful things to say. For example, it rightly criticizes the rigidity of the research council's policy of giving PhD support only to those with "good degrees"—which means upper second or first. But what emerges most clearly is the need for an overall strategy for the earth sciences in Britain. The authors recognize that future postgraduate training is only under the control of individual universities to a marginal extent—and the same could be said of all earth science education. The solution is to formalize this lack of control in a separate subcommittee of the University Grants Committee aided by the research councils. There are, however, other possibilities. The Higher Education Research Unit at the London

School of Economics, for example, has pioneered the study of general problems of higher education within the university itself. The time has surely come for the other units to consider individual disciplines. Or must policy in the earth sciences continue to struggle along from one *ad hoc* decision to another?

100 Years Ago



Natürliche Schöpfungs-Geschichte. Von Dr. Ernst Haeckel. 2te Auflage. (Berlin : Reimer, 1870. London : Williams and Norgate.)

We have, in the above, attempted to give a general idea of what the book is, and how the second edition differs from the first, rather than to enter into any criticism. The first edition has already received the ablest criticism this country could give. We will venture, however, to make one reflection. Had the book been written for scientific men, it would have been read by some with delight, by others with feelings of fretfulness and worry, but by all with more or less of profit. Addressed as it is, however, to an intelligent and cultivated, but still to a general and unlearned public, prone to receive fanciful analogies as real reductions to simpler laws, and to confound together fond imaginings with sound and fruitful hypotheses, we very much doubt whether it may not turn out to be an engine rather of mischief than of good. Genealogies there must be, doubtless, and many, doubtless, also may in time be made out. In cases like the pedigree of the horse, the evidence already seems wonderfully strong; and it would be simple presumption to fix a limit beyond which we cannot hope for success. Still, by their nature, genealogies are like castles of cards in the shape of inverted pyramids, with each tier less safe and less sound than the one above it. A very little memoir may disturb one of the lower stages, and then a whole pile comes down with a run. They are not the kind of things to put before learners as the strongholds of science. Some readers would learn in the first half of this volume to love Darwinism better than biology, and before they had finished the second half, would love Haeckel better than either. Others would pass rapidly through a disbelief in Haeckel and distrust of Darwin to a state of complete doubt about biology in general. Worst of all would be the effect on such minds as that of a speaker at one of the meetings of the Biological Section of the British Association at Liverpool, who said he had believed for many years in spontaneous generation, in natural selection, in the evolution theory, and in most views of a similar kind, and who seemed ready to believe anything and everything except the old truth, that truth is very hard to get, but very precious when it is gotten.

M. F.

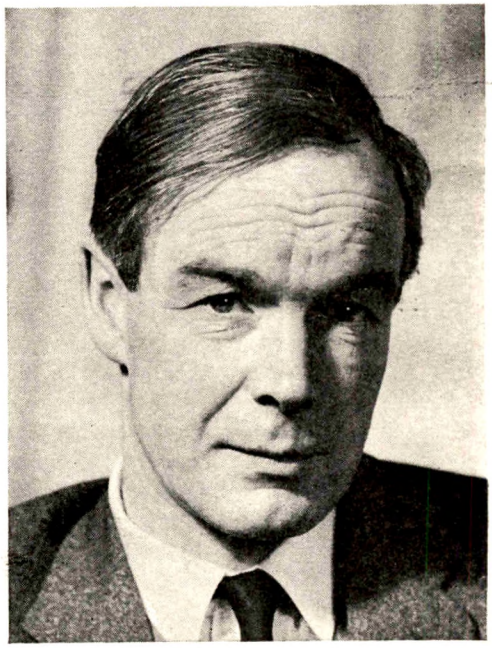
From Nature, **3**, 102, *December* 8, 1870.

OLD WORLD

ROYAL SOCIETY

Looking Outwards

THE election of Professor Alan Lloyd Hodgkin, Plummer professor of biophysics at the University of Cambridge, as President of the Royal Society was announced after the anniversary meeting of the society on November 30. Professor Hodgkin, aged 56, a Royal medal winner in 1958 and joint Nobel laureate for physiology in 1963, is a widely respected biophysicist, best known for his fundamental research into the nature of the transmission of the nerve impulse. His most recent work has been directed towards elucidating the movement of metal ions across the nerve axon membrane as a succession of nerve impulses travel along the nerve fibre.



Professor A. L. Hodgkin. (Photograph by Ramsey and Muspratt Ltd.)

The annual report of the council and the final address by Lord Blackett, the retiring president, both emphasized the Royal Society's concern with, and involvement in, the international aspects of science especially in relation to Europe. Lord Blackett, in reviewing the year's activities, underlined the growing spirit of cooperation between the Society and the government in matters of international science policy. The European Exchange Scheme, started in 1967 and developing rapidly, is a fine example of this interplay between science and state. When it became clear that the original budget of £400,000 a year which the Society had found from private sources would be insufficient to support the fullest development of the programme, finance was arranged with the Department of Education and Science so that the Society was able to match sums made available by organizations in other countries. This scheme has made possible a total of 551 fellowships or study visits during the years 1967, 1968 and 1969.

The second part of Lord Blackett's address diverged from the purely scientific into the more sensitive area of the role of modern science and technology in improving the welfare of mankind. Stemming from a proposal made at the 1966 meeting of the International Council of Scientific Unions (ICSU), of which the Royal Society is a member, a study group was formed with the ponderous title of "Committee on Science and Technology in Developing Countries—Costed". The first fruits of this move are now apparent in the form of the draft of a book entitled *The Role of Science and Technology in Developing Countries*, written by Graham Jones, a chemist and administrator, under the guidance of Lord Blackett. What seems to have emerged from this study is that technology which may be meat to a rich country may equally be poison to a poor one. Although methods of production in under-developed countries must be improved, it is dangerous to provide the means of increasing the gross national product unless steps are taken to ensure that unemployment does not become a concomitant social problem. Regardless of how this conclusion may be received it is clear that the writing is on the wall for the suggestion that the work of the Royal Society ends at the horizons of the purely academic world.

EUROPEAN SPACE

Frustration in ESRO

from a Correspondent

IT seems that the diplomatic initiative of the European Space Conference (ESC) "hard-core" launcher countries—essentially, France, Germany and Belgium—was by no means exhausted by the failure of the ruse to raise $35 million, chiefly to support further work on the ELDO launcher (see *Nature*, **228**, 596; 1970, and **228**, 706; 1970). At what was to have been the final meeting this year of the ESRO Council to approve budgets for 1971, the French delegation last week frustrated the deliberations on the applications satellite budget which should have just begun to swing European work in this field into motion. The scientific satellite budget went through; this covers the funding for the HEOS-A2, TD-1 and ESRO-4 satellites, and preparatory work on the COS-B and GEOS satellites selected to follow on.

The Council had put to its member countries a draft applications satellite budget for next year of $11 million. Instead of either voting down or accepting this sum, the French delegation offered to pay its share (of about 20 per cent), but on only $1 million. This, of course, is a derisory amount, and the other delegations consequently reserved their positions on the applications programme. A decision on the draft applications satellite budget has now been postponed for a new meeting of the ESRO Council on December 22.

There can be little doubt that this manoeuvre was France's retaliation for the non-launcher members of the European Space Conference, and Britain in particular, not going along with the ELDO Europa 3 project at the November 4 Brussels meeting of the ESC. France has the biggest stake in Europa 3; Britain is identified closely with European applications satellites. Professor Hermann Bondi, until next autumn Director-General of ESRO, will have a busy month attempting to salvage the situation in time for the December 22 Paris meeting. He succeeded over the perhaps even more intractable problem of the TD satellite cancellation, so he may well find a way out of this difficulty. He is personally deeply convinced of the importance of pursuing an applications satellite programme in Europe.

PSYCHIATRY

Boost for Biochemistry

"BIOCHEMICAL studies within the broad framework of neurobiology offer a tangible approach to important aspects of mental functioning." That is the conclusion of a committee established by the Medical Research Council under the chairmanship of Professor D. A. K. Black (Manchester), which has just issued its report *Biochemical Research in Psychiatry: Survey and Proposals* (HMSO, 6*s*).

The theme of the report is that the problems of mental illness have not in the past attracted a share of research effort commensurate with their importance in medicine and in society. The biochemical approach, in particular, has been hampered by the complexities of the organization of the nervous system and by difficulties in recruiting suitably qualified research workers. Another contributory factor is the comparative isolation of many of the hospitals which cater for mental illness.

But because of recent scientific developments, the committee feels that circumstances have now changed so as to make such biochemical analysis opportune. The report suggests that the MRC should encourage more strongly the neurobiological approach to psychiatric problems and argues that the observation of correlates between abnormal behaviour and underlying changes in the nervous system is an important (albeit long term) approach to understanding behavioural disorder.

The report recommends that the career structure in this field should be improved and that the MRC should therefore contribute to a coordinated scheme for providing training and more senior posts. There should be additional support for strengthening existing units and for forming new teams to work in this area. The multidisciplinary character of the subject implies that this would be best exercised in a university with departments already established in the relevant supporting subjects.

The MRC is now studying the general recommendations of the report with a view to formulating specific policies. It seems likely that changes in the pattern of MRC expenditure will result (the recommendations of the last such report, on radiobiology, that support should be increased for radiotherapy but decreased for radioprotection were very largely carried out. There must therefore be a slight change in the balance of expenditure, for given limited funds, an increase in support for neurobiology must imply cuts elsewhere. Although these cuts have not yet been decided, the MRC is hopeful that their effect will be more limited than was at first anticipated.

This report marks a departure in MRC policy—it is the first time that such a policy review has been published in this manner (although policy reviews have been held frequently, and commented on in the annual report). The intention of this publication is to make the objectives of the MRC more widely known, and thus perhaps to encourage more interest in this field.

Comparable reports on other selected areas will probably be issued; a committee, for example, is at present examining MRC support for human physiology.

FRANCE

Instrument Development

THE French government, especially the Ministry of Industrial and Scientific Development, is seriously concerned about the state of the scientific instrument industry in France. The balance of payments in the French instrument trade has been deteriorating over the past few years (about £43 million of imports against only £25 million of exports in 1968, for example). The Organization for Economic Cooperation and Development drew attention to this disquieting fact as early as 1968, when the instrument industry was surveyed for its possible role in the technological gap. In fact, the Délégation Générale à la Recherche Scientifique et Technique (DGRST) has been struggling with this problem since 1964, when the delegation was part of the Prime Minister's office, the struggle having taken the form of financial aid for research and development done by a few selected instrument builders. But this so-called "concerted action" has yielded meagre results. Of the twenty-three subsidies paid by the state, amounting to £1·5 million, five can be considered successful, five more show positive results and three have been only partially successful. The trouble is not that state-supported research and development is intrinsically unfruitful but that the sowing determines the harvest. Research projects considered for funding through the DGRST are carefully screened, but although this organization requires market analyses of the products to be subsidized, the market studies often have proved to be erroneous. With the whittling down of the French research budget in the past two years, there has been a commensurate rise in the challenge presented by the American instrument market which, according to a Stanford Research Institute study of the market outlook, will continue to be 2·5 times greater than instrument sales possibilities in western Europe (including Britain) over the next five years.

Before the French Sixth Plan gets under way in 1971, the French government has decided to remodel, strengthen and supplement official aid to the scientific instrument industry. After a year of redefining the industry's responsibilities, a new interministerial guidance committee met on November 13 to spell out a "funding strategy". Following the strictest rules of continental bureaucracy, this committee seems to include no researchers (instrument users) or manufacturers (the suppliers), but only civil servants (who handle the cash). The DGRST, now a component of the Ministry of Industrial and Scientific Development, is represented, as is the Centre National de la Recherche Scientifique. But the committee's chairmanship (a task so essential to technocracy) is the responsibility of the same ministry's Direction des Industries Mécaniques, Electriques et Electroniques (DIMEE), an organization whose habit it is to become involved in matters where research and development plays a purely imaginative role.

The DGRST's aid to instrument research and development was the government's principal form of assistance during the current quinquennial Fifth Plan, now ending. Those concerned wish to see state fun doubled for the Sixth Plan. Marketing support will the secret weapon of the new committee, authors of green paper titled *Action Instruments de Mesure*. F exports, instrument makers will be urged to raise th sales to 20 per cent from the present 10 per cent annual turnover. For the domestic market, a centraliz purchasing mechanism is envisaged to encourage sta institutions (which make more than 50 per cent instrument purchases) to "buy French".

OIL POLLUTION

Unit Shows Its Paces

THE first results from the Field Studies Council's n oil pollution research unit near Milford Haven we presented this week at a symposium organized by t Zoological Society. During the past three years the has been much needed progress from the woeful sta of ignorance that existed at the time of the Torr Canyon disaster about the effects of oil on marine li and for this the unit can take some of the cred It was set up in the wake of the Chryssi P. Gouland episode when 250 tons of crude oil were released the shores of the Haven, now the home of Britain largest oil port and site of not infrequent oil spillag The unit's work is supported by the Institute of Petr leum and the World Wildlife Fund and although it h existed on £9,500 since it started in 1967, these grar are a rare example of cooperation between indust and conservationists. Much of the unit's work has be concerned with the effects of oil on salt marshes, t physiological effects of oil on plants and the effects detergent cleaning on rocky shores.

One of the more useful aspects of the symposiu was that oil was considered in relation to other pollu ants. The most notable contribution was that of t unit's director, Mr E. Cowell, in which he put forwa a rather refreshing view on the problems of oil pollutic Like others, he was anxious about alarmist reporti in the press: it is high time, he said, that the effects oil pollution should be put into some kind of perspecti in relation to the other environmental threats, partic larly those which are less obvious. He therefc suggested a six point scale of pollutants as a basis f allocating them some order of importance. He definec pollutant as anything which, having been introduc into the environment, subsequently causes a change the ecosystem and placed inert "additions" such polythene bottles in category I of his scale. Next car the environmental contaminants which cause sor biological change but which are not considered serio Oil pollution from refinery effluents and the prese level of radioactive waste could fall in this categor Ecologists would become anxious when hazards fr category III are rife; these would include chlorinat hydrocarbons and oil slicks. The public shows anxie at about stage IV when organisms begin to die and t ecosystem becomes unstable. The methyl mercu contamination of the Japanese rivers would come category V, the stage at which official action wou be automatic. Lastly, catastrophic pollution such that from nuclear, chemical and biological warfa would fall in category VI.

In Dr Cowell's opinion, the oil industry now pla only a tiny part in the total pollution of the wor ecosystem—a tribute, he says, to the vigilant an

pollution measures adopted by 80 per cent of the world's oil industry. At the same time it would be unwise to underplay the threat of oil pollution to marine life, even if damage is only on a local scale: many colonies of water fowl are still rapidly disappearing through the effects of oil and not even the remotest penguin remains unscathed.

CERN ACCELERATOR

Social Justification

THE chief justification for building the new 300 GeV accelerator at CERN is a social and political one, Dr J. B. Adams, the director of the project, told a meeting of the European Atlantic Group in London last week. With the governments of the twelve member countries of CERN now engaged on the task of deciding whether or not to support the new venture, estimated to cost £100 million, Dr Adams sought to place the new machine in the perspective of two important ideals—the development of university education in Europe and the need for some centre of European collaboration which could be seen to weather the storms that have scarred so many other European ventures.

Failure to build the new accelerator would have serious consequences for the universities of Europe, Dr Adams said. A significant part of the teaching capacity in physics departments lies with high energy physicists whose teaching is influenced by their involvement at the forefront of research in high energy nuclear physics. This would suffer if Europe dropped out of the high energy arena. The existence of broadly equivalent facilities at CERN, in the United States and in the Soviet Union means that many experiments take on a multinational character, with physicists from Europe gaining the right to use facilities elsewhere in return for outside participation at CERN. This partnership in high energy physics would soon disappear, Dr Adams said, if the new accelerator were not built at CERN. The 200 GeV machine at Batavia, Illinois, which should be completed within the next eighteen months, already has a waiting list of eighty experiments but the machine only has a capacity for about ten experiments a year.

Dr T. G. Pickavance, a director of the Science Research Council, explained that the cost to Britain over the eight year construction period would be about £2·5 million a year. The present contribution to CERN of £7 million would be increased to £9 million instead of dropping to £6·5 million. If any one country decided to opt out of the project it could still go ahead in principle, Dr Adams explained, as the existing 28 GeV proton accelerator would still be available for experiments by the non-contributing countries.

Dr Adams also emphasized the important part played by such a project in providing an advanced market for new technological goods. This is most likely to apply to the superconducting magnets which will be installed in the new machine if the present design hurdles can be overcome. The glaring absence of any overriding criterion by which to judge whether or not the new accelerator should be built, however, must place the fate of the 300 GeV machine very much on a knife-edge. But if the accelerator is to be built, this should be known by the time the CERN council meets next on December 18.

Parliament in Britain

Rolls-Royce

THE government, in March 1968, agreed with Rolls-Royce to supply 70 per cent of the launching aid for the RB 211 engine if either McDonnell Douglas or Lockheed came forward with three airlines ready to order aircraft with Rolls-Royce engines. But on March 27, Rolls-Royce asked Mr Anthony Wedgwood-Benn, then Minister of Technology, for £20 million because Lockheed had decided to go ahead with development of the TriStar airbus with orders from only two airlines. Moreover, the company said that unless the money could be promised within five hours, it would have to withdraw altogether from the race to secure the engine contract. Five and a half hours later, Mr Benn telephoned Rolls-Royce and said that the government would guarantee the company £9 million and Lockheed accepted the Rolls-Royce tender on the basis of that guarantee.

This extraordinary account was given last week by Mr Anthony Wedgwood-Benn during a debate on the government's recent decision to give a further £42 million to Rolls-Royce to launch the RB 211 engine. Proposing a motion which deplored the government's lack of consistency and of effective accountability in its industrial policy for the private sector, Mr Benn criticized the winding up of the Industrial Reorganization Corporation. The disappearance of the IRC, he said, means that there is no supervision over enormous sums of public money.

Mr John Davies, Secretary of State for Trade and Industry, maintained, however, that the decision to provide further launching aid for the RB 211 is in no way contradictory to the government's industrial policies. Rolls-Royce is not only a supplier to airlines throughout the world, but it is also at the very basis of Britain's defence procurement programme, Mr Davies said, and there is a long history of government support in the aircraft industry. Mr Davies also argued that the accountability of Rolls-Royce to its shareholders should provide sufficient control, and that the independent examination by accountants that the government has instituted should ensure extra safeguards. (Debate, November 23.)

CS Gas

MR IAN GILMOUR, Under Secretary of State, Ministry of Defence, said that CS smoke, as used in training, has no effects on the environment. The government, he said, has made extensive studies of the chemical and biological effects of CS smoke, which have shown that it has no effect on domestic animals and birds in the concentration that builds up in training. The amount that might fall on vegetation would also be very small, and would rapidly be dispersed by dew or rain. (Oral answers, November 26.)

Hepatitis

MR GORDON CAMPBELL, Secretary of State for Scotland, said that nineteen people died in Scotland from infective hepatitis and one from serum hepatitis last year. A further 976 patients were discharged from hospital with a diagnosis of infective hepatitis and twenty with serum hepatitis. A committee, under the chairmanship of Lord Rosenheim, is at present looking into the hazards involved in the treatment of chronic renal failure. (Written answers, November 24.)

The Biologist's Dilemmas

Do scientists have either the means or the power to influence the direction along which science advances or the uses to which scientific discoveries are put? In particular, should scientists now be worrying about genetic engineering, and all that the term implies, or are the possibilities so remote that they can conveniently be dismissed to the back of the mind? These were the two chief questions which occupied participants in the conference on the social impact of modern biology, held last week under the auspices of the British Society for Social Responsibility in Science. It is no surprise, nor should it be too great a disappointment, that very few answers emerged from the twenty-three hours of talk and discussion that the meeting provided.

The concept of social responsibility in science is in itself so wide-ranging that the meeting predictably rambled from issue to issue, without really getting anywhere. On one level, the concept embodies the moral dilemma of the individual scientist who participates in research whose results could be grossly misused. One speaker, for example, posed the question: what should a scientist do if he comes up with convincing evidence to show that one race is genetically more intelligent than another—should he bow to the dictates of his conscience as a scientist, and publish his findings, or should he keep quiet? On another level, social responsibility in science raises considerations of the role of science in society, and there were those at the meeting who argued that science cannot be discussed separately from the social system in which it is practised. This led some to suggest that changing the organization of science will not make scientists more socially responsible unless the social system itself is changed, and others to argue that the ethics of science are so far removed from the ethics of governments that scientists should seek to extricate themselves from the apparatus of the state.

But both these viewpoints to some extent beg the question of what scientists should be doing now to stave off some of the more alarming prospects conjured up by so-called genetic engineering. The consensus at the meeting was that although the prospects are still remote, possible consequences of research in molecular biology should now be borne in mind. The galaxy of talent laid on to address the meeting—nine Fellows of the Royal Society and three Nobel Laureates—ensured that the hall was filled and, as one member of the BSSRS unashamedly put it, that the meeting received wide press coverage. The papers presented fell into two chief categories. On the one hand, the present state of molecular biology was outlined and its social implications were aired, and on the other, the relationship between science and society was discussed.

The foundations for much of the discussion about genetic engineering were laid by Professor W. Hayes (Edinburgh), who outlined some of the chief concepts in molecular biology, and this theme was taken up by Professor M. R. Pollock and Professor G. H. Beale (Edinburgh). Both argued that the "horror stories" of genetic engineering are completely out of the question, at least in the foreseeable future, but, as Professor Beale put it, "it is no use smugly glossing over the possible harmful effects" of research in molecular biology. According to Professor Pollock, however, the real problems to tackle are those which involve eliminating some of the more common genetic mutations in man. "It is not too early to start thinking", he said, "of ways in which mutations such as those which cause gross mental deficiency and genetic haemophilia should be tackled." But it is a long step from defining the problems to devising a technique for solving them.

Professor Beale brought up the question of the dilemma facing scientists over the suggestion that males with XYY chromosomes may have a tendency towards psychopathic behaviour. If the theory is correct, there is a danger that perfectly normal males with XYY chromosomes will be wrongly labelled as potential psychopaths, and registration of a person's chromosomes will only reinforce such mislabelling. According to Professor Hayes, however, misconceptions about the relevance of XYY to psychopathic behaviour are based on quite inadequate information, and the chief problem is to prevent such suggestions from being taken as fact.

A salutary reminder of the limitations of research in molecular biology was given by Professor J. Watson (Harvard). "There is certainly enough money for applied cancer research", Professor Watson said, "and there will probably always be an excess of money in this field", yet "nobody should have any illusions that there will be a rapid answer". Professor Watson forecast that research during the next ten years may lead to an understanding of the chief biochemical differences between cancer cells and normal cells, but a cure for cancer still lies in the very distant future. He pointed out, for example, that if the cause of a particular cancer is a mutation in a single gene, it could take as much as one hundred years to pinpoint that gene.

As far as the funding of cancer research is concerned, Professor Watson said that a legislator always wants a solution to a particular problem immediately, preferably within his own term of office, and it is difficult to convince him that probably three decades of science are required to solve the cancer problem. But money will always be available for cancer research and Professor Watson argued that although much of it will be wasted, "if only 10 per cent were spent sensibly, it would be a greater percentage than that of most of the money spent in the United States".

A field of research which has been particularly prominent in all the speculation about genetic engineering is that of fertilization outside the body. Dr R. G. Edwards (Cambridge), whose research led last year to the controlled fertilization of a human egg *in vitro* and who is aiming, with Dr Patrick Steptoe, to implant such a fertilized egg back into the uterus to develop to term, discussed the implications

of his work. The crucial question, Dr Edwards maintained, is to balance the short-term benefits of such research against the possible consequences of genetic engineering. There is little doubt, he said, about the possible benefits. His research is likely to lead, in the relatively near future, to a technique which will enable women who have blocked Fallopian tubes to have a baby. It may also lead, in the more distant future, to a treatment for sex-linked genetic disorders, by selectively implanting sexed blastocysts back into the uterus.

But the methods employed by Dr Edwards may themselves lead to abnormal foetuses, and this raises the question of whether or not the foetus should be aborted. "There is no clash between scientific and medical ethics", Dr Edwards argued "if the rights of the patient and the foetus are safeguarded at all times". It should be up to the patient to decide whether or not the pregnancy should be terminated, and it is important at all times to balance the rights of two stages of foetal development. For example, use of the intrauterine contraceptive device implicitly balances the right of the blastocyst against the rights of the parents. Such a concept, Dr Edwards argued, obviates the necessity to define a point in foetal development at which it is considered that life has begun. Familiar arguments about whether or not life begins at conception or at birth cloud the issue.

But it was left to the audience to point to some of the more bizarre consequences of genetic engineering and the so-called test-tube baby. It was generally regarded that many of the speakers had skated round the more controversial problems, and the suggestion put forward by Professor G. Pontecorvo (ICRF) that the meeting was "a dreadful display of smugness and self-congratulation" received considerable support. "We are deluding ourselves", Professor Pontecorvo said, "if we regard genetic engineering as science fiction". He predicted that, within four or five years, molecular biologists would be capable of minor genetic modification aimed at preventing some genetic disorders.

What, in any case, can the individual scientist do to prevent the more bizarre and frightening developments in science from taking place? Or, more pertinently, can the social consciences of individual scientists somehow be fused into a coherent campaign for social responsibility in science? Here, the interrelationships between science and society cannot be ignored, and this was the other chief theme running through the conference. Debate on these issues was necessarily more vague and less coherent, and suggestions ranged from a self-policing system in the scientific community to excommunicate scientists engaged on, for example, warfare research, to radically altering society itself. The responsibility of the individual scientist to communicate his research findings, together with their possible implications to the public, was also often mentioned but inadequately discussed.

Professor J. Monod (Pasteur Institute) fired the first shots in the debate about science and society. He argued, during the opening session of the conference, that the values of science and the values of society are incompatible—science is founded on an objective valuation of knowledge, while society rests on a foundation of beliefs and ethics. But, Professor Monod maintained, the development of science itself relies on a series of value-judgments by scientists, and it is essential that these values be made explicit both to the scientist and to the layman. "Society feeds off science like a junkie", he suggested, and he foresaw many problems for society if it accepts scientific discoveries without at the same time adopting some of the objective values of science as well. It was also Professor Monod who asked, but did not answer, the question of what a scientist should do if his research produced results which were clearly open to misuse.

Professor Monod's thesis that scientific values and the values of society are incompatible was taken up by Professor J. Bronowski (Salk Institute), in the final lecture at the conference. Professor Bronowski argued that it is the duty of the scientist always to tell the truth. "Science", he said, "is an endless search for truth, and those that devote their lives to it must accept a stringent discipline. For example, they must not be a party to the hiding of truth, for any end whatever. . . . Science therefore rejects all those devices of expediency by which men who seek power excuse their use of bad means for what they call good ends". Herein lies the burden of Professor Bronowski's message, that the ethics of science and the ethics of governments are so disparate that scientists should "disentangle themselves from the apparatus of state".

"A pervasive moral distortion, a readiness to use any means for its own ends, warps the machinery of modern government", Professor Bronowski argued, and because most of the finance for scientific research now comes from government sources, the scientist is increasingly having to conform to the goals of governments in his activities. He therefore faces "a choice between two moralities: the morality of science and the morality of national and government power", and his only hope of maintaining his integrity lies in disassociating himself from government machinery. Pressed by a questioner to define how this should be done, Professor Bronowski suggested that scientists alone should control the funds for their own research, because only in this way could science be directed along the paths leading to a search for truth, and still remain free from the corruption of power.

But this élitist argument did not appeal to many of the participants in the meeting, who argued that science and its values can never be separated from the values of the society in which it is practised. In a closely argued lecture, for example, Dr R. M. Young (Cambridge) maintained that value-free science is a myth. Drawing on the examples of the debate about evolution in the nineteenth century, and of Lysenko genetics in the Soviet Union, Dr Young concluded that "science is no substitute for morality or politics nor is it independent from them". Moreover, "ideology is an inescapable level of discourse and is needed (in the first instance) to debate conflicting ideological positions and (in the last instance) to face and resolve the inevitable conflicts between needs and goals of men in the appropriate way".

The meeting was therefore swept by opposing winds. The debate was often muddled and incoherent, jumping from issue to issue, with participants putting forward well-worn arguments. But the chief value of the meeting was that the issues facing many biologists now and during the next ten years were given an airing, and there will also be a chance to read the full proceedings in a less confused and heady atmosphere when they are published next year.

NEW WORLD

Fulfilment Lags Promise in Biology

by our Washington Correspondent

A MASSIVE increase in the scale of government support for the life sciences is urged in a survey issued this week by the National Academy of Sciences. The gist of the argument is that in all the nationally important problems on which biology can be brought to bear—whether in curing disease, controlling population, improving agriculture or safeguarding the environment—the present biological research endeavour is being depressed below its full capability by a severe shortage of funds.

The new report*, the last in a series of surveys of various scientific disciplines, is the work of a committee with Dr Philip Handler as chairman in the days before he became president of the National Academy of Sciences. Based in part on a couple of questionnaires sent out in mid-1967 to more than half of the 24,000 biological research workers in the United States, and to more than 1,000 heads of academic departments, the report provides a wealth of statistical insight into how biologists spend their time and money, what instruments they need, use or lack, and what they would do with more money if they had it. From this information together with an appraisal of the present frontiers of biological research, Dr Handler's committee has arrived at a number of general conclusions concerning both the directions that should be pursued by pure and applied researchers and the ways in which the organization and support of biological research should be remoulded to support these pursuits most efficiently. To a large extent the proposed remoulding is simply a call for more money, and the committee has furnished what critics may regard as a shopping list, but what may more usefully be seen as an ordering of priorities. Among the chief proposals of the National Academy of Sciences committee are the following:

● SUPPORT OF ACADEMIC RESEARCH. Federal government funds for research in the last financial year were "approximately 20 per cent less than required to ensure that the nation's truly qualified academic life scientists are fully and usefully engaged". The committee urges that this deficit be made up as soon as possible and that thereafter "the research-support system grow at a rate commensurate with the ability of the system to utilize such funds efficiently and wisely". The committee believes that a 12–15 per cent growth rate is desirable.

● INSTITUTIONAL SUPPORT. Graduate and professional education and research should be supported by the federal government but the practice of paying faculty salaries out of research grants constitutes a "most undesirable distortion of the internal arrangements of academic institutions". For this reason programmes of block support should be instituted to enable universities to pay all faculty staff out of funds controlled by their own administrators.

● GRADUATE EDUCATION. The budget for graduate education in the present financial year is a serious underestimate of the need. "It is our hope that appropriations for these programmes will be doubled over the course of the next five years." The National Science Foundation should expand its training grant programme along the lines of that conducted by the National Institutes of Health.

● UNIVERSITY CURRICULA. The committee notes that the pace of research has long since outstripped the pace of educational reform in the life sciences; a new curriculum containing the core of material central to all biological sciences should be prepared and presented to all undergraduate students majoring in biology.

● DEPARTMENT OF AGRICULTURE. The committee regards as unfortunate the failure of the Department of Agriculture to support biological research conducted outside the agricultural schools and its own in-house laboratories; the Department should set up a broad research grant programme similar to that administered by the National Institutes of Health.

● HEALTH RESEARCH. Within the budget of the National Institutes of Health, research for improving methods of delivering health care should not be allowed to grow at the expense of funds for basic research.

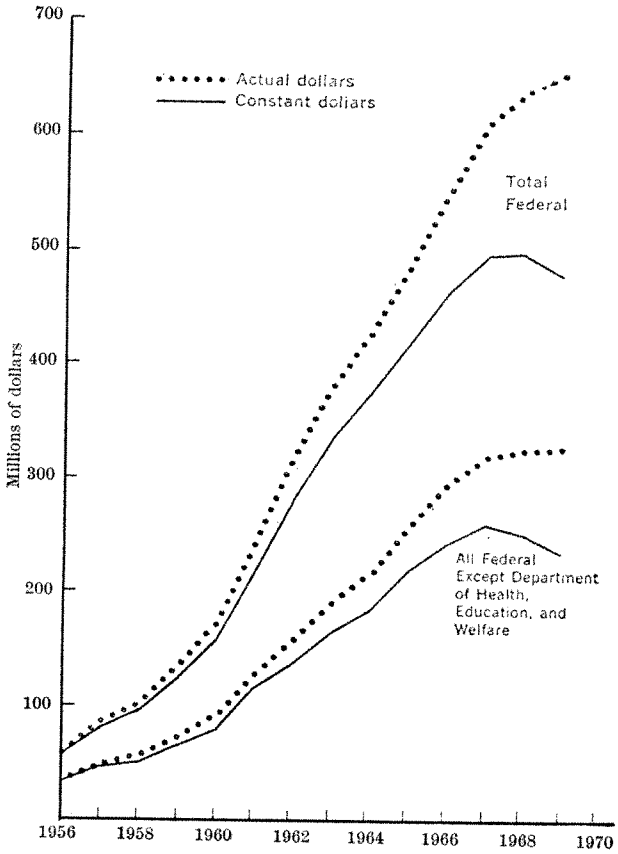


Fig. 1. Federal support for research in the life sciences expressed in absolute dollars (continuous line) and in 1965 dollars (broken line).

*_The Life Sciences_ National Academy of Sciences, 1970. Pp. 526.

Fig. 2. Reasons given for limitations of research programmes

Limiting factor	Academic scientists		Non-academic scientists	
	Number	Per cent	Number	Per cent
Grand total	7,007	100	3,076	100
Space	3,529	50	1,277	42
Budget for:	5,399	77	2,187	71
Supplies	1,779	25	371	12
Equipment	2,806	40	803	26
Professional staff	1,554	22	957	31
Technicians	3,353	48	1,454	47
Clerical-Administrative	1,169	17	404	13
Student stipends	1,742	25	196	6
Postdoctoral stipends	1,621	23	339	11
Computer time	245	3	100	3
Travel	1,329	19	661	21
Insufficient research time due to:	3,429	49	1,118	36
Heavy teaching responsibilities	1,851	26	75	2
Service	848	12	371	12
Administration	1,913	27	867	28
Unfilled funded positions for:	1,446	21	725	24
Professional staff	759	11	430	14
Technicians	832	12	432	14
Clerical-Administrative	149	2	62	2

● EQUIPMENT. The National Institutes of Health and the National Science Foundation should set aside funds (about $25 million a year) to pay for particularly expensive or unique instruments. The two agencies should also be prepared to grant $100 million a year for constructing laboratories in biological and medical research laboratories (the committee has identified a backlog requirement of about $150 million for construction of laboratory space). Funding agencies should be increasingly prepared to countenance demands for computer use in grant requests, an activity which will soon be consuming some $50 million a year. Money is also needed to sustain museums and upgrade marine biology stations.

The prospects of finding the money for any of these recommendations in the budget now under preparation are dim, to say the least. The Academy's reports covering physics, chemistry and astronomy have not yet been implemented in any significant way and, as Dr Handler conceded this week, science does not have a very high priority at present with the Office of Management and Budget. But even if the specific recommendations should be ignored, the Academy's efforts at delineating the social system in which life scientists work will still be of value, even if only as a benchmark with which to measure the future state of the system.

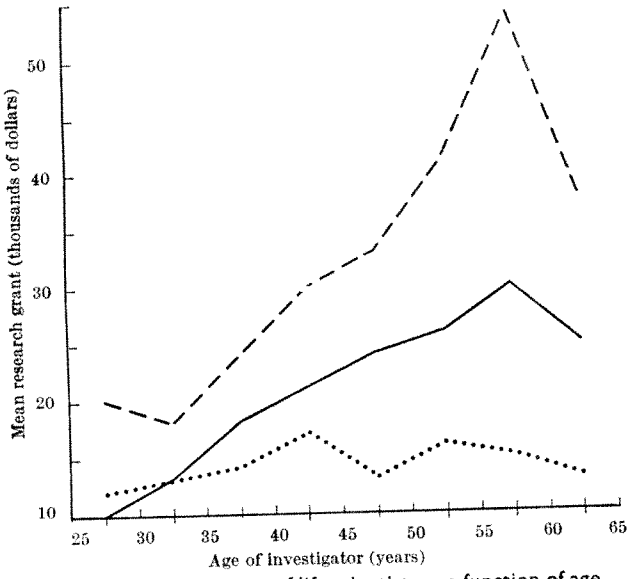


Fig. 3. Research support of life scientists as a function of age. ----, National Institutes of Health; ———, all sources; . . . . , National Science Foundation.

Overall federal support of the life sciences, when expressed in terms of constant dollars, started to fall in 1968 (see Fig. 1). The Academy's questionnaires were sent out too early to record the full impact of this decline, and the inadequacies already apparent in the latter half of 1967 have presumably worsened accordingly since then. For example, the questionnaires identified serious constraints on individual research programmes with respect to laboratory space (cited by 50 per cent of respondents), budget for professional staff (reported by 40 per cent), and other factors related to the level of funding such as time available for research (see Fig. 2). From these statements and others the Academy committee has arrived at the estimate (quoted above) that the deficit between current support of life science research and a level commensurate with current capability is between 20 and 25 per cent.

Both individual scientists and chairmen of academic departments reported unmet needs for a variety of major instruments, with electron microscopes being the first priority of 9 per cent of respondents, followed by amino-acid analysers (top of the list for 8 per cent) and small specialized computers (7 per cent). But instruments rank comparatively low on the overall list of first priority needs to improve current research; of a thousand chairmen of academic departments nearly 300 reported that funds to pay additional faculty were their first requirement, and a quarter rated top priority to stipends for predoctoral students.

Support of predoctoral students is viewed with particular concern by Dr Handler's committee, which states that stipend levels have failed to keep pace with the rising cost of living. The committee also deplores the failure of certain agencies—the Department of Agriculture, the Atomic Energy Commission, the Department of Defense and NASA—to make any significant contribution towards the cost of training PhDs.

Other notable features of the report include the following findings:

● Seven per cent of all budgeted positions were unfilled in 1967, ranging from 5 per cent for schools of agriculture to 8 per cent in medical schools.

● Some 38 per cent of all postdoctoral fellows in the life science research community are not of American origin, and about one sixth of these had obtained their doctoral degrees within the United States; the committee regards this proportion as "not seriously excessive" in view of the contribution of foreign postdoctorals to research progress.

● More than 30 per cent of those responding to the questionnaire indicated their research is very seriously hindered by insufficient personal training in various non-biological disciplines such as chemistry, computer science, mathematics and statistics.

● The average life scientist employed in an institution of higher learning devotes about half his time to research, between 10 and 20 per cent to administration, a quarter to a third of his time in teaching and the rest to "assorted other responsibilities" such as patient care.

UNEMPLOYMENT

No Niche for Aerospace Industry

by our Washington Correspondent

DESPITE the chronic unemployment afflicting the defence and space industries, the Administration has no intention of creating a favoured niche for such companies in the environmental protection business. In hearings held last week before the House Subcommittee on Conservation and Natural Resources to consider how the skills of aerospace companies could be transferred to cleaning up the environment, government spokesmen made it clear that there would be no large-scale federal support for the research necessary to effect such a transfer, and that if the aerospace industries cannot fend for themselves in open market competition, the Administration will observe John Clare's improved commandment, "Thou shalt not kill, but needst not strive officiously to keep alive".

According to a survey published last month by the Aerospace Industries Association, total employment in the aviation and space industry will have dropped by some 16 per cent in the twelve months ending next March, from 1,238,000 to 1,044,000.

Some of this unemployment, which it has long been foreseen would attend the inevitable reduction in military and space spending, could perhaps be alleviated if the aerospace companies were successfully to transfer their skills to the problems of environmental pollution. In its simplest terms, as stated by Representative Henry Reuss in opening last week's hearings, this proposal boils down to thinking that the answer to deterioration of the environment is to "have the same people who got us to the Moon go to work on cleaning up pollution".

One of the troubles with this theory is that the market for anti-pollution technology has remained strangely small, despite the incessant sounds of movement against pollution in Congress, and so far the aerospace companies seem in any case to have captured a rather exiguous share of what business is going. A possible reason for this failure was suggested by Mr Murray L. Weidenbaum, Assistant Secretary of the Treasury for Economic Policy, who despite his former employment as corporate economist for the Boeing Company, had some distinctly unflattering remarks to make about the aerospace industry's attempt at diversification. "There has been a great deal of naïveté in attempting to sell the systems ability of the aerospace industry as a cure-all for public sector needs," he told Congressman Reuss's subcommittee last week. "One industry president has been quoted as saying, 'Creating a system to warn a field army the enemy has launched an attack of germ warfare is basically no different from creating a system to control juvenile delinquency'. Or, to parody Gertrude Stein, a system is a system is a system."

The upshot of this way of thinking is that the various diversification projects of the aerospace industry, ranging from powered wheelbarrows and canoes (which exploited transportation skills) to wall panelling and coffins (which exploited the ability to fabricate light metals) have typically led to meagre profits or outright losses. "Personally", Mr Weidenbaum remarked, "I believe that the focus of the present inquiry needs to be reversed. The national interest is not well served by viewing the question as to how to employ the aerospace industry or any other single industry in solving pollution and other environmental problems. Rather, we need to start with the problems to be solved and then see what technology and other capabilities are needed." The public interest would best be served by letting the aerospace and other companies compete for the emerging environment market, and if the federal government were to allot any relocation allowances, Mr Weidenbaum would prefer to see them go to veterans returning from Vietnam rather than out of work aerospace employees who in general "have been one of the highest paid groups in our society".

The substance if not the cutting edge of Mr Weidenbaum's views was echoed by the President's Science Adviser, Dr Edward E. David, who told the subcommittee in roundabout terms that the Office of Science and Technology is not contemplating any large-scale research programme to help the aerospace industry diversify into the anti-pollution business. Dr David mentioned as an incentive for industry to invest in pollution control technology the possibility of taxing polluters on the theory that the nation's air and water are common property and anyone who discharges wastes into them should pay for the privilege, only to add that he was "too new on the job to have formed an opinion on this option". In any case, to judge from a written appendix to Dr David's testimony prepared by the staff of the Office of Science and Technology, it appears that the OST has yielded precedence on matters of environmental research to the recently instituted Council on Environmental Quality: "Our role with respect to environmental research", the statement explains, "involves a special relationship with the Council on Environmental Quality, which has overall responsibility for environmental concerns at the Executive Office level. OST, as in the past, will be consulted with respect to the research and development initiatives of the agencies and from time to time undertake special reviews and analyses, bearing particularly on the basic science underlying environmental decisions and on the provision of technological alternatives for solving environmental problems."

By limiting his inquiry to the environmental issue, Congressman Reuss seemed to be offering at once too narrow and too diffuse a solution for a real enough problem. A bill proposed over a year ago by Senator Edward Kennedy stands a better chance of tackling unemployment at its roots, though less of winning the favour of the Administration; his Conversion Research Education and Assistance bill would assign $450 million over a three year period for converting people and resources from defence to civilian problems. The bill would establish as national policies that "scientists should have continuing opportunities for employment, in positions commensurate with their professional and technical skill. Second, federal funding for civilian research and development should be raised to a level equivalent to defence-related research and development. Third, the total federal investment in science and technology should continue to grow annually in proportion to increases in our gross national product." The programmes envisaged by the bill would be administered by the National Science Foundation, the Department of Commerce and the Small Business Administration.

CONSERVATION

Axe Falls on Hickel

by our Washington Correspondent

CONSERVATIONISTS lost one of their firmest friends in the Administration last week with the summary firing of Secretary of the Interior, Walter J. Hickel. Mr Hickel, a former governor of Alaska who campaigned effectively for President Nixon in the 1968 elections, was sacked on the eve of Thanksgiving, and six of his closest aides at the Department of the Interior followed him the next day.

Mr Hickel's departure has been only a matter of time ever since his leaked letter at the period of the Kent State shootings in May which criticized President Nixon for being out of touch with students. The strong constituency he quickly built up with conservationists may be one of the factors that have delayed his dismissal until now. Mr Hickel became Secretary of the Interior two years ago to the bitter opposition of the conservationist lobby and after personally humiliating hearings before the Senate Interior committee. Having made his fortune in real estate and construction in Alaska, Mr Hickel seemed unlikely to be sympathetic to environmental issues, an impression borne out by some of his remarks at the time. He had no obvious qualifications for the Interior post and President Nixon's campaign pledge to appoint a Western governor seemed to be the chief factor behind his elevation.

Shortly after he took up office, Mr Hickel must have seen the light about ecology, or at least realized that preservation of the environment was the political rallying cry of the future. With the sudden zeal of the converted, he proceeded to confound his former foes and friends by deciding a number of important issues in a manner quite contrary to what each was expecting of him. After the blow-out at the Union Oil well in the Santa Barbara channel, a few weeks after his appointment, Mr Hickel ordered a cessation of offshore drilling operations in the region and a moratorium on new leasings until stricter regulations had been developed. He had the Chevron Oil company prosecuted when its oil well in the Gulf of Mexico ran out of control in March this year and more recently he instigated suits against companies polluting public waters with mercury effluents.

Mr Hickel's final act in office was his announcement last week that the United States would import no more products made of whale and that eight species of whale—the fin, sei and sperm (these are the main commercial species) together with the bowhead, blue humpback, white and grey whales—were to be placed on the list of endangered animals. Insofar as the United States imports nearly a third of the world's whale products, this decision should be of some help in saving whales from extinction.

Had Mr Hickel stayed in office three days longer, his views on the Alaska pipeline would have been known. The proposed pipeline, which would carry oil from the North Slope strikes down to the ice-free port of Valdez on Alaska's southern coast, has been opposed by conservationists on the basis that if built underground the heat of the oil would melt the permanently frozen soil, causing erosion, earth slides, and the destruction of the fragile tundra, while if built above ground the pipeline would obstruct the migration paths of the caribou. The oil companies involved on the North Slope have already bought some 600 miles of pipe.

A decision on the pipeline—there are also native land claims and a conservationist injunction to be settled before any construction can begin—will now be delayed until after Mr Hickel's successor has taken office in the new year. He is Mr Rogers C. B. Morton, a Representative from Maryland now serving his fifth term and chairman of the Republican National Committee. Mr Morton holds no particular record as saviour of the environment but is said to be much devoted to fishing. On a recent rating prepared by the League of Conservation Voters in which Representatives were scored for their voting record on ten environmental issues, Mr Morton unfortunately received minus 14 points out of a maximum of plus 28 (pro-environment) and a minimum of minus 28 (anti-environment).

RESEARCH FUNDING

Mansfield Champions Science

by our Washington Correspondent

A STRONG statement in favour of keeping the national research effort at least up to its present strength was made by Senator Mike Mansfield, the Majority Leader, during the Senate debate on appropriations for the Department of Health, Education and Welfare. Senator Mansfield, author of the now defunct amendment that restricted the Pentagon's research to matters with obvious military relevance, said in the Senate debate on November 20 that "for the Federal Government to act in a responsible manner, the civilian Federal agencies that sponsor basic research should have their budgets increased at least by an amount reducted from the Department of Defense. In fact, the amounts should also reflect the loss of the research purchased because of inflation".

Commenting on the bill's provision for research at the National Institutes of Health—the recommendation is for a $129 million or 12 per cent increase over the sum requested by President Nixon—Senator Mansfield said he believed these additional sums were "perfect examples of money well spent and the re-allocation of resources through the civilian sector which might otherwise have been channelled through the Defense Department. I would hope that the additional moneys recommended by our committee and earlier by the House of Representatives will be expended so that the great pressures on the research community in this country will be alleviated". Final action on the bill depends on reconciliation of the House and Senate versions, both of which include considerably more for medical research than requested by the Administration. Only the Senate version, however, includes provision of $15 million for the conversion of the biological warfare facilities at Fort Detrick to civilian use.

SELENOLOGY

Moon Revealed by Apollo 12

by our Astronomy Correspondent

It is now a little more than a year since the last Moon landing, and NASA marked the anniversary by gathering together some of those responsible for the instruments left behind on the Moon, and one of the astronauts who put the instruments there, Alan Bean. But with the possibility of further cancellations in the Apollo series being openly recognized, the event was not as cheerful as it might have been. Dr Frank Press of MIT, who was there to provide a summary of what has been achieved by the Apollo instrument package, said that it would be a pity if the exigencies of the moment were to deflect the United States from the further exploration of the Moon. The meeting also caught NASA employees looking over their shoulders at the Soviet successes with Luna 16 and 17. Alan Bean for one poured some cold water on the Soviet attempts to explore the Moon from the safety of a control room on the ground: "Apollo 11 brought back 50 pounds of rocks. We brought back 75 pounds. And the Russians on their last one brought back two-tenths of a pound. If the Russians were bringing back 50 and 75 pounds and we were bringing back two-tenths of a pound, think how you'd feel. I don't think we'd be so doggone excited as we (*sic* they ?) are about their two-tenths of a pound".

All the experimenters had fascinating tales to tell, but it is the results from the seismometer which have captured the imagination. It is now clear, for example, that tidal strain is causing moonquakes when the Moon is at its nearest to the Earth, an effect which has been looked for on the Earth many times without success. If the only strain present was due to the tidal effect the slip would be first in one direction, and then the other, but oddly the indications are that the slip is always in the same direction. This is interpreted to mean that there is another source of strain within the Moon whose release is triggered once a month by tidal effects, and Dr Gary Latham (Lamont-Doherty Geological Laboratory) and his collaborators have recognized nine locations around the Apollo 12 station where the strain is being released. Each location gives a set of signals that are identical in every respect, and there are nine sets of these matching records, one of which appears to originate within the Fra Mauro crater close to a set of rilles and not far from the Apollo 14 landing site. Fortunately with moonquakes being no stronger than between magnitude 1 and 2 on the Richter scale, there seems to be no threat to the Apollo 14 crew.

What the source of the strain can be is unknown. The total energy release amounts to only 10^{-12} that of the annual energy release by terrestrial earthquakes, however, and this seems to rule out the possibility that the interior can be molten except in a few minor pockets. It could be, Latham speculated, that the Moon is expanding slowly as a result of radiogenic heating. Other possibilities being considered are that a residual effect from large impacts is being observed, or that local hot spots are responsible.

A second type of moonquake which does not fit the monthly pattern has been attributed to the impacts of meteorites, and the frequency and strength of these moonquakes should lead eventually to an estimate of the amount of rock-sized fragments in space. As a preliminary indication Latham and his group say that kilogram-size meteorites strike the region up to 200 km from the seismometer on average once a month.

What do the experiments say about the interior constitution of the Moon ? The key here comes from the seismometer records following the two artificial impacts. The famous reverberations are taken as indicating that the mare is made up of loosely consolidated rubble that becomes increasingly compacted with depth down to at least 20 km, the greatest penetration reached by the artificial signals. This of course rules out a lunar equivalent of the Mohorovičić discontinuity, but leaves several possibilities for the origin of the surface material—pulverization of the original rock by impacts; removal of the original rock by a large impact, followed by flooding of the crater with lava and subsequent pulverization; or even that the rubble represents material swept up from the solar system during the final stage of accretion of the Moon.

Information about depths greater than the 20 km penetration of the seismic signals comes from the magnetometer which indicated a magnetic field of 35 gamma, only 10^{-3} that of the terrestrial field at the equator but more than people expected assuming a cold Moon. This appears to be a paleomagnetic field which could have originated during a conjectured close approach into the Earth's magnetosphere, or by the dynamo process which in turn implies that the Moon at one time had a hot interior. More important for an understanding of what the interior of the Moon is like at present is the comparison of the field measured at the surface of the Moon with that recorded by the space probe Explorer 35 in orbit around the Moon. The comparison indicates that the solar magnetic field is generating eddy currents inside the Moon, detected by the surface magnetometer, and the decay of the currents gives values for the conductivity and temperature. According to Dr Charles P. Sonett (Ames Research Center) reporting for the magnetometer team the data fit a model in which the temperature is increasing into the Moon by 2 or 3 degrees per kilometre. Sonett suggested a core temperature of perhaps 1,000° C, and if this is not exceeded at the centre then none of the rock need be molten.

The other experiments left by Apollo 12—the solar wind spectrometer and the suprathermal ion detector—refer to the environment around the Moon, chiefly the solar wind, but measurements taken following the impact of the Apollo 13 Saturn 4B stage might have something to say about the constitution of the lunar surface layers. As well as plotting the time variation of the solar wind, particularly as the Moon swings through the Earth's magnetotail, the two instruments picked up what seemed to be swirling clouds of ions shortly after the rocket impact. Oddly enough, the impact was downwind of the instrument package. The suprathermal ion detector recorded the cloud twenty seconds after the impact as a burst of ions with energies up to 500 eV. The acceleration mechanism is something of a mystery. Within a minute or two of the impact the cloud was observed by the spectrometer. According to Dr John Freeman (Rice University) the origin of the ion clouds is less likely to be the remaining fuel in the rocket's tanks, than gases such as helium, argon and krypton held in the surface layers of the Moon.

NEWS AND VIEWS

Footprints of Human Cancer Viruses?

THE discovery that RNA tumour viruses contain polymerase activities capable of reversing the familiar direction of genetic transcription, by using RNA as a template for the synthesis of double stranded DNA, has from the beginning promised an extraordinarily rich harvest for cancer researchers, and, after only six months, the first fruits are being gathered. At the recent Lepetit colloquium on the biology of tumour viruses (*Nature*, **228**, 609; 1970) several groups announced, after weeks of rumours and anticipation, the detection of reverse transcriptases in human leukaemic cells. This week, *Nature* includes what seems to be the first detailed account of this momentous discovery.

On page 927, Gallo, Yang and Ting report that extracts of the leukaemic lymphoblasts of three patients with acute lymphoblastic leukaemia contain an RNA dependent DNA polymerase activity. This activity cannot be detected in extracts of normal lymphoblasts taken from forty-eight healthy people, even after the cells have been stimulated to divide by exposure to phytohaemagglutinins. The extracts which Gallo and his colleagues have made are only partially purified and the RNA dependent DNA polymerase activity is admittedly very low; further, their assay conditions are clearly very far from optimal. But in spite of these limitations they have been able to characterize some of the properties of the polymerase. It is dependent on magnesium ions, on the presence of all four deoxyriboside triphosphates and on the addition of an RNA template, either rat liver RNA or the synthetic RNA poly rA·poly rU. Moreover, N-dimethylrifampicin, a derivative of rifampicin which Green and his colleagues have shown inhibits the RNA dependent polymerase activity in RNA tumour viruses, inhibits the polymerase activity in the extracts of leukaemic cells, although complete inhibition is only achieved with very high concentrations of the antibiotic. Finally, the product made by the reverse transcriptase in leukaemic lymphoblasts is resistant to hydrolysis by ribonuclease and alkali but is degraded by deoxyribonuclease.

All these characteristics point to one conclusion, namely, that the activity Gallo and his colleagues have detected in the leukaemic cells is essentially identical to that in the avian and mammalian RNA tumour viruses. Any doubts about this were removed by the more extensive investigations reported at the colloquium by Spiegelman and his colleagues, who, using a better assay system and more purified extracts, had then detected a polymerase activity, dependent on synthetic RNA/DNA hybrids, in extracts of cells from nine leukaemia patients and have since considerably increased this tally. But what is the significance of these findings? Clearly they raise the hope that a sensitive assay for reverse transcriptases may prove to be, at the very least, a useful tool for the diagnosis of leukaemia and perhaps other forms of cancer, if the cells of solid tumours are also found to contain these activities. Furthermore, should it prove that reverse transcriptases only occur in tumour cells of adults, it may be possible to exploit their presence in chemotherapy.

The discovery of these enzyme activities in human leukaemic cells can be interpreted also as suggestive evidence that at least some human cancers have a viral aetiology. So far the search for human, or for that matter primate, RNA tumour viruses has proved fruitless. The events of the past six months, however, should not only add impetus to the search but also provide new experimental approaches to the problem. For example, Todaro and his colleagues announced at the Lepetit colloquium that antibodies against the group specific antigens of murine sarcoma viruses inactivate the reverse transcriptases not only in the murine sarcoma and leukaemia viruses, but also those in the corresponding rat, cat and hamster viruses. The outcome of experiments to test whether these antibodies inactivate the polymerase activities in human leukaemic cells are awaited with interest. Should the result be positive and should significant inhibition be detected, the obvious implication will be that human leukaemia viruses do exist. And it would be no great surprise if the only footprints these putative human cancer viruses leave behind as a clue to their existence consist of the reverse transcriptases, for, as Aaronson, Todaro and Rowe have shown, when murine sarcoma

virus transforms certain mouse cells growing in tissue culture the transformed cells neither bud progeny virus nor contain any detectable viral antigens. Whether these murine cells gain the two polymerase activities as a result of transformation has yet to be determined.

Unlike a great deal of current research into tumour viruses and their interactions with cells, which in the short term at least seems to have all too little relevance to cancer as a clinical disease, the work on reverse transcriptases may quite soon find clinical applications. Pollack, Wolman and Vogel, who on page 938 of this issue of *Nature* report an analysis of the correlation between the malignancy and the chromosome count of spontaneously and virally transformed cells grown in culture, suggest that their work may also have clinical interest. Pollack and his colleagues find that an increase in the number of chromosomes in a transformant correlates with a loss of several of the characteristics of the transformed state. It is as if an increase in the number of chromosomes somehow suppresses the transforming effect of a resident viral genome in a transformed cell, and the parallels between this observation and the suppression of malignancy by fusing a malignant and a non-malignant cell, which Harris and his colleagues have reported, are obvious enough.

Pollack and his colleagues report, for example, that mouse cells of a line transformed by SV40 have a median chromosome count of 75. Revertant cells selected from this line exhibit many of the properties of untransformed cells—like untransformed cells they grow to a low density in culture, they are not agglutinated by plant agglutinins and they are less malignant than transformed cells—although they retain the SV40 genome, and these revertants have a median chromosome number of 118. Further, cells with the phenotypic characteristics of transformants back selected from the revertants have a median chromosome number of 72. In other words, when revertants shed chromosomes they return to the transformed state.

The molecular mechanism by which an increase in the number of chromosomes leads to the selective inactivation of the transforming function of tumour viruses are at present obscure although it is open to experimental analysis. But irrespective of the mechanism by which hyperploidy may suppress malignancy, Pollack *et al.* point out that their observations may explain why relapses so often follow remissions during the chemotherapy of human cancers. In essence they suggest that many chemotherapeutic drugs now in use are known to have, or may well have, the potential to induce hyperploidy in cells. It may well be that although such drugs kill most cancer cells they induce the formation of a reservoir of hyperploid revertant cells. Such revertants would have growth properties similar to undiseased normal cells and they might well survive the killing effect of further doses of the drug. At the same time they might act as a persistent source of back revertants, cells with a low chromosome number and high malignancy. Such back revertants would proliferate once chemotherapy was stopped and so change remission into relapse.

PROTEIN STRUCTURE

Facts and Prediction

from our Molecular Biology Correspondent

Some little time has now passed since the notion was first seriously mooted that it might be possible to "explain" the structure of globular proteins, in the sense that the native (by implication lowest-energy) conformation could be uniquely predicted from the sequence, by taking into account only a modest number of long and short-range interactions. Encouraged by a degree of success in calculating the stablest structure of homopolypeptides, and beguiled by the glitter of their objective, a number of groups of workers followed this *ignis fatuus* into a theoretical morass, in which they have for the most part now been lost to view. True that some interesting lessons emerged. Scheraga and his colleagues (Lewis *et al.*, *Proc. US Nat. Acad. Sci.*, **65**, 810; 1970) have recently, for instance, calculated the tendency towards α-helix formation in random chains corresponding in sequence to the globular proteins of known structure. Such calculations (which some theoreticians might even feel it is jumping the gun to contemplate at this stage) demand some intrepid assumptions, but the strategy is interesting, and the results so far even somewhat encouraging: those parts of the chain, it seems, which are most likely to be α-helical tend also to be helical in the native structure. That is to say interactions between different parts of the chain in the compact globular molecule do not override the secondary structure that the sequence alone tends to impose within each segment.

This is not perhaps a startling result, but tangible none the less. Atassi and Singhai (*J. Biol. Chem.*, **245**, 5122; 1970) have prepared fragments of myoglobin, and examined their conformation by optical rotatory dispersion. All contain an appreciable proportion of α-helix, but much less than in the protein. Thus a 32-residue fragment from a long helix in the protein is no more than one-third helical in water. Earlier work also suggests that this is a very general situation, and that inter-segment interactions are of extreme importance.

The travails of the theoreticians lend added point to experimental attempts to come by the rules of chain folding by way of non-covalent recombination of fragments. This approach has been pursued to great effect in Anfinsen's laboratory, especially on staphylococcal nuclease. In the latest paper, Chaiken and Anfinsen (*ibid.*, 4718) have synthesized a series of N-terminal fragments for recombination with the C-terminal residuum, comprising residues 49–149. The fragment 6–47, for example, recombines to give a substantially active enzyme. Fragments 9–47 and 10–47 also regenerate activity, but as judged by proteolytic resistance of the product, and the fluorescence change, which shows the extent to which the one tryptophan is engulfed in the refolded structure, they bind much less strongly. Shorter fragments, from 11–47 to 18–47, give no active product. Even here, however, their presence promotes the association of the residual chain with antibodies to the native enzyme, so that at least in the ternary system features of the native structure must be present in tangible concentration. Evidently a few residues, notably 9 and 10, are involved in conformationally crucial interactions, which direct reassociation with refolding.

Ripples, meanwhile, are still spreading from the first successful experiment on recombination of fragments by Richards, who regenerated activity from the S-peptide (residues 1–20) and the residuum (S-protein) of the pancreatic ribonuclease chain. It was later found possible to remove several residues on either side of the join without serious detriment to the recombination. Liu, Gutte, Moore and Merrifield (*ibid.*, 5169) have now started at the other end of the chain. Removal of the last six amino-acids (119–124), which include three active site residues, leaves an inactive protein. It will recombine with a synthetic C-terminus, 14 residues long, with revival of activity. The eight superfluous overlapping residues evidently do not get in the way, but when 4 or 5 residues only are detached from the C-terminus, there is evidently some hindrance. An active species can also be produced by mixing S-peptide, the C-terminal peptide and a central core of truncated S-protein.

A special conformational role for the C-terminus emerges from the work of Taniuchi (*ibid.*, 5459), who has pursued the observation that ribonuclease deprived of its last four residues is inactive, but not conformationally disordered. In this form, however, the chain will not refold correctly from the reduced state: evidently random disulphide pairings occur, a vital element of information having been lost with the C-terminus. This supports once more the notion that the chain will not assume its native topology during biosynthesis until it is essentially complete.

TUMOUR VIRUSES

Mutants in the News

from our Cell Biology Correspondent

As the experiments of Burger and Noonan (*Nature*, **228**, 512; 1970) prove, changes in the surface of cells, which can be assayed by the binding of plant agglutinins, seem to play an important part in maintaining a cell in the transformed state. It is as if a tumour virus or other transforming agents cause a permanent exposure of the surface layer containing the agglutinin receptors and as a result the cell cannot stop dividing. If this is the case, mutants of tumour viruses, which have lost the capacity to transform, should fail to cause the exposure of the surface layer in infected cells.

Happily Benjamin and Burger (*Proc. US Nat. Acad. Sci.*, **67**, 929; 1970) report just that result. They have measured the capacity of two agglutinins, wheat germ agglutinin and concanavalin A, to agglutinate Swiss mouse 3T3 cells infected with a mutant polyoma virus NG18. This mutant is one of a suite of host range mutants isolated by Benjamin which I discussed a few weeks ago (*Nature*, **228**, 414; 1970). It can only replicate in Py-3T3 cells, 3T3 cells already transformed by polyoma virus, and it fails to transform either abortively or stably normal hamster or rat cells in culture. As anticipated, 3T3 cells infected with NG18 and three similar mutants are not rendered agglutinable as a result of infection. 3T3 cells infected with wild type polyoma virus, on the other hand, support the replication of the virus and are rendered agglutinable. Experiments with inhibitors of DNA synthesis prove that, in 3T3 cells infected with wild type virus, the membrane change assayed with the agglutinins depends on the induction of DNA synthesis. Further, several experiments with various cell and virus combinations which have been reported by various groups indicate that cellular rather than viral DNA synthesis is required for the exposure of the agglutinin surface layer. But the induction of cellular DNA synthesis, although necessary, is not sufficient to induce the surface change, because NG18 induces cellular DNA synthesis in 3T3 cells but does not render the cells agglutinable. This mutant therefore uncouples the induction of DNA synthesis and the surface change. Either an additional function, which wild type virus can provide but NG18 cannot provide, is also necessary in addition to the induction of DNA synthesis, or the DNA synthesis induced by NG18 differs somehow from that induced by wild type virus.

Clearly, although the mechanism of the induction of the surface change has still to be elucidated, these experiments with NG18 strengthen the suggestion that the exposure of the agglutinin sites is functionally correlated with a loss of growth control. These experiments also draw attention again to the paradox that although host DNA synthesis seems to be necessary for the surface change, the exposure of the agglutinin sites seems to lead to DNA synthesis, for, when resting, untransformed cells are treated with trypsin, the agglutinin sites are exposed and the cell then replicates its DNA and goes through at least one round of mitosis. Presumably in the cell cycle, exposure of the sites leads to DNA synthesis and division, and DNA synthesis is a necessary but not sufficient prerequisite for the subsequent exposure of the agglutinin sites and yet another round of synthesis and division. In this scheme a transforming agent is envisaged as a master switch which ensures that the surface sites are always exposed and the cell is always dividing.

While work with mutants of polyoma virus is progressing rapidly the first temperature sensitive mutants of SV40 are being characterized. Kit *et al.* (*J. Virol.*, **6**, 286; 1970), for example, describe the properties of an SV40 mutant defective in some function, other than the formation of viral capsids, which is required late in the replication cycle of the virus. It is to be hoped that a concerted effort will be made to select mutants of SV40 defective in the function(s) necessary for transformation, because SV40 has one great advantage over polyoma virus for the experimenter; the SV40 genome, unlike the polyoma virus genome, can readily be rescued from a transformed cell.

GENETICS

Neurophysiology of *Drosophila*

from a Correspondent

GYNANDROMORPHS, or sexual mosaics, are individuals in which a portion of the body is composed of mutant cells of one sex, in contrast to the rest of the body which is composed of normal cells of the other sex. The division line between normal and mutant parts can occur in various orientations. Mutants of five different genes on the *X* chromosome of the fruit fly, *Drosophila melanogaster*, that cause abnormalities in visual function have been tested by the construction of gynandromorphs in which the mutant part of the

body was male. All of these visual mutants have been found to be autonomous, that is, the mutant eye always functions as mutant tissue, independent of the amount of normal tissue present elsewhere. Thus, at least in this series of mutants, the primary causes of the abnormality in the mutant eyes are within the eye and not elsewhere in the nervous system or brain.

Hotta and Benzer (*Proc. US Nat. Acad. Sci.*, **67**, 1156; 1970) selected mutants of *Drosophila*, carrying the required *X*-linked recessive mutation plus marker genes, and they crossed these flies with females of a strain that often loses the *X* chromosome spontaneously during mitosis. About 10 per cent of the female progeny were found to be mosaics. By counter current distribution, the mutant males were selected to be negatively phototactic and gynandromorphs were selected which had one eye of the normal genotype (that is, positive phototaxis) and the other eye of the mutant type (deficient visual function).

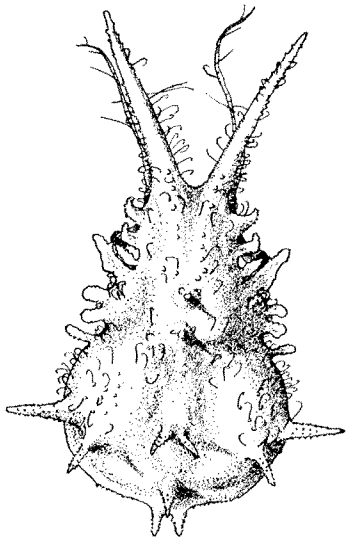
Several of the gynandromorphs were tested for their phototactic response. All the composite flies expressed the visual deficit of the mutant, and parabiosis with a normal half of a fly did not remove the deficit. The response to a short flash of light was measured in these gynandromorphs with the aid of electroretinograms (ERG). The ERG can be tested separately in each eye of a mutant fly. ERGs obtained from the female eye, in which the normal allele is present, were the same as for a normal fly. The ERG recorded from the male eye, in which the recessive mutation was uncovered, was defective in the same way as in a non-mosaic fly. As before, the normal half in parabiosis with the mutant half did not compensate for the defect in the mutant. For flies with heads which had normal and mutant portions, the same result was obtained in every case. Thus, these mutations are clearly autonomous and reside within, or close to, the eye. In six eyes in which the line of mosaic cells occurred within the eye, there seemed to be a high degree of autonomy between those segments of the eye that were normal and those that were deficient. Whatever the genetic defect in these flies, it is clearly expressed only in the mutant parts.

Similar results have been reported by Ikeda and Kaplan (*Proc. US Nat. Acad. Sci.*, **67**, 1480; 1970), who have studied the behavioural phenotype caused by a sex-linked, single gene mutation in gynandromorphs of *Drosophila*. The gene, *hyperkinetic*, controls a leg-shaking action during the administration of ether anaesthesia. In their studies the male tissue of the gynandromorph was hemizygous for *hyperkinetic* whereas the female tissue was heterozygous. The characteristic neural activity caused by the *hyperkinetic* gene can be recorded only in the presumptive male region of the thoracic ganglion. Motor regions of the male side were found to function independently of their counterparts on the female side of the fly and they were unaffected by the genotype of other parts of the body. Thus, expression of the *hyperkinetic* gene is genetically autonomous, and each side of the ganglion is independent of the other. There is also a high correlation between the external genotype and the genotype of underlying neural tissue of the thorax, but the correlation between external and internal genotype is not absolute.

Although these studies are incomplete, a start has been made in the application of the techniques of bacterial genetics to the problems of understanding the role of the brain and nervous system in higher organisms. It will be interesting to observe the results of future studies using mutants with a lesion which can be mapped at a site distant from the sensory organ.

CRUSTACEA

New Spider Crab



Among the benthic invertebrates dredged up some time ago off Rottnest Island, Western Australia, was this strange looking animal which D. J. G. Griffin now describes as a new species of spider crab, *Chlorinoides occidentalis* (*Records of the Australian Museum*, **28**, 65; 1970). The illustration shows the carapace and rostrum of the male holotype which is 17·1 mm long.

TRYPANOSOMIASIS

No Room for Complacency

from a Correspondent

HUMAN trypanosomiasis has been, statistically speaking, a scarce disease in Africa for some years, giving the general impression that it is fully under control. But this is not so; there are probably a million cases of sleeping sickness in the Congo (Kinshasa) at the present time. This gave added interest to the research reported at the annual seminar of the Trypanosomiasis Panel of the Overseas Development Administration, held in London on November 12.

Although contributions covered the whole range of current research on trypanosomes and their tsetse fly vectors, two new developments are particularly worth noting. The first, reported in a series of papers by Dr B. A. Newton, Mr J. K. Burnett and Dr G. A. M. Cross (Molteno Institute, Cambridge), concerns the differing, but constant, DNA from different strains of the same species of trypanosome. Particular attention has been paid to the DNA of the *T. brucei* group, which contains the species causing sleeping sickness, for it may provide information on differential resistance, or sensitivity, to trypanocide drugs.

The approach being considered is that of comparing the metabolism of normal and mutant bloodstream and culture forms, and observing the sequence of metabolic changes during transformation from bloodstream to culture forms. It has been found that different

strains of *T. brucei* differ in the readiness with which they transform, as well as in the ease with which they can be maintained in serial subculture. Current work includes comparing the metabolic patterns of a highly pleomorphic and an apparently monomorphic strain. At the same time it has been observed that naturally occurring and drug-induced mutations which lead to loss or modification of the kinetoplast can only be tolerated in the bloodstream forms of *T. brucei*.

The second new development is the elucidation of the mechanics of animal trypanosomiasis in Latin America. In Venezuela, dairy cattle are protected at present by administering a general treatment against a wide range of diseases, with no discrimination against trypanosomiasis, the epidemiology of which is little known. Now, work reported by Dr M. T. Clarkson (University of Liverpool) has shown that the disease is transmitted by various biting flies, and spread largely because all the cows and their calves are brought in for milking each day, and confined in a small space in conditions which are ideal for transmission of the disease. Further study on the epidemiology of *secodera*, as the disease is called in Latin America, could lead to changes in the organization of the dairy industry which the Food and Agriculture Organization is helping to build up in these countries.

Several gaps in present knowledge also became evident at the seminar. There is a need for much more study of the feeding habits of different species and strains of tsetse. The concentration of "natural history" in the field is being increasingly supplemented by more laboratory studies, such as those of Mr D. A. Turner (London School of Hygiene and Tropical Medicine) and Mr John Brady (Imperial College Field Station). At present it is still a matter of working out the methodology for more detailed studies of visual, olfactory and other reactions which apparently lead different strains to feed on different hosts.

There is also a need for cytogenetic research on tsetse, similar to that done on mosquitoes and *Similium*, which is revealing that strains with different chromosome "architecture" feed on different hosts.

OCEANOGRAPHY

Currents and their Dynamics

from a Correspondent

PROBLEMS which have been worrying oceanographers for some time were aired at a discussion meeting on ocean currents at the Royal Society on November 19. In much of the ocean the currents are so variable that it is often hard to detect and thus to calculate correctly the mean current. This difficulty was impressed on oceanographers through some observations made some ten years ago by Dr J. C. Swallow of the National Institute of Oceanography. The general view of ocean circulation then as now was that the strongest currents (10 to 100 cm s^{-1}) were found near the surface and the mean currents in deep water were relatively weak (about 1 cm s^{-1}). By tracking the movements of floats which stay at a pre-determined depth, however, transient currents of around 20 cm s^{-1} were found. These seem to be associated with eddies which are about 100 km across and which cause currents to vary on a timescale of the order of months.

Because the eddies are so strong relative to the mean flow, it is important to know whether they are significant in the dynamics of the mean circulation. The experience of meteorologists may be pertinent. At one time, their models of the circulation of the atmosphere took no account of eddies (cyclones, and the like), until it was discovered that these are so fundamental in the circulation that any model which neglected them was irrelevant. Oceanographers are asking if such might also be the case with the ocean circulation.

Against this background theories of ocean circulation and of variable currents were discussed by Dr A. R. Robinson (Harvard), Professor M. J. Lighthill (Cambridge), Dr A. E. Gill (Cambridge) and Dr P. Welander (Gothenburg). Wind-driven ocean circulation models provide a good qualitative understanding of the principal ocean currrents of the world, like the Gulf Stream and Kuroshio, and why they are found on the western edges of the ocean basins. In present models, estimates of the rate of flow of these great streams depend on friction coefficients, but such coefficients may be inappropriate. For example, they imply that energy is continually being removed from the stream by eddy processes, whereas there are indications that eddies may often give up energy to the stream instead. Theoretical and observational studies of Gulf Stream eddies are in progress, and will throw light on the processes involved, but it also is important to understand the role of eddies in other parts of the ocean.

In the past ten years, technological developments of self-recording, moored current meters and of trackable floats have greatly helped in the direct measurement of currents. The results of such measurements were discussed by Dr N. P. Fofonoff and Dr F. Webster (Woods Hole Oceanographic Institution) and Dr W. J. Gould and Dr J. C. Swallow (National Institute of Oceanography). The energy spectrum shows peaks at semi-diurnal (tidal) periods and inertial periods (a half pendulum day) and also shows a great deal of energy at longer periods. The information about the longer period motion (weeks or more) is less comprehensive because of practical requirements of servicing and maintaining instruments, yet these long-period motions are probably the most significant for the mean circulation.

An afternoon's discussion was aimed towards deciding what could be done observationally to improve the present unsatisfactory situation. Unlike meteorologists, oceanographers do not have a great network of stations at which observations are continually made. They are forced to plan experiments of very limited scale and duration. The significant eddies in the ocean may be small and the timescale large, so that a closely spaced array of measuring devices may be required to be in operation for long periods (for example, about a year). This would require a considerable concentration of resources, and so an international Mid-Ocean Dynamics Experiment (MODE) has been proposed. The discussion centred on the type of information that would be required even to design an effective experiment, or series of experiments. Are the major processes highly intermittent or not? How can the new technologies of current-meter mooring, float-tracking, bottom-pressure recording and of radiometery (surface temperature observation by aircraft or satellite) best be utilized and how can more conventional hydrographic methods and data from

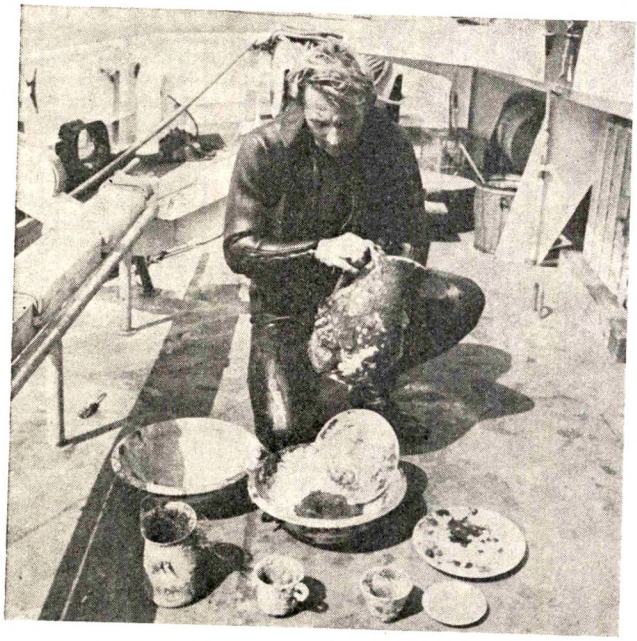
island tide-stations be exploited? The answers to these and other questions will require sophisticated observational programmes. Scientists of the Soviet Union (unfortunately none could be present at the meeting) have an imaginative series of "polygon" experiments in operation, involving the deployment of an antenna-like array of current meters in a 100-mile square of ocean. American and British scientists are planning a PREMODE experiment in which participating groups plan to carry out independent projects in the same area at the same time so that the results will be complementary. The "polygon" and PREMODE experiments signify a new approach to the study of ocean currents and their dynamics. Perhaps there will be fundamental changes in our thinking over the next ten years.

ARCHAEOLOGY

Export Pottery Revealed

from our Geomagnetism Correspondent

THE unmarked wreck of a nineteenth century cargo vessel containing British pottery has been discovered in Delaware Bay between Lewes, Delaware, and Cape May, New Jersey, only thirty-nine feet below the surface. It was found by two ships of the US National Oceanic and Atmospheric Administration (NOAA), the Rude and the Heck, during a routine wire-dragging of the bay in search of submerged navigational hazards. The ship's anchor, of woodstock type weighing about 3,500 pounds and measuring about six feet across the flukes and eight feet along the shank, indicates that the wreck was that of a sea-going vessel, probably British.



Some of the pottery found in the wreck. (Photograph by courtesy of NOAA.)

Scuba divers found the wreck laid open on the bottom. Most of the cargo was embedded in coral and silt; but thousands of pieces of British-made pottery, neatly packed, were exposed in a pile about ten feet high and thirty feet across. According to Jefferson Miller, curator of ceramic history at the Smithsonian Institution's National Museum of History and Technology, the white, undecorated pottery found in the wreck was manufactured from 1850 onwards, but no later than 1876. He identified it as the products of Powell and Bishop, a Hanley, Staffordshire, pottery firm which operated between 1876 and 1878; J. W. Pankhurst and Co., also of Hanley, 1850–82; and W. and E. Corn of Longport, Staffordshire, about 1864–94.

Most of the chinaware was English "ironstone", a heavy durable earthenware intended for everyday use. One exception was a hand-enamelled cup with simple multi-coloured flowers manufactured between 1850 and 1900. The cargo also included pitchers and washbowls.

SUPERFLUIDITY

Flow in Solids

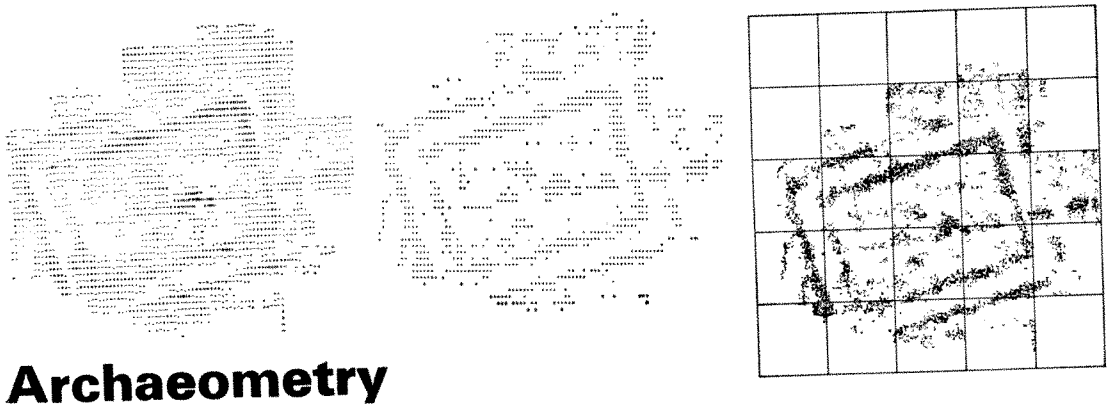
by our Solid State Physics Correspondent

THE speculation by A. J. Leggett (*Phys. Rev. Lett.*, **25**, 1543; 1970) that it may be possible to observe in certain solids a mild form of superfluidity is not as far fetched as it might seem. There is no suggestion, of course, that a solid can exhibit the astonishing flow properties which allow liquid helium-4 to pass through holes so minute that even many gases are barred from entry. The identity of a solid hinges, after all, on its rigidity and atomic order. But what Leggett is proposing is that certain insulating solids may be in a sufficiently similar quantum state to that in superfluid helium to show a diminution in the moment of inertia of the solid which can be detected in an experiment.

The onset of superfluidity in liquid helium is associated with the Bose condensation of the fluid at about 2·2 K. A recent suggestion that a finite fraction of the atoms in a Bose solid may reside in the zero momentum state alerted Leggett to the possibility that a milder strain of the same superfluid condition may also manifest itself in these solids, and he has calculated the lower limit of what he calls the superfluid fraction in such a solid. If all the atoms are localized at given atomic sites, superfluidity cannot occur, but if atoms can swap places in the lattice, the solid should exhibit some drop in its rotational inertia from the classical value. This swapping is quite in keeping with the concept of a solid, providing the atomic sites themselves are essentially fixed.

Leggett suggests that the most favourable material in which to observe the effect should be solid helium, although practical difficulties may mean that other substances are more suitable. He suggests two experiments in which the superfluid property could manifest itself. If the solid is formed into the shape of a ring, the moment of inertia of the ring should be below that expected in a classical calculation. This could be tested in some dynamic experiment. The sample would have to be retained at a very low temperature throughout. A second test would be to rotate the solid and bring it into impact with some other body. If the solid had the superfluid property there should be some residual angular momentum. Leggett points out that it is not surprising that nobody has stumbled on the effect by accident because the conditions required to observe it are quite exacting.

Computer-produced diagrams from measurements taken by a magnetic detector, clearly showing the outline of archaic remains beneath modern topsoil at a Roman site in the Rhineland.



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Generation of Radio Waves in Pulsars

by
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Nuffield Radio Astronomy Laboratories,
Jodrell Bank

Pulsars generate radio waves by an unknown process which gives the highest volume emissivity known in astrophysics. The radiation forms a beam the width and polarization of which are independent of frequency. This article assembles the observational facts which any theory of emission must explain.

THE Crab Nebula pulsar, NP 0532, is the only pulsar of the fifty-five known in which any radiation has been detected outside the radio range of wavelengths. In this article I shall set out the requirements of a theory to explain the radio emission, concerning which a great deal of experimental detail is known; but it is proper to ask first whether the same mechanism is responsible for the X-ray, optical, infrared and radio radiation of NP 0532. The spectrum of this radiation is shown in Fig. 1, including the long wavelength radiation previously ascribed to a continuously radiating discrete source by Hewish and Okoye[1,2].

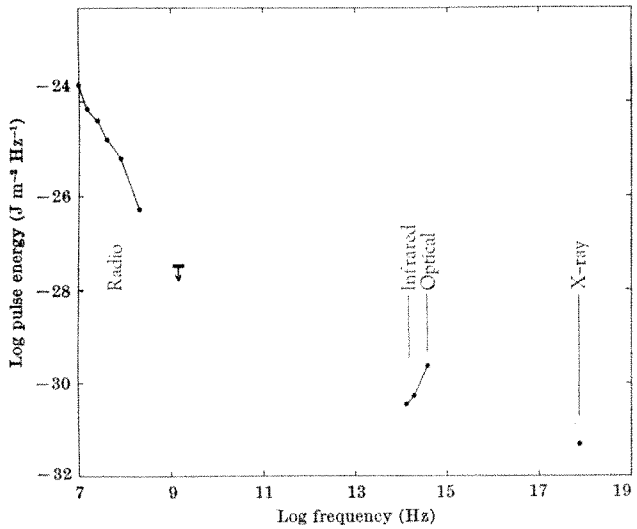


Fig. 1. Spectrum of the radiation from NP 0532. The points at frequencies below 100 MHz refer to the discrete source discussed by Bridle[2]; other points are taken from Bradt *et al.*[1].

The Crab Nebula itself radiates a continuous spectrum throughout this range by the synchrotron process, but it seems unlikely that the pulsar radiation has a similar continuous spectrum. In particular, there seems to be a minimum between the radio and the infrared, which has been interpreted by Shklovsky[3] as a boundary between two different radiation processes. There may also be differences between the radio and optical pulses concerning their polarization[4] and variations in intensity[5]. It is therefore assumed that no single process operates continuously over the whole spectrum; nevertheless there is close similarity between the integrated pulse profiles at all wavelengths, which surely indicates a common point of origin and a common beaming mechanism.

The radio pulses from a typical pulsar have the following properties. (1) A precise periodicity. The pulses occur during a time interval of between 1 and 4 per cent of the period. The duration of this "pulse window" bears a remarkably constant ratio to the period, since the periods range over a ratio of 100:1. (2) The pulses are often considerably shorter than the pulse window. For those pulsars where individual pulses can be observed, their duration is between $\frac{1}{2}$ and 3 per cent of the period. (3) Multiple pulses can occur. More than one pulse can be seen within a single pulse window. In CP 1133 pulses appear substantially independently in two overlapping regions of the window (Fig. 2). In AP 2015 and several other pulsars two or three pulses may appear in a single window, with a well defined time interval between them[6]. (4) Pulses vary greatly in power, but at least a factor of 10 within a few pulses or even between successive pulses[7]. (5) There are trains of related pulses; successive pulse windows may contain pulses which are recognizably similar in strength, width or structure. Successions of up to 10 or more related pulses are observed in some pulsars, such as CP 1919[8]. (6) Successive appearances of similar pulses drift earlier in the pulse window at a rate typical of individual pulsars[6]. The drift may carry an identifiable pulse from one window to another some hundred or so periods later, as in MP 0031[9]. (7) The shape of pulses is variable. Symmetrical pulses, like Gaussian functions, are observed in many pulsars; others have more complex and variable shapes of pulse which might be described as the sum of several Gaussian curves. Fine structure, down to 0·1 per cent of the period, has been observed, as for example in CP 0950[10]. (8) Individual pulses, and especially those that have a simple Gaussian shape, are highly polarized[11]. The polarization is in general elliptical, with an axial ratio and a direction of major axis which varies through the pulse. The polarization often reaches 100 per cent, including occasionally a completely circular polarization[12]. Fig. 3 shows some pulses from CP 0328 recorded with a polarimeter at 408 MHz, in which the measured fractional polarization $(V^2+Q^2+U^2)^{\frac{1}{2}}/I$ varies generally from 0·5 to 1·0. Separate pulse components may have quite different polarizations, as seen for CP 1133 in Fig. 2. (9) The sum of some hundreds of pulses, superposed by adding successive periods, forms an integrated profile which is characteristic of an individual pulsar. The profile

usually has a single smooth maximum, although it may contain two or more peaks[13]. (10) The Stokes parameters of successive pulses can also be added to obtain an average polarization. This is usually predominantly plane, for the circular components of different pulses tend to cancel. The position angle of the integrated plane component rotates through the pulse window by an angle between 0° and 180° (refs. 12, 14). (11) The spectrum of the radio pulse extends for some pulsars at least from 40 MHz to 4,995 MHz, but few points are available to define the average spectra of most pulsars. At frequencies greater than 300 MHz the average flux density of most pulsars decreases rapidly, at least as the square of the frequency. Some pulsars have a flatter spectrum at lower frequencies; CP 0328 seems to have a broad peak at about 500 MHz, witb a decrease below 300 MHz. (12) The shape of the integrated profiles of most pulsars is markedly insensitive to frequency, apart from a lengthening at lower frequencies due to interstellar scintillation[15,16]. The relative heights of the several peaks in the profile of AP 1237+25 are, however, different at frequencies an octave apart[17], while the separation of the double peak of CP 1133 varies roughly as the fourth root of the frequency[18]. (13) Detailed comparison of individual pulses recorded simultaneously at widely separated frequencies is difficult, largely because of the effects of interstellar scintillation. Fig. 4 shows a new recording of individual pulses of

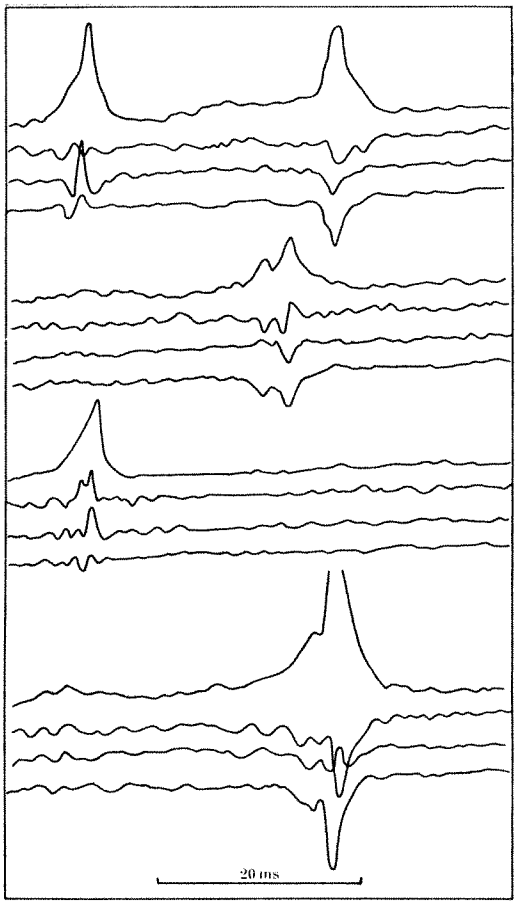


Fig. 2. Four individual pulses from CP 1133, recorded with a polarimeter at 408 MHz. For each pulse the Stokes parameters I, V, Q, U are recorded. The pulses tend to occur at two separate times about 20 ms apart; the sum of many pulses produces the well known double peaked profile.

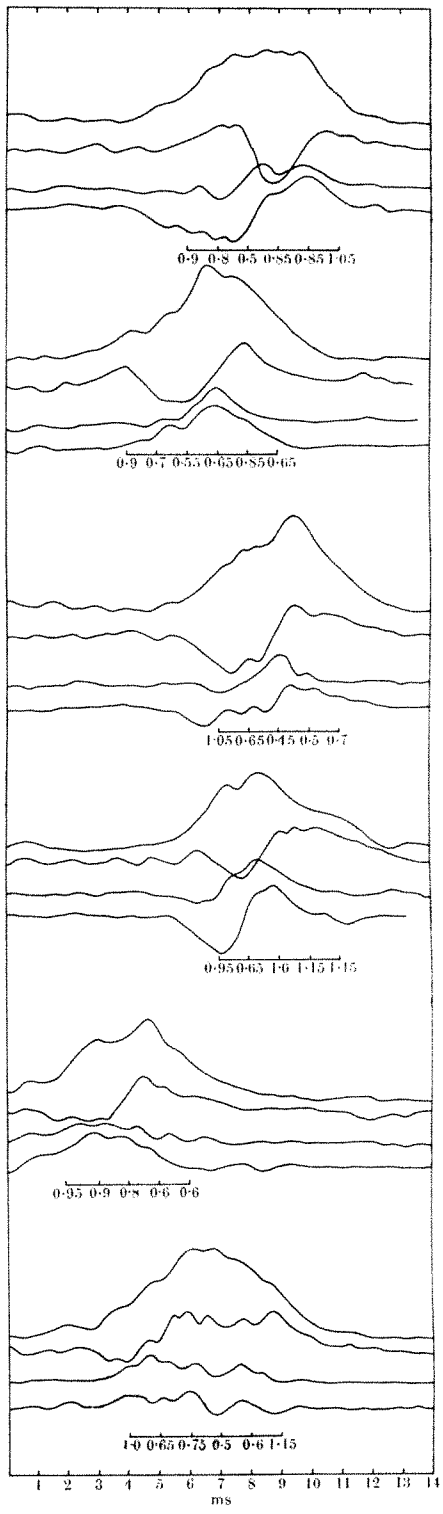


Fig. 3. Six pulses from CP 0328, recorded with a polarimeter at 408 MHz. For each pulse the Stokes parameters I, V, Q, U are recorded. The fractional polarization $(Q^2+U^2+V^2)^{\frac{1}{2}}/I$ is shown at intervals of 1 ms through each pulse.

CP 0328, at frequencies of 408 MHz and 240 MHz. Only the I and V components are shown, because differences in the linear components are to be attributed to Faraday rotation in interstellar space[19]. Within the noise level of the recordings the pulse shapes are seen to be identical. (14) Within several months the intensity of some pulsars changes by considerable factors, as measured on a single

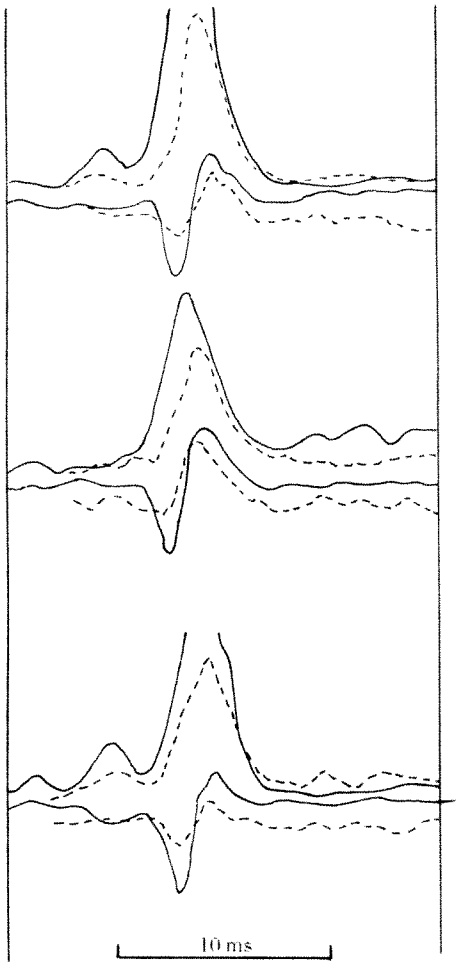


Fig. 4. Three pulses from CP 0328, recorded both at 408 MHz and 240 MHz. The upper traces record intensity (I) and the lower traces record the circularly polarized component (V). Full line—408 MHz (bandwidth 330 kHz); broken line—240 MHz (bandwidth 100 kHz).

frequency[20]. It is not yet clear whether this variation affects the whole radio spectrum simultaneously.

The extent and detail of this information are so great that one might expect the mechanism of the radiation to be already obvious. There is general agreement that the pulses are due to a beaming effect in a rotating source[21,22], and it was natural to suggest a mechanism similar to synchrotron radiation[23,11]. The problems in such suggestions concern the very high intensity, the spectrum, the high polarization, and the frequency invariance of the polarization and beamwidth.

High Intensity

From the precise periodic reappearance of the pulse window, we can deduce that the source of the radiation is attached to a rotating neutron star, presumably by magnetic field lines. It therefore lies within the distance $c\omega^{-1}$, where ω is the angular velocity of the star, and its radius r is less than $c\omega^{-1}$, which is called the radius of the "velocity of light cylinder". The narrowness of the pulse in relation to the period suggests that the source extends over only a very small range of longitude, probably a distance less than $0{\cdot}02\ c\omega^{-1}$. It may, of course, be more extended radially. I shall assume that the source is roughly spherical, with radius $0{\cdot}05\ c\omega^{-1}$, and calculate on this basis the volume emissivity, using as examples NP 0532 and CP 0328.

A further assumption must be made about the directional properties of the source, which may radiate preferentially in a plane containing the observer; it is also possible that the observed peak pulse intensity is enhanced by a relativistic beaming effect[13]. I shall calculate first the pulsed flux density at the surface of the source, and then the volume emissivity in terms of the power averaged over one period. Again it is necessary to make an assumption about the total solid angle of sky illuminated by the source; here I shall assume arbitrarily that the fan beam of the source sweeps across a solid angle of one tenth of 4π. Thus if the distance of the source is D, the peak flux density is S_p, the equivalent pulse width is w, and the period P, the peak flux density S_{ps} at the source, is given by

$$S_{ps} = S_p \left(\frac{D}{r}\right)^2$$

and the volume emissivity Q is given by

$$Q = 0{\cdot}1\ \frac{3S_{ps}}{r} \cdot \frac{w}{P}$$

The total power flux and the total volume emissivity have been calculated by using a bandwidth factor of 200 MHz; this factor is poorly determined at this time.

The electric field E_s in the radiation at the surface of the source is given by $E_s^2 = 377\ Q_{\text{total}}$, using SI units. The distance of CP 0328 is taken to be 1 kpc, and that of NP 0532 as 2 kpc. Peak values of flux density at 100 MHz for NP 0532[24], and at 400 MHz for CP 0328[25], are approximate, because little account has been taken of interstellar scintillation in observations so far.

Coherent Electron Motion and Synchrotron Radiation

In spite of the crudity of the assumptions which have been made about the size of the source, there seems no escape from the very high values of emissivity and flux density in Table 1, even though the rate of power generation per unit volume in NP 0532 is comparable with that in the core of a nuclear power generator. Some reduction may be allowed if the beaming is a relativistic effect of the rapid orbiting motion of the source[13], but this only reduces the volume emissivity by a factor less than 100, and the field strength by a factor less than 10. The brightness temperature of NP 0532, using the tabulated value of S_{ps}, reaches 2×10^{28} K, which exceeds the brightness temperature of an electron gas with energy $\varepsilon\ m_0c^2$ by a factor $4 \times 10^{18}\ \varepsilon^{-1}$. Evidently, a coherent electron motion is involved. Further, the high field strength E_s, observed for radiation with wavelength of order 1 m, ensures that all electrons acquire a relativistic energy, such that $\varepsilon \gg 1$, merely by the existence of the radiation field. The conditions of radiation therefore must involve the coherent motion of high-energy electrons, with the attendant difficulties of non-linear theories of wave propagation and interaction.

Table 1. PARAMETERS OF TWO PULSARS

	S_p ($Wm^{-2}Hz^{-1}$)	S_{ps} ($Wm^{-2}Hz^{-1}$)	S_{total} (Wm^{-2})	E_s (Vm^{-1})	Q ($Wm^{-3}Hz^{-1}$)	Q_{total} (Wm^{-3})
NP 0532	10^{-23}	6×10^{6}	$1{\cdot}2 \times 10^{15}$	7×10^{8}	$0{\cdot}8$	$1{\cdot}6 \times 10^{6}$
CP 0328	10^{-24}	3×10^{2}	6×10^{10}	5×10^{6}	2×10^{-6}	400

Early attempts to explain the radio emission were based on the synchrotron mechanism. It was noted that an organized stream of electrons would radiate a beam the angular width of which might correspond to the pulse

width[23], and it was later found that the polarization varied across individual pulses in a way which resembles that of synchrotron radiation[11]. The combination of the independence of pulse width on frequency, and of the high degree of polarization, however, now makes this theory unacceptable. Indeed, no simple radiation process which can produce these two properties together has yet been suggested. A simple geometrical explanation of the beam is required, as opposed to an electrodynamic radiation process. For example, it has been suggested[14] that electrons are constrained to flow along a bundle of magnetic field lines, the directions of which define the beam. In common with other synchrotron explanations, this theory cannot, however, explain the existence of a high degree of polarization over a wide range of frequencies. A simple geometrical explanation of the beam is required, in which the angular width and the polarization distribution are determined by factors which are independent of wavelength.

Wave Amplification

A more promising line of theory ascribes the high intensities to a wave amplification process in which plasma waves are coupled to the electromagnetic field[26]. Here a high degree of polarization is to be expected, for it only requires one polarized mode of propagation to be amplified preferentially for almost complete polarization to be obtained from an initially unpolarized wave. The coupling is high at frequencies near the gyro-frequency, and for an assembly of highly relativistic particles it seems possible for coupling to extend over a considerable range of frequencies. It would, of course, be necessary to show that the polarization is independent of frequency over a wide range. This kind of theory does not of itself explain the beaming of the radiation.

The narrow beam of radiation may conceivably be produced in an unpolarized or partially polarized state, by some mechanism which gives a beam width independent of frequency, and with a subsequent amplification by interaction with plasma waves. The original beam might then be defined by the flow of electrons along magnetic field lines[14]. Then the polarization induced by the plasma waves must follow the complex patterns which are observed, and which now represent differences over small angular widths in the relative amplification of different modes. These processes must produce a polarization which does not depend on frequency; this may raise serious difficulties, since the width of any small scale angular dependence seems necessarily to involve a relation between wave frequency and plasma or gyro frequencies.

The only other suggestions for the formation of a narrow beam depend on the high relativistic velocity of the emitting region of the pulsar atmosphere, which is now required to be located close to the "velocity of light cylinder" and which is nevertheless coupled tightly to the pulsar through the magnetic field of the pulsar. If it is thought that the relativistic velocity is itself the cause of the radiation[27], then the difficulties of beamwidth and polarization must again be contended with. If, on the other hand, the relativistic velocity is considered to be producing a beaming effect in a source which is radiating more nearly isotropically[13], then a purely geometrical condition for beamwidth has been found and the radiation mechanism is easier to understand. In this latter case the wave amplification theory must amplify selectively a wave which is generally elliptically polarized, with an axial ratio that varies only slowly with wave direction; for example, as the cosine of the angle between the field and wave directions. The emergent polarization would then vary with direction roughly as does the polarization radiation at the gyro frequency (cyclotron radiation). An amplification process, however, might be expected to select a purely circular or purely linear mode, and it remains to be shown how a plasma can radiate or amplify with a suitable angular distribution of elliptical polarization.

Spectrum and Fluctuations

On any theory the source of the radiation must contain a high concentration of energy in the form of coherent plasma oscillations; probably these will be concentrated at the gyro frequency. The source is located within a region defined by the pulse window. Within this window the spectrum of the radiation from all locations is everywhere wide, and everywhere nearly but not quite the same, as seen by the small variations in shape of the pulse window. The field direction, which must define the state of polarization, varies across the region so that its aspect appears to the observer to change smoothly by an angle between zero and 180° for different pulsars.

The excitation of small parts of the region varies rapidly, usually within a single rotation period. The excitation may travel from one part of the region to another, sometimes with an organized motion which carries it in the forward direction of rotation. An identifiable excitation can travel right through the region and persist for a complete circuit of the pulsar, reappearing in the emitting region. Since the magnetic field in the emitting region appears to be stable, it seems that this excitation must be in the form of a travelling concentration of plasma waves.

The description of the phenomena of the radio emission, and the immediate deductions about the nature of the source, naturally represent a selection of those observations and theories which seem to me at this time to be the most important and deserving of attention. The references could not be made comprehensive, and apologies are due to many authors whose work may have been touched on without due acknowledgement.

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Structure and Activity of Acetylcholine

by
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New York,
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Conformational analysis of several agonists and antagonists can be used as a basis for the definition of structural parameters necessary for the range of activity of acetylcholine.

THE classical experiments of Dale[1] established that the pharmacological actions of acetylcholine (Ach) outside the central nervous system could be divided into two categories, nicotinic and muscarinic, each defined by the effects of characteristic agonists and antagonists and based on the actions of nicotine and muscarine. These two pharmacological classes can also be separated on physiological and anatomical grounds. The nicotinic transmission sites are predominantly at neuromuscular junctions and in autonomic ganglia, whereas the muscarinic sites are mostly in parasympathetic target organs. More recently some muscarinic synapses have also been described in autonomic ganglia[2]. There have been numerous attempts to explain in structural terms the different, non-overlapping specificities of drugs representing each class, and to identify the properties of acetylcholine which account for its activity at both types of junction. According to Chothia[3], the nicotinic and muscarinic receptors are considered to interact respectively with two different sides of acetylcholine when the transmitter is in a conformation which is observed in one crystal structure. We offer an alternative proposal.

We have examined Dreiding and CPK space-filling models of compounds which affect nicotinic and muscarinic junctions and have attempted to identify common structural features in each series. We have focused attention on molecules containing elements of structural rigidity because rigidity restricts conformational options and reduces the ambiguity in stereochemical assignments for functional groups. Our findings indicate that the nicotinic and muscarinic specificities of cholinergic receptors can be understood in terms of interactions based on two different combinations of functional groups in the transmitter molecule. Within each category common structural features are shared by agonists and antagonists. This is reassuring, for they are thought to compete for the same receptor site[4-6]. In addition, there are some molecular differences between agonists and antagonists which may be correlated with activity.

Nicotinic Agents

We propose that the specific binding of nicotinic agents to their receptor is mediated by two elements: (1) a coulombic interaction involving the alkylammonium moiety, and (2) a hydrogen bond which depends on an acceptor group in the drug and is formed approximately 5·9 Å from the centre of the positive charge (Fig. 1). This structural pattern is common to a family of fully or partly rigid molecules that are active as agonists or antagonists only at nicotinic junctions; in acetylcholine the corresponding distance can be reached only if the carbonyl oxygen serves as the H-bond acceptor.

The alkaloid cytisine (Fig. 2) is pharmacologically identical and virtually equipotent with nicotine[7]. The fused ring system makes cytisine a rigid molecule in which conformational uncertainty is restricted to the six-membered ring containing the secondary amino group. We assume that this six-membered ring exists predominantly in the more stable chair form. In this conformation the centre of the positive charge is 5·9 Å from the proposed H-bond acceptor site on the amide oxygen.

Nicotine, the prototype agonist at autonomic ganglia and neuromuscular junctions, is not as rigid as cytisine, but does not have the conformational freedom which might be implied by a non-space-filling representation of its structure. The possible conformations are restricted by steric interactions between protons on the pyrrolidine and pyridine rings. From the crystal structure[8], magnetic resonance and dipole moment data[9], and from space-filling models, the conformation shown in Fig. 3 seems to be the most likely. In this the centre of the charge is 5·9 Å from the centre of the van der Waals surface of the pyridine nitrogen (the proposed H-bond acceptor site).

Two points should be noted about nicotine. It contains an asymmetric carbon atom and therefore exists in two optically active forms; *l*-form occurs naturally in tobacco. The non-superimposable mirror image—the *d*-form—should retain the distance between the charged nitrogen and the free electron pair on the pyridine nitrogen. If these two elements are indeed the determinants of nicotinic specificity, *d* and *l*-nicotine should have identical properties. In fact, there is no pronounced difference between them. The estimates for the relative potencies of the two isomers range from 1 (ref. 10) to 8 (ref. 11).

Because of the presence of the asymmetric carbon atom, and because all the atoms of nicotine are incorporated into ring systems, the *d* and *l*-isomers, even at first glance, seem to be quite different. Closer examination reveals that if one superimposes the planes in *d* and *l*-nicotine which are formed by the cationic centre, the unshared electron pair and the centre of the pyridine nitrogen, then only one additional atom (the carbon

para to the pyridine nitrogen) is spatially homologous in the two enantiomers. Thus, if the charge and pyridine nitrogen are important for activity of the molecule, the similar potency of *d* and *l*-nicotine argues strongly against any direct contribution from the carbon skeleton. We conclude that the role of the carbon skeleton is simply to provide proper stereochemical localization of the functional groups which determine specificity.

Strychnine and Other Antagonists

Strychnine (Fig. 4) is best known as a powerful convulsant which acts directly on the central nervous system. In isolated neuromuscular preparations[12] and in ganglia[13], however, it behaves as an antagonist of acetylcholine. Strychnine has a tertiary amino nitrogen which is positively charged at neutral *p*H; it contains in addition an amide grouping and an ether oxygen. Quaternization of the nitrogen yields N-methylstrychnine with enhanced curare-like activity[14]. N-Methylstrychnidine

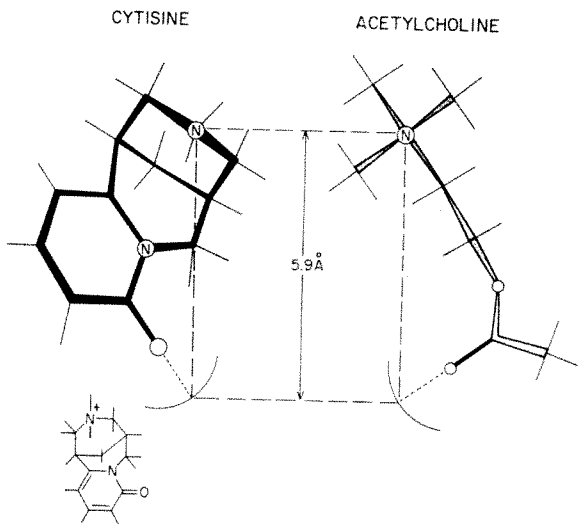


Fig. 2. Cytisine, see text.

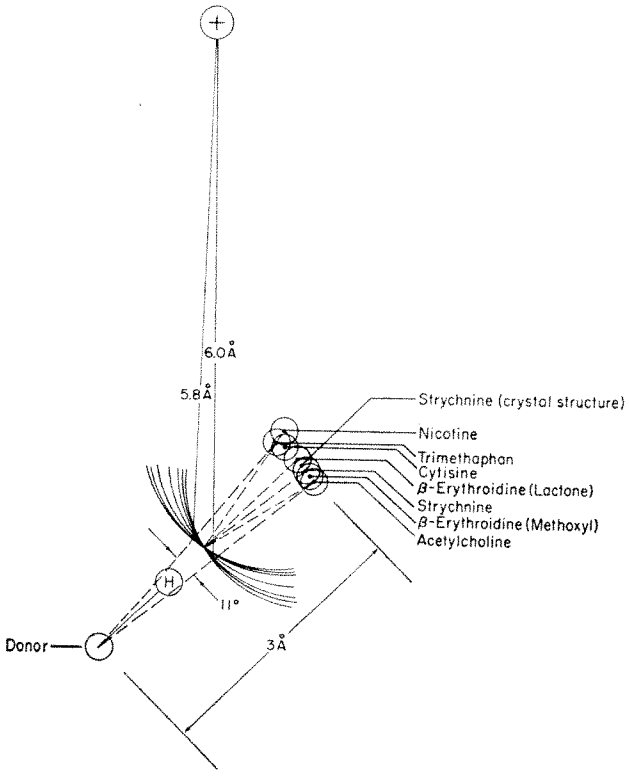


Fig. 1. Composite diagram of nicotinic agonists and antagonists, illustrating the range of H-bonding geometry envisaged for some of the rigid and partly rigid nicotinic agents considered in the text. After a preliminary study of the individual drugs had established that the separation of the proposed H-bond acceptor, and a point on the van der Waals the molecules were compared as follows. The structure of each molecule was drawn to scale in a plane defined by the charge centre, the nucleus of the proposed H-bond acceptor, and a point on the van der Waals surface of the acceptor. The point selected for this can be considered the "centre" of the van der Waals surface; it is the point of intersection of the van der Waals surface and the radius that is in the plane formed by the covalent bonds to the acceptor atom and which bisects the angle between these bonds. When carbonyl oxygens are envisaged as H-bond acceptors, the radius that intersects the centre of the van der Waals surface is obtained by extending the line that represents the C=O bond. The arcs in Figs 1–6 represent the intersection of the van der Waals surfaces of the respective acceptor atoms with the plane of the diagram. For each molecule a point on this arc was located corresponding to a distance 5·9 Å from the charge centre. The different molecules were then compared by superimposing these points and the charge centres, and the positions of the respective nuclei of H-bond acceptors were recorded. Assuming that the distance between the H-bond donor and acceptor nuclei is 3 Å, and that the donor is positioned as shown, the distortion of the proposed H-bond is less than ±6° for the drugs which were examined.

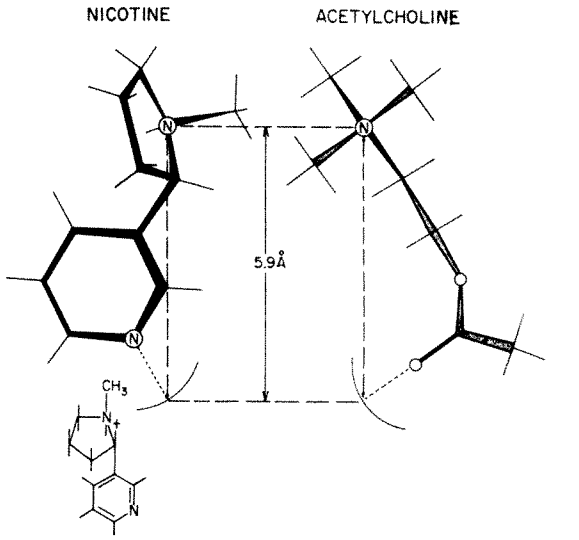


Fig. 3. Nicotine. The *l*-enantiomer is shown here. The distance from the centre of the charged pyrrolidine nitrogen to the centre of the van der Waals surface of the pyridine nitrogen is 5·9 Å.

is identical with methylstrychnine, except that the amide oxygen is no longer present; however, N-methylstrychnidine remains a potent curariform agent[14]. Thus the amide oxygen cannot be responsible for this activity.

The distance from the centre of the charged nitrogen to the van der Waals surface of the ether oxygen can be estimated with some confidence because of the rigidity imposed by the structure of the alkaloid. From model building this distance is estimated to be 5·9 Å. This value is in satisfactory agreement with both the results of X-ray analysis of the strychnine crystal structure[15] and the distance between the homologous functional groups in nicotine and cytisine.

Beta-erythroidine (Fig. 5) is a potent nicotinic antagonist, and has, like strychnine, a rigid caged structure that limits the conformational freedom of individual functional groups. Erythroidine contains a tertiary amino nitrogen which is positively charged at physio-

logical *p*H, and three oxygen atoms, all of which can act as H-bond acceptors. For two of the oxygen atoms the distance from the potential H-bonds to the centre of the positive charge is 5·9 Å. The potent nicotinic antagonism of erythroidine could in part result from this aspect of its structure, which provides two potential binding combinations which can mediate stereochemically satisfactory interactions with the receptor. This possibility is supported by the fact that removal of the methoxyl group or opening of the lactone ring reduces the potency of erythroidine, but only by a factor of 2 or 3 (ref. 16).

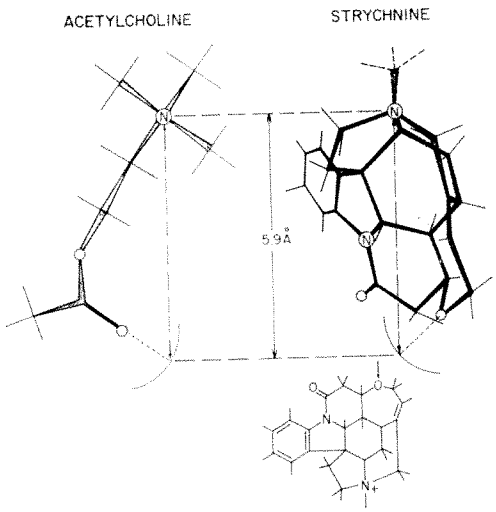


Fig. 4. Strychnine, see text.

Trimethaphan (Fig. 6) is another nicotinic antagonist in which the proposed structural determinants of specificity are situated in a rigid ring system. In this case the cationic grouping is a sulphonium ion, and an H-bond can be formed at a distance of 5·9 Å from the centre of the positive charge; the ureido oxygen atom is visualized as the presumptive H-bond acceptor.

Some other nicotinic agents should also be considered. Although their conformations are not fixed by rigid caged structures comparable with those which occur in strychnine and erythroidine, the distribution of functional groups in the potent nicotinic agents lobeline, benzoquinonium and muscarone would readily permit these drugs to interact with the receptor in the manner proposed for Ach.

The biological activity of a family of substituted toxiferines supports the postulated formation of an H-bond at the Ach binding site on the nicotinic receptor. It is generally accepted that the two cationic centres of toxiferines interact electrostatically with the anionic groupings of neighbouring Ach binding sites. It is therefore interesting that the stepwise addition of two hydroxyl groups onto the toxiferine nucleus is associated with successive two to three-fold increases in activity[17]. These hydroxyl groups are free to rotate, and to assume a position which permits the oxygen to act as an H-bond acceptor 5·9 Å from the charge centre. This type of interaction at the Ach binding site could account for the increasing potency which accompanies progressive hydroxylation of the toxiferine nucleus.

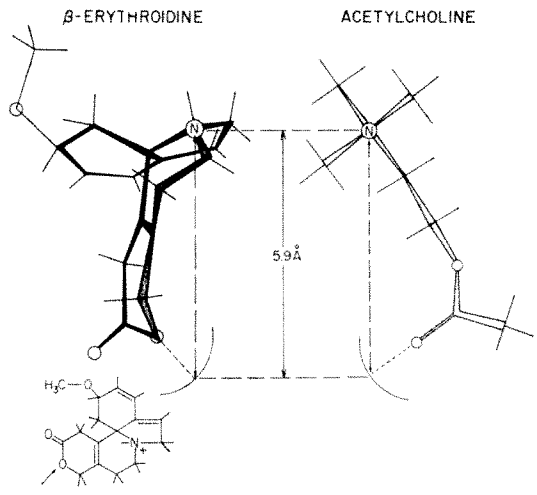


Fig. 5. Here the distance between the H-bonding site on the lactone oxygen and charge centre is shown. A separation of 5·9 Å between the charge centre and an H-bonding site on the methyl ether oxygen is also possible.

Like toxiferine, *d*-tubocurarine is considered to complex primarily with the anionic components of adjacent Ach binding sites. The tubocurarine molecule contains numerous oxygen atoms and its crystal structure (unpublished work of T. D. Sakore, S. S. Tavale and H. M. Sobell) indicates that two of these can serve as H-bond acceptors at positions that are stereochemically compatible with those proposed for the other nicotinic agents.

The limited pharmacological knowledge of the alkaloid anagyrine suggests that this compound too is a nicotinic agonist[18]. Anagyrine is a derivative of cytisine and is identical with it in terms of the relationship between the charge centre and H-bond acceptor.

The nicotinic agents we have considered differ markedly in structure and in physico-chemical properties, but they have one set of common structural features: a cationic centre and a potential H-bond acceptor 5·9 Å apart. Thus the Ach binding site is visualized to contain

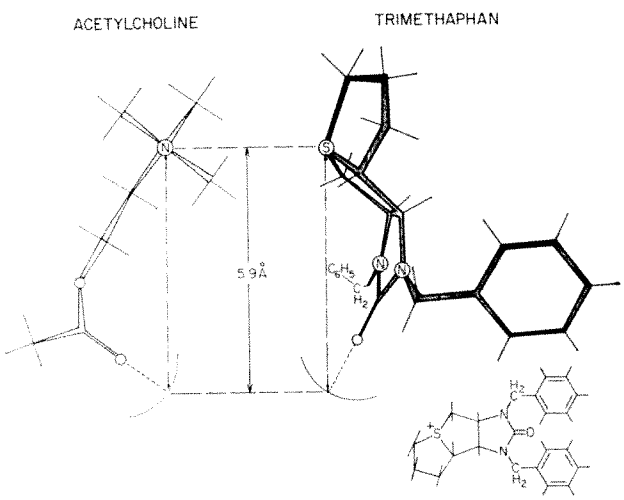


Fig. 6. In trimethaphan it is a sulphur atom which is charged. The distance from its centre to the centre of the van der Waals surface of the ureido oxygen is 5·9 Å.

both an anionic centre and an H-bond donor group the precise nature of which remains to be established. In Ach, the formation of an H-bond approximately 5·9 Å from the positive charge can be mediated only by the carbonyl oxygen; no conformation permits the ester oxygen to assume the position required for such an interaction. We therefore propose that the interaction of Ach with nicotinic receptors is stereospecifically determined by a coulombic interaction involving the quaternary nitrogen, and by the concurrent formation of an H-bond, for which the carbonyl oxygen acts as acceptor.

It is well known that organic cations, such as TMA (tetramethylammonium ion) and DMPP (dimethylphenylpiperazinium ion), which contain no potential H-bond acceptor, mimic the effects of acetylcholine. They do so especially well at synapses in autonomic ganglia. We shall consider the action of these substances later.

Muscarinic Agents

Pfeiffer[19] was the first to recognize the structural homologies among drugs active at parasympathetic sites. With the inadequate structural information then available, he formulated a proposal to account for the activity of these agents that was qualitatively confirmed in a subsequent analysis by Beckett[20], and ours is in accord with these.

The most potent muscarinic agonists and antagonists have a common set of structural elements which differs from, and does not overlap with, that found for the nicotinic series. In the muscarinic series the functional groups which determine interaction are (*a*) a quaternary ammonium grouping or its equivalent and (*b*) an unshared pair of electrons which can mediate the formation of an H-bond at approximately 4·4 Å from the centre of the positive charge. In addition, the interaction at muscarinic sites is strongly reinforced by a suitably located alkyl residue; the position of this residue can correspond to that of the methyl group in the acetyl moiety of Ach. These conclusions are derived from a consideration of four agonists and one set of antagonists.

F-2581 (Fig. 7) is the most potent muscarinic agent known[21]. The two oxygen atoms form part of the ring system and are symmetrically placed with respect to the positive charge, itself directly attached to the ring. These structural properties limit the separation of the positive charge and potential H-bond to the range of 4 to 5 Å.

Furmethide (Fig. 7) is another potent muscarinic agonist. In it the single oxygen atom is fixed in the unpuckered ring, minimizing conformational variations. The maximum distance which can be attained between the centre of the van der Waals surface of this oxygen and the charged quaternary nitrogen is about 4·4 Å. The addition of a methyl group to C-5 of furmethide increases the muscarinic potency by a factor of 10 (refs. 22 and 23). The position of this methyl group can easily be matched by the acetyl methyl group of Ach. This is one of numerous examples which show that a properly placed hydrophobic group enhances muscarinic activity and points to the involvement of the acetyl methyl group of Ach in muscarinic interactions.

The third compound which helps to establish the distance of about 4·4 Å is the classic muscarinic alkaloid, pilocarpine (Fig. 7). This contains two oxygen atoms which can act as H-bond acceptors, and two nitrogen atoms. Because of the restrictions imposed by the ring structure of pilocarpine, no combination of a single charged nitrogen and one H-bond acceptor site can be any closer than 4·3 Å. Because the maximum possible separation of the homologous groups in furmethide is 4·4 Å, we conclude that the muscarinic receptor interacts with Ach to form an H-bond between 4·3 and 4·4 Å from the cationic centre.

Meprochol (Fig. 7), another significant muscarinic agent contains only one oxygen, and it is analogous to the ester oxygen of acetylcholine. The muscarinic potency of meprochol is equal to that of acetylcholine[24]. Because meprochol has no potential H-bond acceptor which could assume the position of the carbonyl oxygen of acetylcholine, the latter cannot be essential for potent muscarinic activity.

It is also interesting that the more active remaining muscarinic drugs, such as muscarine, arecoline, 2-methyl-

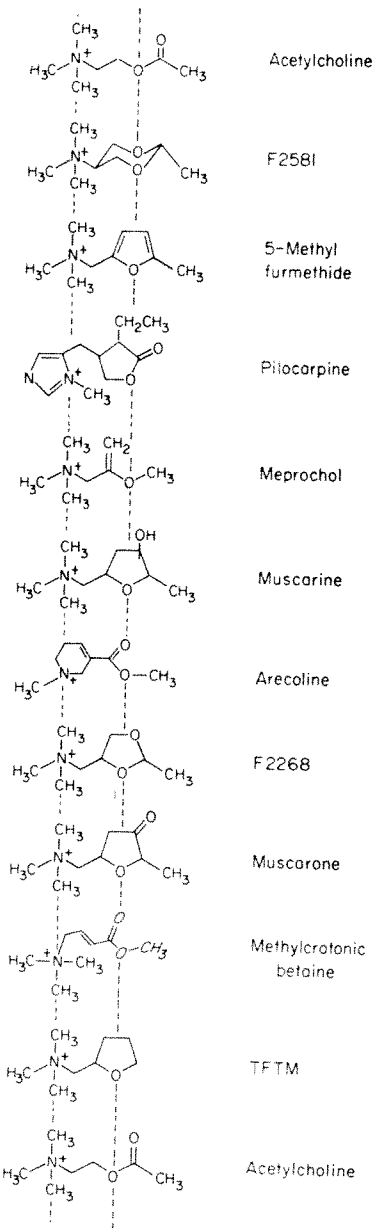


Fig. 7. All the drugs shown here contain an oxygen which, in terms o the distance between it and the charge centre, can attain a position equivalent to that of the ester oxygen in acetylcholine. Most of the more active drugs contain a methyl group which can assume a position equivalent to that of the acetyl-methyl group of acetylcholine.

4-dimethylaminomethyl-1-3-dioxolane (F2268) (ref. 25), muscarone, methylcrotonic betaine[26] and tetrahydrofuranyl-trimethylammonium ion (TFTM)[27] (Fig. 7) contain a potential H-bond acceptor 4·3 to 4·4 Å from the cationic centre. The most potent of these also have a methyl group in a position which can be assumed by the acetyl-methyl group of acetylcholine. Several of these compounds do not contain a potential hydrogen bonding function which is homologous with that of the carbonyl oxygen of acetylcholine: these are meprochol, methylfurmethide, TFTM and F2581.

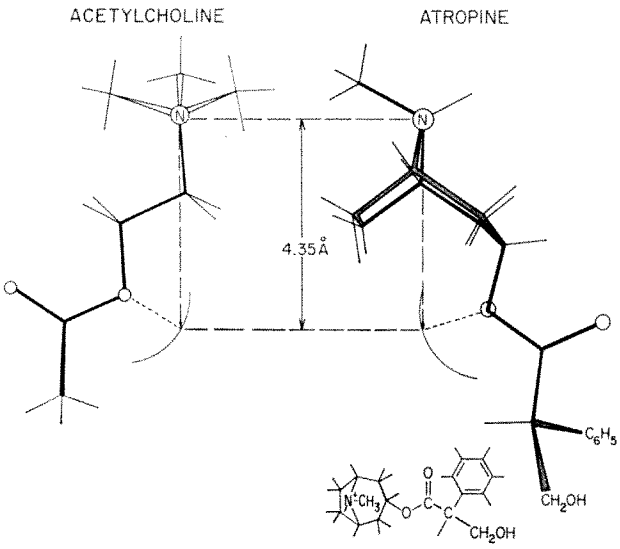


Fig. 8. Atropine, see text.

The potency of muscarine is markedly attenuated when any asymmetric centre is epimerized[28,29], and the same is true for acetyl-β-methylcholine[30,31]. The high degree of stereospecificity shown by the activity of these compounds is consistent with the view that optimal interaction with the muscarinic receptor normally involves three functional groups of the drug, in contrast to the two which seem to suffice at nicotinic junctions. This stereospecificity might also permit structural analysis which could identify the conformation of Ach that is responsible for its action at muscarinic sites[32]. But the sensitivity to epimerization which is reflected in the potency of the muscarine isomers contrasts with properties of several closely related compounds, that is, F2268, muscarone and 4,5-dehydromuscarine[20,25,26]. In some of the latter the muscarinic potency is much less strongly influenced by stereochemistry, and in others the pattern of activity of the various diastereoisomers does not coincide with that of the muscarines. These inconsistencies suggest that conclusions regarding the precise stereochemical requirements of the muscarinic receptor should be deferred. Beckett[20] has proposed one scheme for resolving this question. Additional insights may develop as further information about the properties of these molecules in solution becomes available.

In the muscarinic antagonist atropine (Fig. 8), the separation of the charged nitrogen and the H-bonding site on the ester oxygen is confined to a narrow range which includes the 4·4 Å distance observed in muscarinic agonists.

Deptropine (Fig. 9), like atropine, is a cholinergic antagonist at muscarinic sites. The oxygen which is the only potential H-bond acceptor in deptropine is stereochemically identical with the ester oxygen of atropine.

Like Ach itself, several drugs have both nicotinic and muscarinic actions. These include the γ-crotonyl betaines[26], muscarone, arecoline and pilocarpine. All of these, like Ach, contain distributions of functional groups which could mediate the formation of H-bonds at distances both 5·9 Å and 4·4 Å from the charge centre.

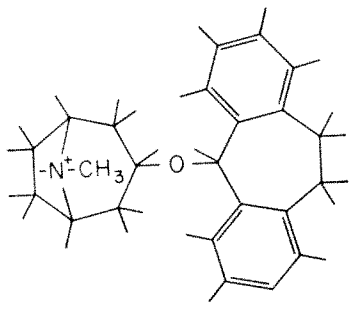


Fig. 9. Deptropine, see text.

As we have indicated, there are structural features common to agonists and antagonists within each family of cholinergic drugs. No precise expression or set of molecular properties seems to permit differentiation between agonists and antagonists. Increasing hydrophobicity and increasing structural rigidity are usually associated with antagonism. In the nicotinic series a tentative generalization can be formulated which relates to the alkyl substitution on the charge centre: molecules with more than two alkyl substituents larger than methyl are antagonists. One exception is the blocking action of hexamethonium in autonomic ganglia; another likely exception is the rigid alkaloid anagyrine, which is probably a nicotinic agonist. The most consistent structural property of muscarinic antagonists is the hydrophobic character which is conferred on them by large alkyl substituents on the charged nitrogen atom or by aromatic residues in the side chain, or both.

Functional Basis for Receptor Differentiation

The unsubstituted ammonium ion is inert at cholinergic junctions; cations which are active must have some hydrophobic character. The step-wise alkylation of ammonium (or other suitable cations) ultimately permits detectable interaction with the cholinergic receptor, the first expression of which is agonist action. The degree of alkylation which exists in trimethylammonium ion approaches the optimum required for this activity. The increased hydrophobicity that accompanies alkylation with larger substituents still permits interaction with the receptor, but the interaction is then expressed as antagonism.

The pharmacological activity of organic cations such as tetramethylammonium ion and DMPP indicates that the corresponding ammonium grouping in acetylcholine should be considered the true effector structure in the natural transmitter. That being so, if the response of all receptors were related to the charge alone, the activity

of TMA should be the same at all cholinergic sites. In fact, the relative potencies of TMA and Ach vary markedly at different cholinergic junctions; and, in view of the following considerations, these variations might provide some insight into the role of the individual functional groups in acetylcholine as they interact with the separate classes of receptors.

(1) TMA and Ach have equal potencies in autonomic ganglia[33], whereas the relative activity of TMA is lower by a factor of approximately 100 in skeletal muscle[34,35] and by a factor of 1,000 at the muscarinic junctions in smooth muscle[34].

(2) The synaptic delay and rise time of endplate potentials and excitatory post-synaptic potentials at nicotinic sites are much shorter than those of the junctional potentials at the muscarinic synapses in smooth muscle[36,37].

(3) In the rabbit superior cervical ganglion, where a complex series of responses follow preganglionic stimulation, the early, fast response is blocked by the nicotinic blocking agents tubocurarine and dihydrobetaerythroidine. The late, slower and ten-fold longer lasting response is blocked by atropine and is thus muscarinic[2]. Likewise, the slow prolonged responses to iontophoretically applied Ach, of neurones in the central nervous system are, predominantly muscarinic[38,39]. These findings imply that nicotinic junctions are more sensitive and responsive to chemical stimulation than muscarinic junctions.

This functional group analysis and the relative potencies of TMA and Ach suggest the following correlation with the physiological observations. In autonomic ganglia the demands on the transmitter are minimal; a transient interaction with simple, appropriate hydrophobic cations such as TMA is sufficient to depolarize the post-synaptic fibre. At neuromuscular junctions the demands on the receptor–transmitter complex are more exacting: the addition of an H-bond to the coulombic interaction can meet these demands by mediating a more precise and prolonged positioning of the quaternary ammonium group with the complementary receptor structure. At the sluggish muscarinic sites the demands are greatest of all, and the simple cation TMA is much less potent than Ach. At muscarinic junctions a transmitter–receptor interaction based on electrostatic elements alone is not adequate to produce a response efficiently with low concentrations of drug. Superimposition of hydrogen bonding and hydrophobic interactions at these sites should increase the lifetime of the complex and thereby enable the cationic portion of Ach to excite the tissue efficiently. As has been suggested previously, prolonged interaction of the drug and receptor would help to explain the long duration of "slow potentials" and the marked facilitation which is evident in the rabbit colon–pelvic nerve preparation[36].

The view of Ach which emerges from these considerations is that of a moderately hydrophobic cation, with functional versatility which is increased by several accessory structural elements; these are the two oxygen atoms, which can act as H-bond acceptors, and the methyl group which can participate in hydrophobic interactions. The single molecule, Ach, influences the activity of many target tissues whose ultimate response to the neutral transmitter is expressed in many different forms (for example, secretion, muscle contraction and bradycardia). It is likely that there are significant differences in the physical–chemical parameters, and particularly in the time constants of the numerous processes which culminate in the response of the various target tissues to Ach. The limiting factors in the different tissue responses would impose different requirements on the excitatory mechanism in each case. These needs could be met by modifications of the binding site on the receptor which would produce concomitant changes in the receptor–transmitter complex: the latter might include alterations in functional group specificity, in affinity, and in the rate of association and dissociation. We visualize that all Ach receptors interact with, and respond to, the charged quaternary ammonium moiety; the nicotinic receptor, at least at the neuromuscular junction, exploits in addition one accessory grouping—the carbonyl oxygen; the muscarinic receptor interacts with two of the accessory structures of Ach, the ester oxygen and the methyl group. Still other receptor specificities may remain to be characterized, especially in the central nervous system. Such a multiplicity of receptor specificities, which is apparent also in adrenergic transmission, is a reasonable basis for functional and pharmacological diversity in excitable tissues; it would provide a mechanism for qualitative and quantitative modulation of cellular responses to excitation.

We thank Dr Henry Sobell for providing details of the crystal structure of *d*-tubocurarine before publication, and Dr A. S. V. Burgen for helpful and stimulating discussions and for reading the manuscript.

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Evidence for Extraterrestrial Amino-acids and Hydrocarbons in the Murchison Meteorite

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Organic molecules found in meteorites seem to have been formed before the meteorites reached Earth.

THE Oparin–Haldane[1,2] hypothesis of chemical evolution, postulating the formation of organic compounds before the appearance of life on Earth, has been substantiated by experiments in the laboratory[3–5]. Many of the molecules important to living processes can be synthesized in conditions which may have prevailed on a primitive planet[6,7]. The fundamental concept of chemical evolution would be further substantiated by the finding of compounds of biological significance in extraterrestrial conditions.

Recent studies of intergalactic space, using the techniques of radioastronomy, have shown the presence of water[8,9], ammonia[10], hydrogen cyanide[11], formaldehyde[12], carbon monoxide[13] and cyanoacetylene[14], which are considered to be precursors in the formation of many biochemicals. Some evidence has also been put forward for the possible presence of molecules such as porphyrins[15] and polyaromatic compounds[16,17] in the interstellar medium. The lunar samples from the Apollo 11 and Apollo 12 missions provided a further opportunity for the search for carbon compounds in extraterrestrial materials, but the analysis of the fines from Mare Tranquillitatis[18] and Oceanus Procellarum[19] revealed only minute traces of such compounds. No conclusive evidence is available for the presence of any biomolecules indigenous to the lunar surface.

On the other hand, meteorites have been analysed for organic compounds for over a century[20–22]. Berzelius[23] examined the Alais, Wöhler[24] the Kaba and Berthelot[25] the Orgueil, and reported the presence of substances of organic origin. These investigations have continued, and there is general agreement about the presence of polymeric organic matter in carbonaceous chondrites[20]. The inherent potentiality for contamination resulting from the ubiquitous distribution of biomolecules on Earth[26,27] leads us to believe that many of the results reporting the presence of organic compounds in meteorites are inconclusive, but the results of our present investigation seem to resolve some of these ambiguities and provide evidence for amino-acids and hydrocarbons of possible extraterrestrial origin.

The Murchison meteorite fell at about 11.00 a.m., local time, September 28, 1969, near Murchison, Victoria, Australia (lat. 36° 36′, long. 145° 12′)[28]. The parent object broke up during flight and scattered many fragments over an area of about 5 square miles. Most fractured surfaces on the individual pieces have fusion crusts. Notable and distinctive features of many of the individual stones are networks of deep cracks extending into their interiors. Several stones were picked up soon after the fall and many have been collected since that time particularly during February and March, 1970. Many of the stones broke on impact with the ground.

For this study we selected those stones which had the fewest cracks, the least exterior contamination, and which generally appeared to have a massive character. The samples we examined contained 2·0 weight per cent carbon and 0·16 weight per cent nitrogen. Chemically, the Murchison meteorite is a type II carbonaceous chondrite (C-2).

Amino-acids

An interior piece of the meteorite (10 g) was pulverized and refluxed with 50 ml. of triply distilled water for 20 h at about 110° C. The extract was recovered by centrifugation and the residue rinsed twice with 50 ml. of water. The extract and rinses were combined, evaporated to dryness, and hydrolysed with 50 ml. of 6 M HCl for 20 h at about 100° C. This hydrolysate was evaporated to dryness and desalted by ion exchange using Dowex 50 (H^+) followed by Dowex 2 (OH^-). Portions of the recovered product were analysed by conventional ion exchange chromatography (Beckman–Spinco 120), by capillary gas chromatography of amino-acid derivatives (Perkin Elmer 881), and by gas chromatography combined with mass spectrometry (CEC 21-491). The meteorite residue after water extraction was hydrolysed with 6 M HCl for 20 h at about 110° C. This hydrolysate was treated in the same manner as the water extract.

Conventional ion exchange chromatography of the hydrolysed water extract from the 10 g sample revealed a number of peaks having retention times similar to common amino-acids. Five abundant amino-acids suggested by the chromatograms were glycine (6 μg/g), alanine (3 μg/g), glutamic acid (3 μg/g), valine (2 μg/g) and proline (1 μg/g).

The presence of the amino-acids, suggested by ion exchange chromatography, and their enantiomeric distribution were established by gas chromatography of the N-trifluoroacetyl-D-2-butyl esters of the amino-acids[29–32]. In this procedure, amino-acids with an asymmetric centre were transformed to diastereomers and the enantiomeric distribution of these compounds was determined on two different capillary columns—'UCON 75-H-90,000' and 'XE 60'. Fig. 1 shows a representative chromatogram resulting from an analysis of amino-acid derivatives. Peaks of the common amino-acids identified on the chromatogram confirm the identification made by ion exchange chromatography. Moreover, both enantiomers of amino-acids with asymmetric centres are present. The percentage of D-amino-acid enantiomers present was calculated from the gas chromatograms to be about 50 per cent for alanine, 45 per cent for glutamic acid, 40–47 per cent for valine and 40–43 per cent for proline. The presence of 2-methylalanine and sarcosine, amino-

acids not commonly found in biological systems, was suggested by the gas chromatogram.

To obtain an unambiguous identification, the compounds eluted from the gas chromatograph were introduced through a membrane separator into the mass spectrometer. The mass spectra obtained were compared with the spectra of known standards. In this manner the identities of the amino-acids, glycine, alanine, valine, proline and glutamic acid, were confirmed. Furthermore, the identity of each enantiomeric derivative of alanine, valine and proline was established. The D enantiomeric derivative of glutamic acid was confirmed but because of interfering compounds the spectra of the L enantiomeric derivative was equivocal.

The identities of 2-methylalanine and sarcosine were also established by mass spectrometry. Fig. 2 shows the spectra obtained from a standard N-TFA–D2-butyl ester of 2-methylalanine compared with the derivatized compound from the Murchison meteorite sample. Similarly, Fig. 3 illustrates the spectra of a derivatized standard sample of sarcosine and a derivatized compound from the meteorite sample. As in the mass spectra of the N-TFA-*n*-butyl esters of amino-acids[33] the key peaks in the spectra of the N-TFA-D-2-butyl esters of 2-methylalanine and sarcosine are the $(M-C_4H_9COO)^+$ peak and the $(M-C_4H_9O)^+$ peak.

Amino-acids have been found previously in other meteorites[20,34] but because the distributional patterns of these amino-acids are similar to amino-acids in fingerprints, the occurrence of amino-acids in meteorites has commonly been attributed to contamination[20]. The total concentration of glycine, alanine, valine, proline and glutamic acid obtained from Murchison meteorite was about 15 μg/g, which is about twice the total concentration of these same compounds found free in Murray, another type II carbonaceous chondrite[34]. In previous work serine has usually been reported as among the most abundant amino-acid in both meteorites and fingerprints[20]. In this study serine was tentatively identified only by ion exchange chromatography and its concentration was an order of magnitude less than the concentration of glycine. The unique distribution of amino-acids in the Murchison meteorite cannot be explained satisfactorily on the basis of contamination by human hands.

The distribution of the enantiomers of alanine, valine, proline and glutamic acid also supports the idea that the amino-acids from the Murchison meteorite are not recent biological contaminants. Amino-acids in living systems are of the L configuration except in rare instances. In recent sediments the L configuration dominates[32].

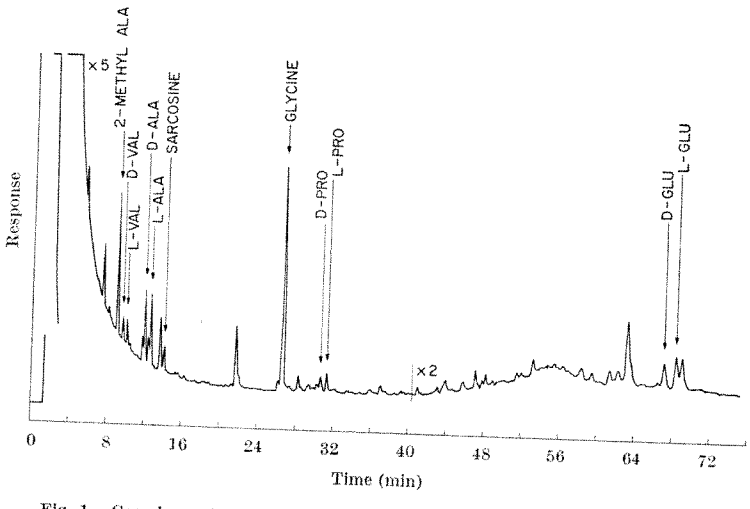


Fig. 1. Gas chromatogram of N-trifluoroacetyl-D-2-butyl esters of amino-acids in acid hydrolysed water extract of a 10 g sample of the Murchison meteorite. Gas chromatography: 0·02 inch × 150 feet capillary column coated with 'UCON 75-H-90,000'; temperature programmed from 100° to 150° C at 1° C/min.

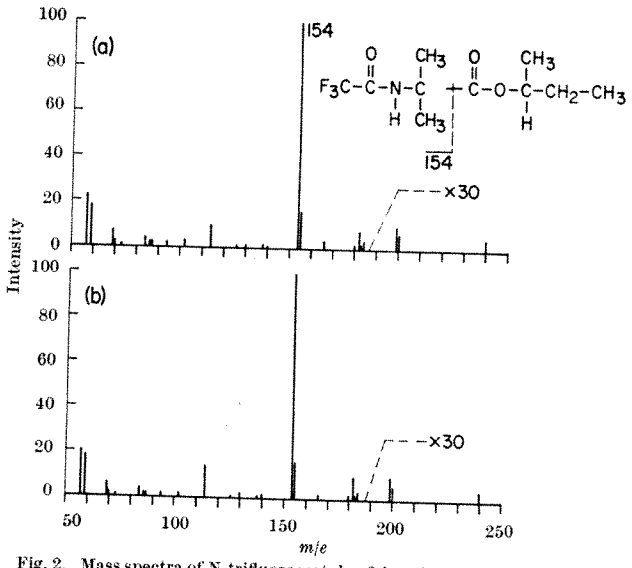


Fig. 2. Mass spectra of N-trifluoroacetyl-D-2-butyl ester of: *a*, 2-methylalanine standard; *b*, compound from the Murchison meteorite.

Studies of amino-acid configurations in other carbonaceous chondrites show that the distribution of enantiomers resembles those of recent sediments and soils[35]. The D and L enantiomers of amino-acids in the Murchison meteorite are almost equally abundant. Slight biological contamination might account for the small dominance of the L isomers of valine, proline and glutamic acid. The distribution of amino-acid enantiomers in Murchison can be accounted for either as a racemic modification of biologically produced amino-acids or as a racemic mixture of abiotically produced amino-acids. The discovery of 2-methylalanine and sarcosine, which are not commonly found in proteins, supports the hypothesis that the whole collection of amino-acids was produced abiotically. In chemical evolution experiments simulating the primitive atmosphere, both sarcosine and 2-methylalanine have been noted among the products[36].

Hydrocarbons

Ten g of the pulverized meteorite were extracted by sonication in a centrifuge tube three times successively, with about 20 ml. benzene–methanol (9 : 1). After centrifugation the extract was evaporated to dryness in a rotary evaporator. This residue was redissolved in 1–2 ml. hexane, which was applied to a 10 g activated silica gel column. The column was eluted with 15 ml. portions of hexane, benzene and benzene–methanol. Activated copper strips were placed in the hexane and benzene fractions and allowed to stand overnight to remove any free sulphur. After the removal of the copper the three fractions were evaporated to about 100 μl. About one-tenth of the hexane fraction was analysed by gas chromatography using a 50 foot 'OV-1' support coated open tubular column in a Perkin–Elmer '880' gas chromatograph. The remainder of the sample was analysed by gas chromatography combined mass spectrometry ('CEC 21-491'). Mass spectra were taken every 3 min during the chromatography.

A second sample (15 g) was similarly extracted. After the removal of sulphur the hexane fraction was analysed by thin layer chromatography using silica gel plates impregnated with silver nitrate to separate saturated alkanes from the unsaturated[37]. Saturated alkanes were eluted from the silica gel with hexane, and one-twenty-

fifth of the eluate was analysed by gas chromatography as before. A small fraction (approximately one-third) was volatilized directly into the source of a 'CEC 21-110' mass spectrometer for a series of high resolution photoplate spectra, and one-third was vaporized directly into the source of the 'CEC 21-110' mass spectrometer to obtain a series of low resolution spectra.

The chromatograms in Fig. 4 indicate that a very complex mixture of alkanes has been isolated from the two meteorite samples. A rough estimate of their abundance can be made by assuming that the arch shaped trace is an equilateral triangle composed of a series of peaks spaced at very close intervals. This estimate suggests that about 350 μg of alkanes were isolated from the 10 g sample and 175 μg from the 15 g sample.

Contamination seems to be limited to a small group of compounds eluted at 210°–249° C. These have been routinely observed in our laboratory and seem to be airborne contaminants.

The distribution pattern of the alkanes isolated from the 10 g sample is a symmetrical trace (Fig. 4) with a peak at 180° C, which corresponds to the elution temperature of n-C_{18}, with a shoulder in the 100°–140° C region. On this shoulder (130° C) is a peak with the retention time corresponding to a saturated n-C_{13} hydrocarbon, and represents about 0·1 μg of hydrocarbons. Other—much smaller—peaks on the chromatogram correspond, in retention time, to n-C_{18} and n-C_{22}–C_{23}. The mass spectra of the various GLC fractions taken at 3 min intervals were very similar and showed a predominance of fragment ions corresponding to the homologous series:

$$(C_nH_{2n-3})^+ > (C_nH_{2n-1})^+ > (C_nH_{2n-5})^+ > (C_nH_{2n+1})^+$$

Mass spectra of fractions eluting from the column at higher temperatures show an increasing predominance of the $(C_nH_{2n}-5)^+$ ion over the $(C_nH_{2n}-1)^+$ ion. The peak emerging at 130° C has not been completely characterized. The mass spectra of the species coinciding with the appearance of this peak on the gas chromatogram shows intense ions at m/e 127 and 182.

The gas chromatographic distribution pattern of the saturated alkanes isolated from the 15 g sample by thin layer chromatography is also a symmetrical trace (Fig. 4), but it differs from the first sample, in that it reaches a maximum at 160° C ($n-C_{16}$ retention time) and does not have a shoulder in the 100°–140° C region. This may result from removal of small amounts of unsaturated isomers by $AgNO_3$ chromatography. The low resolution mass spectra of this alkane mixture resembled those from the first sample. Several predominant molecular ions are present (Fig. 5), suggesting that the hydrocarbons in this particular sample are relatively stable to fragmentation on electron bombardment. These molecular ions (m/e 278, 292, 306) correspond to the bicyclic, homologous series C_nH_{2n-4}. A series of relatively intense peaks at m/e 221, 235 and 263 suggests that the loss of hydrocarbon fragments, ranging from $-CH_3$ to $-C_4H_9$, is a favourable process. This evidence, and the relatively large intensity of the m/e 151 peak relative to m/e 137 and m/e 165, suggests that these hydrocarbons closely

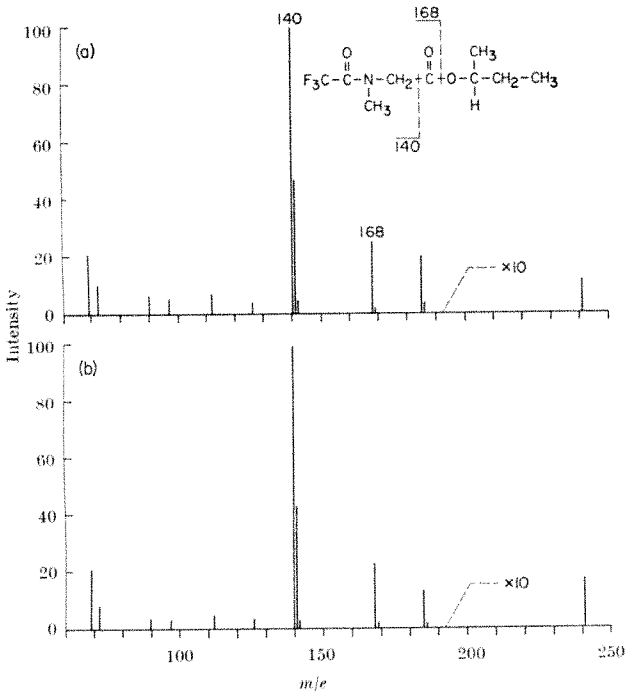


Fig. 3. Mass spectra of N-trifluoroacetyl-D-2-butyl ester of: a, standard of sarcosine; b, compound from the Murchison meteorite.

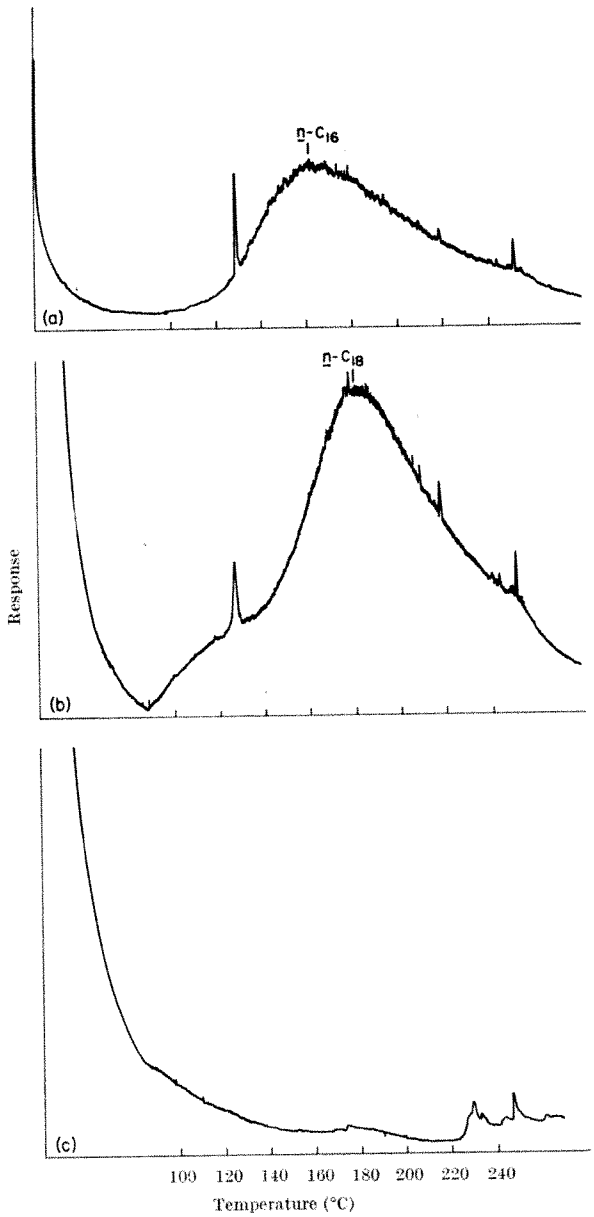


Fig. 4. Gas chromatograms of alkanes isolated from benzene–methanol extracts of two samples of the Murchison meteorite: a, 1/10 of alkane fraction isolated from 10 g sample (attenuation ×50); b, 1/25 of alkane fraction isolated from 15 g sample (attenuation ×20); c, chromatographic blank (attenuation ×20) gas chromatography: 50 feet support coated open tubular column with 'OV-1' liquid phase; temperature programmed from 100° to 240° C at 2° C/min.

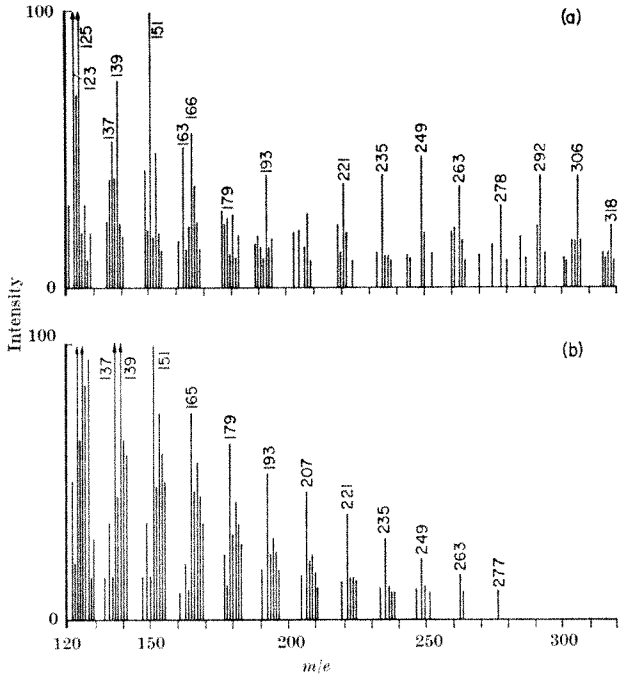


Fig. 5. Low resolution mass spectrum of alkane fractions obtained on volatilization at 125° C from the direct probe of a 'CEC 21–110' mass spectrometer. Ion intensities normalized to *m/e* 151. *a*, Fraction from 15 g sample of the Murchison meteorite; *b*, fraction from spark discharge in methane.

recemble decahydronaphthalenes containing short chain hydrocarbon substituents.

There is a marked resemblance between the gas chromatographic traces of the aliphatic hydrocarbons synthesized by the action of electrical discharges on methane[38] and those from the meteorite. The analysis of these samples by thin layer chromatography on silica gel plates impregnated with $AgNO_3$ (ref. 37) indicates that they consist largely of saturated alkanes. The mass spectral data reveal the same dominant homologous series in both samples (Fig. 5). The similarity between the aliphatic hydrocarbons from the meteorite and the spark discharge material, on these three counts, is suggestive of a possible abiogenic origin for the hydrocarbons in the Murchison meteorite.

Further investigations are in progress to determine the possible presence of aromatic and unsaturated hydrocarbons, carboxylic acids and porphyrins.

Carbon Isotope Distribution

Various carbon compounds were analysed for $^{13}C/^{12}C$ following the technique of Smith and Kaplan[39]. The results (expressed in the conventional manner as $\delta^{13}C$, relative to Peedee Belemnite) are given in Table 1. These results, together with the carbon content (2·5 per cent), place the meteorite as a type II carbonaceous chondrite[39]. The values obtained for the organic solvent extractable material are significantly more enriched in ^{13}C than any previous measurements on such compounds in other carbonaceous chondrites. Extracts of terrestrial sediments and petroleum fall in the range $\delta^{13}C = -25$ to -35 parts per thousand[40,41]. Results obtained from the meteorite confirm the indigenous nature of the carbon and indicate that the amount of terrestrial contamination is insignificantly low.

Table 1

Form of carbon analysed	$\sigma^{13}C$ PDB (parts per thousand)
(i) Total carbon	−7·3, −7·1
(ii) Carbonate	+45·4
(iii) Benzene–methanol soluble compounds	+4·4, +4·8, +5·9
(iv) Insoluble carbon (after solvent extraction and demineralization of silicates with HF)	

The results of our investigation suggest the indigenous nature of amino-acids and hydrocarbons in the Murchison meteorite. The $\delta^{13}C$ values of +4·43 to +5·93 for the extractable organic material of the meteorite fall into a range widely different from terrestrial organic matter, and the presence of the amino-acids glycine, alanine, valine, proline, glutamic acid, 2-methylalanine and sarcosine was unequivocally established. The presence of almost equal amounts of the D and L enantiomers of valine, proline, alanine and glutamic acid minimizes the possibility of terrestrial contamination and suggests a possible extraterrestrial origin. The presence of the two amino-acids, 2-methylalanine and sarcosine, which are not generally found in biological systems, is indicative of a possible abiogenic synthesis. The gas chromatographic pattern of distribution of hydrocarbons and their mass spectrometry fragmentation pattern similar to that obtained by an abiotic synthesis in the laboratory support the contention that the organic molecules identified here are abiotic and possibly extraterrestrial in origin.

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RNA Dependent DNA Polymerase of Human Acute Leukaemic Cells

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An RNA dependent DNA polymerase analogous to that of RNA tumour viruses has been found in lymphoblasts of leukaemic patients but not of normal donors. The enzyme can use an RNA template from mammalian cells to synthesize DNA.

RECENT reports by Temin[1] and Baltimore[2] that an RNA dependent DNA polymerase activity is present in oncogenic RNA viruses, now confirmed and extended in other laboratories[3-5], provide a mechanism by which an RNA virus may insert stable genetic information into a host cell genome.

The aetiology of human acute leukaemia is not known, but a role for RNA oncogenic viruses in human neoplasia has been proposed for several reasons[6]. Although RNA virus particles have not been clearly associated with human leukaemia, we have examined human leukaemic cells for the presence of an RNA dependent DNA polymerase because: (1) it is possible that RNA virus particles are regularly present in human leukaemic cells but cannot be detected by ordinary means. The presence of a unique enzyme might be a more sensitive index. (2) The virus particles may never be formed but the viral genome would be integrated and undetected, yet functional in the host cell. The enzyme could be required for subsequent formation of additional viral DNA used in infection of other host cells. (3) Information flow from RNA to DNA raises interesting questions regarding gene amplification during biochemical cytodifferentiation. This mechanism could have considerable implications for cell growth and differentiation[7], and because human leukaemia has been considered a disorder of cell differentiation, it may also have implications for leukaemogenesis[8,9].

Choice and Preparation of Cells

Several considerations influenced our choice of cells. First, acute leukaemia was selected rather than the chronic form because the characteristics of the former cell type are more malignant and less often contaminated with other types of leucocytes. In leukaemia of an acute "blastic" type, a population of almost 100 per cent blasts can be obtained directly from a patient. Second, the lymphoblastic type was chosen rather than the myeloblastic (granulocytic type) because the latter are more likely to be associated with other more differentiated cells of the myeloid (granulocytic) series. These cells contain abundant lysosomes with high nuclease activities, making any RNA analysis or polymerase assay extremely difficult[9]. Third, proliferative lymphoblasts can also be obtained from normal human volunteers. This is achieved by transformation of normal peripheral blood lymphocytes to lymphoblasts with a mitogenic agent. Fourth, the polymerase activities of tumour cells are generally higher than those of normal adult organs. A much greater content of various polymerases would be more likely to lead to a spurious interpretation of a unique polymerase in such cell types. For this reason, we would expect a better controlled comparison between normal and neoplastic cells of comparable DNA and RNA polymerase activities.

The simple use of peripheral blood leucocytes, which consist primarily of fully mature non-proliferating granulocytes and lymphocytes, cannot be considered as controls for leukaemic blast cells, particularly in view of the fact that these cells have minimal or no detectable DNA dependent DNA polymerase activity[9]. On the other hand, after 72 h of stimulation of normal human lymphocytes with phytohaemagglutinin (PHA), DNA synthesis is maximal[10]. In addition, Loeb *et al.*[11] have reported a 30 to 100-fold induction of DNA polymerase at this time, so that activities reach levels comparable with neoplastic cells, and Hausen *et al.*[12] have reported an induction of RNA polymerase in lymphocytes stimulated with PHA. We have confirmed both these findings (unpublished results). Fifth, human cells obtained directly from peripheral blood instead of human tissue culture cell lines were chosen for these initial investigations because they obviously are a more true reflexion of the disease. Furthermore, there is much less chance of contamination with microorganisms or of developing mutations not relevant to leukaemogenesis.

The leukaemic cells utilized in this study, therefore, were peripheral blood lymphoblasts obtained from three patients with acute lymphoblastic leukaemia (ALL). In each, the number of lymphoblasts was more than 100,000/mm^3 of blood. Two patients were untreated and the third received hydroxyurea for one day. Normal lymphocytes were obtained from the peripheral blood lymphocytes of forty-eight normal donors.

The lymphocytes were separated from other blood cells, as previously described[13], except that an additional nylon column chromatographic step was carried out to obtain more pure cell populations (more than 98 per cent lymphocytes). These cells were incubated with the mitogenic agent and harvested after 72 h, as previously described[13]. In our conditions, at 72 h the number of cells transformed to lymphoblasts and the rate of DNA synthesis are maximum[10]. After terminating the incubation, the cells were extensively washed with 0·15 NaCl and used for polymerase assays.

RNA dependent DNA Polymerase Activity

Nucleic acid "free" preparations were made by gentle manual homogenization (Ten-Broeck) of purified lymphoblast pellets in 3 volumes of 25 mM Tris-sulphate buffer, *p*H 8·3; 1 mM $MgSO_4$; 6 mM NaCl; 4 mM dithiothreitol; and 0·1 mM EDTA. The samples were centrifuged at 15,000 r.p.m., and the supernatants and pellets separated. The pellets of membranes and nuclei were washed with the same buffer with gentle homogenization. After centrifugation the wash (second supernatants) was combined with the first supernatants and the nuclei–membrane pellets removed.

Nucleic acids were removed from the supernatant fractions by successive precipitations with $MnCl_2$ and

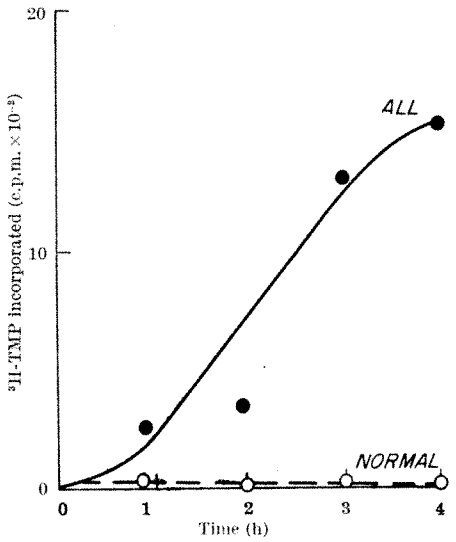


Fig. 1. Kinetics of ^{3}H-TMP incorporation into TCA precipitable materials. Reaction mixtures in a final volume of 1 ml. contained: 0·295 mg protein from the nucleic acid "free" crude extract (see text); 50 μmoles Tris-HCl buffer, *p*H 8·3; 6·0 μmoles MgAc; 20 μmoles of dithiothreitol; 60 μmoles of NaCl; 0·8 μmole each of dATP, dCTP, and dGTP; 10 μCi [^{3}H-methyl]-TTP (New England Nuclear, 7·7 mCi/μmole); and 40 μg of rat liver RNA. (Later experiments revealed that concentrations of RNA lower than 10 μg/ml. were more effective for template activity.) Samples were incubated at 37° C and were withdrawn at intervals and the reactions terminated by the addition of 0·5 ml. of cold 0·08 M Na-pyrophosphate with 200 μg of yeast nucleic acid added as a carrier prior to precipitation of macromolecules by adjustment to 12 per cent TCA. The precipitates were then washed extensively with cold 5 per cent TCA on Millipore filters, dried, and dissolved in 2·0 ml. of Beckman Bio-solv No. 3. 10 ml. of 'Omnifluor'-toluene were added, and the samples were counted in a liquid scintillation counter. ●—●, Activity of RNA dependent DNA polymerase from ALL lymphoblasts; ○ - - - ○, activity of lymphoblasts from PHA stimulated lymphocytes of normal donors. Counts were corrected for endogenous templating activity at each point. Heated controls (85° C for 5 min) incubated for the same period showed no significant non-specific trapping of radioactivity. The rat liver RNA was prepared by repeated phenol extraction and ethanol precipitation of the supernatant obtained after homogenization of liver slices (General Biochemical Co.). The commercial preparation was treated with deoxyribonuclease and the enzyme removed by phenol extraction and DEAE cellulose chromatography. The fractions of RNA eluting at 0·7 M NaCl were combined and after precipitation with ethanol this RNA was used as the template.

protamine sulphate. The final supernatants were the nucleic acid "free" preparations. Subsequent purifications were performed by elution of these fractions from DEAE-cellulose and gel filtration columns. The final fraction, designated the gel filtration preparation, was purified between 200 and 270-fold. More detailed information on the method of purification will be described elsewhere.

No RNA dependent DNA polymerase activity was detected in any of the preparations of normal lymphoblasts obtained from a total of 48 normal donors. Fig. 1 shows a comparison of the kinetics of incorporation of ^{3}H-TMP by nucleic acid "free" preparations from human ALL lymphoblasts (Patient 1), and one of the normal lymphoblasts in a DNA polymerase assay dependent on a rat liver RNA. The activity in the ALL lymphoblasts cannot be maximum because later experiments with other preparations revealed that RNA and TTP concentrations were not optimum. But with optimum conditions, activity could again not be demonstrated in the normal lymphoblasts.

A small endogenous reaction with both the leukaemic and normal lymphoblasts was observed with these nucleic acid "free" preparations, apparently due to contamination with DNA dependent DNA polymerase and a small amount of DNA in this relatively crude extract. A lag in kinetics was observed during the first hour of incubation similar to the reaction described by Baltimore with Rauscher mouse leukaemia virus RNA dependent DNA polymerase[2]. This might be due to interference in the process of template binding to enzyme.

Fig. 2 shows the kinetics of incorporation of ^{3}H-TMP in the presence of rat liver RNA by a more purified enzyme (G-100 gel filtration following initial steps described above) from human ALL lymphoblasts (Patient 2). This preparation is free of DNA as judged by diphenylamine assay, and unlike the less purified preparation there is no polymerase activity in the absence of an RNA template. An additional difference is the absence of lag in the initial period of incubation. This was also noted in another human acute leukaemic G-100 gel filtration preparation (Patient 3). The RNA dependent DNA polymerase activity of Patient 3 (c.p.m./μg protein/h) was approximately the same as Patient 2. Also shown in Fig. 2 are the results obtained when the "template" was pre-incubated for 1 h with ribonuclease (11 μg/ml.). Ribonuclease treatment completely removed the "template" activity of the RNA preparation. In other experiments with a human tumour cell line, 90 per cent inhibition of RNA template activity was obtained with as little as 0·2 μg/ml. of ribonuclease (observation of R. G. Smith and F. Herrera in our laboratory).

As described above and shown in Table 1, the reaction depends completely on the addition of rat liver RNA. The reaction required Mg^{2+} and deoxynucleoside triphosphates.

The Template is RNA

We have excluded the possibility that rat liver RNA might have been contaminated with a trace amount of DNA despite the initial treatment with deoxyribonuclease (see legend to Fig. 1 for purification of the RNA). The rat liver RNA was pre-incubated with deoxyribonuclease followed by subsequent removal of the enzyme as described in Table 2. Extensive treatment with deoxyribonuclease again showed no effect on the template activity of the purified RNA. But after pre-incubation with ribonuclease all activity was lost (Fig. 2). The reason for this might be that ribonuclease, a basic protein, could inhibit the polymerase reaction by interfering with the enzyme-template complex formation. Similar to the approach used by Baltimore[2], the effect of lysozyme, another basic protein, was also examined. No effect of lysozyme was found on the ALL enzyme with rat liver RNA as template even with a 10-fold increase in concentration (Table 3).

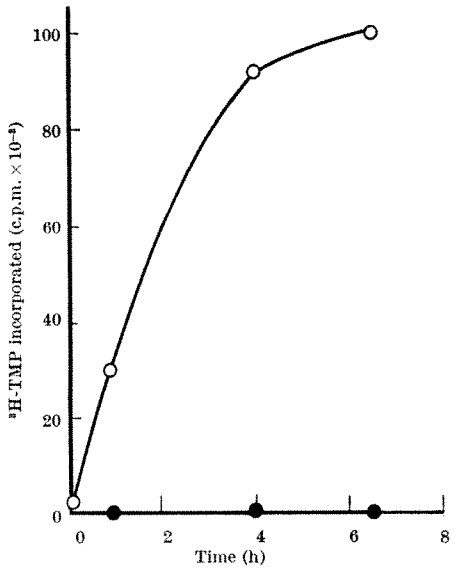


Fig. 2. The effect of ribonuclease hydrolysis on rat liver RNA template activity with the G-100 gel filtration partially purified RNA dependent DNA polymerase from ALL lymphoblasts. Rat liver RNA (20 μg) was incubated with 11 μg of highly purified pancreatic ribonuclease (crystalline, Worthington) at 37° C for 60 min in 1 ml. of 10 mM Tris-HCl, buffer *p*H 7·3, with 5 mM sodium acetate, prior to assay for its template activity. The assay system was the same as described in Fig. 1 except that 25 μCi/ml. of ^{3}H-TTP was used. ○, Kinetics of RNA dependent DNA polymerase with the untreated rat liver RNA template; ●, activity with the ribonuclease digested rat liver RNA as template.

Further evidence that the template is RNA rather than a DNA contaminant was obtained by assay for DNA. Two mg of the rat liver RNA was precipitated with perchloric acid and assayed for reactivity with diphenylamine. No deoxyribose was detected (Table 2).

We estimated the size of the rat liver RNA template by gel filtration (G-100) and sucrose gradient (5 to 25 per cent) centrifugation for 16 h at 105,000*g*. The results showed that approximately 85 per cent of the active template moved coincident with 4S RNA, while the remaining 15 per cent was RNA with a molecular weight greater than 150,000 (Table 2).

It is possible that an RNA dependent DNA polymerase is present in mammalian cells to amplify RNA species and does not have specificity for viral RNA. For most of these experiments, therefore, mammalian RNA was chosen as template. But, to verify further that the enzyme responds to RNA, the synthetic ribopolynucleotide poly rA·poly rU (10 μg/ml.) was tested, and it showed activity comparable with the results shown in Fig. 2 with rat liver RNA (see Table 1).

That the product is DNA is suggested by the incorporation of deoxynucleoside triphosphates into an acid precipitable form. The nature of the product was further investigated by determining the effects of various hydrolytic agents. Table 3 shows (1) that the reaction product is completely hydrolysed by deoxyribonuclease, (2) but it is not hydrolysed by ribonuclease or trypsin, and (3) it is alkaline resistant. Experiments are in progress to characterize it further.

Table 1. NATURE OF RNA DEPENDENT DNA POLYMERASE REACTION

Reaction mixture	c.p.m./ml. minus background
Complete, with rat liver RNA 40 μg/ml.	4,149
Complete, with rat liver RNA 10 μg/ml.	5,806
Complete, with poly rA · poly rU 10 μg/ml.	9,760
Without RNA template	0
Without $MgCl_2$	0
Without dCTP	405
Without dATP	622

The assay system was similar to that described in the legend to Fig. 1, except that 22·5 μg of the G-100 gel filtration partially purified enzyme was used. Incubations were carried out at 37° C for 2 h. Endogenous incorporation (no RNA template) was taken as the background and consistently gave values close to 200 c.p.m. Recent experiments have shown that maximum activity is obtained with concentrations of template less than 10 μg/ml.

Table 2. PROPERTIES OF THE MAMMALIAN RNA TEMPLATE

Determinations	Comments
Ribonuclease sensitivity 11 μg/ml.	100 per cent
Lysozyme sensitivity 100 μg/ml.	None
Deoxyribonuclease sensitivity 200 μg/ml.	None
Diphenylamine assay of deoxyribonuclease treated RNA template	No deoxyribose
Sucrose (5 to 25 per cent) equilibrium centrifugation	Major fraction has coincidence with 4S marker. Also small amount of high molecular weight component
DEAE-cellulose chromatography	Coincidence with 4S marker
Amino acid (^{14}C-aspartic acid or ^{14}C-serine) acceptance	0·4 pmoles per μg RNA

Assay systems were identical to those described in the previous table except ^{3}H-TTP was increased to 40 μCi/ml. RNA template concentration was 50 μg/ml. Controls (no treatment) gave 5,480 c.p.m. The increase in counts compared to previous experiments is attributed to the increase in ^{3}H-TTP. The ribonuclease sensitivity was determined by pre-incubation of the template RNA with ribonuclease for 1 h at 37° C and the reaction continued for 2 h. Deoxyribonuclease sensitivity was determined by pre-incubation of the RNA template with deoxyribonuclease at 37° C for 1 h. The template and deoxyribonuclease were separated by repeated phenol extraction and ethanol precipitation followed by DEAE-cellulose column chromatography with an NaCl step gradient of 0·15 M, 0·35 M, and 0·7 M. The RNA fraction, eluting at 0·7 M NaCl, was precipitated with ethanol and then assayed for template activity. Lysozyme sensitivity was determined by co-incubation of human lysozyme in the reaction mixture. Sucrose gradient centrifugation was performed in an SW-25 rotor at 105,000*g* for 16 h. For the diphenylamine assay 2 mg of the deoxyribonuclease treated RNA was precipitated with cold TCA and assayed by the method of Burton[14]. Test of the rat liver RNA template for amino-acid acceptance was carried out with partially purified rabbit liver aminoacyl-tRNA synthetases. The reaction mixtures and methods of preparation of the synthetases were previously described[8]. The aminoacyl-tRNA synthetases used in these assays were limiting. Thus the amino-acid acceptance by the RNA was not a maximum.

Table 3. ENZYMATIC DIGESTIONS OF ^{3}H-TMP-LABELLED PRODUCT

Treatments	c.p.m./ml. minus background
Control, none	12,260
Ribonuclease, 2 mg/ml.	11,640
Trypsin, 1 mg/ml.	11,080
* NaOH, 0·5 N	7,670
Deoxyribonuclease, 4 mg/ml.	560

RNA dependent DNA polymerase reaction was carried out for 4 h as described in Fig. 1. The sample was then divided into 5 aliquots and treated with either water (control), ribonuclease, deoxyribonuclease, trypsin, or NaOH (0·5 N). The reaction was then continued for an additional 1 h. * The adjustment to 0·5 N NaOH terminated the reaction early and hence yielded lower counts.

Inhibitors of ALL Polymerase

M. Green (personal communications) has found that the rifampicin analogue N-demethylrifampicin, but not rifampicin itself, inhibits the RNA dependent DNA polymerase of the oncogenic RNA virions. The partially purified human ALL RNA dependent DNA polymerase is also markedly inhibited by N-demethylrifampicin and inhibited less by rifampicin (Table 4). If the presence of the enzyme is, in fact, unique to leukaemic or other neoplastic cells, its inhibition by these agents has obvious implications to chemotherapy.

Table 4. INHIBITORS OF ACUTE LEUKAEMIC LYMPHOBLAST RNA DEPENDENT DNA POLYMERASE

Treatment	Per cent of control
None	100
Rifampicin	
50 μg/ml.	102
200 μg/ml.	81
460 μg/ml.	60
N-demethylrifampicin	
50 μg/ml.	60
200 μg/ml.	42
300 μg/ml.	37
460 μg/ml.	0

The assay was identical to that described in Table 1.

Although the significance of this enzyme to the leukaemogenic process remains to be proven and a viral RNA active in templating the enzyme has yet to be demonstrated, the finding of an RNA dependent DNA polymerase in ALL lymphoblasts and the failure to find the same activity in normal lymphoblasts is consistent with a viral etiology.

On the other hand, the RNA template dependency of this polymerase could implicate a gene amplification role of the enzyme in biochemical cytodifferentiation, which could prove to be the basis of leukaemogenesis. Although differences in enzymes of human leukaemic cells compared with comparable normal cells have been sought for many years, there is little if any evidence for any qualitative differences. But there is suggestive evidence by molecular hybridization studies[15] and by comparative column chromatography[8] for the presence of different RNA species in human leukaemic cells and in normal cells. The presence of an RNA dependent DNA polymerase in leukaemic cells might be implicated in these differences.

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Variability of Interchain Binding of Immunoglobulins

The variability of the interchain binding of light and heavy chains (the different human immunoglobulins is a puzzling feature of the covalent structure. The following articles describe the intercha binding of the three subclasses of mouse IgG and of the huma IgD class.

Interchain Bridges of Mouse IgG2a and IgG2b

THE four-chain structure of two heavy and two light chains, usually linked by disulphide bridges, is basic to immunoglobulin molecules, but differences in the number and position of the interchain bridges have been noted in the study of molecules of different human IgG[1] and IgA[2] subclasses. Thus the number of interheavy chain bonds in human IgG molecules may be two (in IgG1 and IgG4) to five (in IgG3); these bridges may be close to the bond to the light chain (in IgG1) (refs. 1, 3, 4)

Mice have three major subclasses of IgG (IgG1 or Igl IgG2a or IgG; and IgG2b or IgH) the biological properti of which have been reviewed[7]. Only IgG2a and IgG2 molecules fix complement, whereas only IgG1 molecul give positive passive cutaneous anaphylactic reactio when tested in a homologous (mouse skin) system. He we report studies on the sequences around the intercha disulphide bridges of two mice IgG2a (MOPC 173 ar 5563) and a mouse IgG2b (MOPC 141) myeloma prote obtained from transplanted tumours. The protei were purified from serum provided by Drs A. Mun (MOPC 141) and Fougereau (MOPC 173 and 5563).

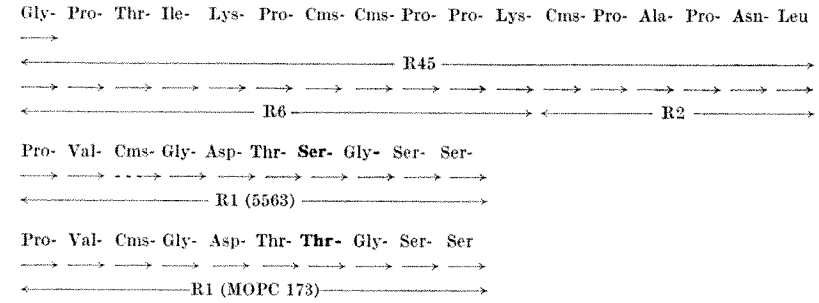


Fig. 1. Sequences of carboxymethylcysteine containing peptides obtained by partial reduction and carboxymethylation of IgG2a proteins 5563 and MOPC 173. The peptides were obtained by digestion with pepsin and trypsin. The sequence of peptide R1 was in fact established using the cysteic acid derivative isolated in diagonal experiments. Cms: carboxymethylcysteine sulphone.
Arrows indicate results of Dansyl-Edman procedure. Broken arrow indicates that the identification was uncertain.

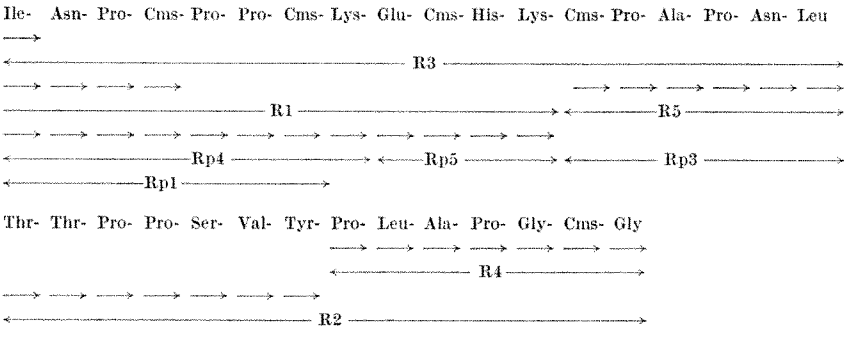


Fig. 2. Sequences of Cem-containing peptides obtained by partial reduction and carboxymethylation of IgG2b protein MOPC 141.

or probably more than 100 residues distant in linear sequence from it (in IgG2, IgG3 and IgG4 molecules[1]). These differences are in contrast to the otherwise close similarity between molecules of different subclasses[5,6], and may be important in mediating the different biological activities of the subclasses.

Interchain bridges were characterized by parti reduction and labelling with ^{14}C-iodoacetic acid[8], and t the diagonal procedure. In each case pepsin/trypsi digests were prepared and subjected to paper electr phoresis at *p*H 6·5, and selected peptides were purifie (before and after oxidation) at *p*H 3·5. After parti

reduction and labelling, the protein was re-reduced and alkylated with cold iodoacetic acid[9] to maximize the yields of carboxymethyl-cysteine containing peptides.

Labelled peptides obtained from IgG2a proteins 5563 and MOPC 173 are shown in Fig. 1; there was another peptide which was identified as the C-terminal sequence of the κ light chain[10]. The two myeloma proteins gave identical results except for a substitution of a serine for a threonine residue in peptide R1. This substitution may be a genetic difference between the two strains of mice on which the tumours were grown, serine being present in C3H mice instead of threonine present in Balb/c. Peptide R1 contains the half cystine residue involved in the binding of light chains. This was clearly shown in experiments using the diagonal procedure for the elucidation of disulphide bridges. The other half cystine residues present in peptide R45, R2 and R6 were present in the diagonal experiments in the absence of other cysteine containing peptides indicating that they are involved in interheavy chain binding. Six labelled peptides were obtained from the partial reduction of IgG2b protein MOPC 141. One was identified as derived from light chains by its characteristic electrophoretic mobility[10], and the others are presumably derived from heavy chains. Their sequences were established by the Dansyl–Edman method (Fig. 2). The sequence of peptide R3 was further studied after digestion with papain which gave rise to the peptides Rp1, Rp4 and Rp5 of Fig. 2.

The partial sequence of the latter peptide was established by Dansyl–Edman as

Pro-Leu-Ala-Pro-Gly-Cya(Asp,Thr_2,Ser_2,Gly_2)

It seems then that IgG2a molecules contain three interheavy chain disulphide bridges and IgG2b contains four. It is still possible, however, that in each case one pair of half cystine residues forms a very labile intrachain link. The sequences obtained are compared with homologous sequences from human and rabbit immunoglobulin molecules (Fig. 3). The comparison between the two mouse proteins is particularly interesting for this shows that, as in the human subclasses, there is wide variation in sequence in this area and that gaps are required to preserve homology. The comparisons are compatible with the concept that human and mouse IgG subclasses evolved recently and independently of one another[6].

Human and mouse IgG1 molecules (see communication on page 932) are so far unique in having the interheavy–light bond only a few residues distant from the hinge region. Other immunoglobulins (probably including a human IgM protein[12]) have the bond situated at the beginning of the constant region. The cystine residues are not in exactly homologous positions in the mouse (position 132) and human (131) IgG proteins, but the two different positions are homologous to two adjacent cystine residues (of which one or other binds the light

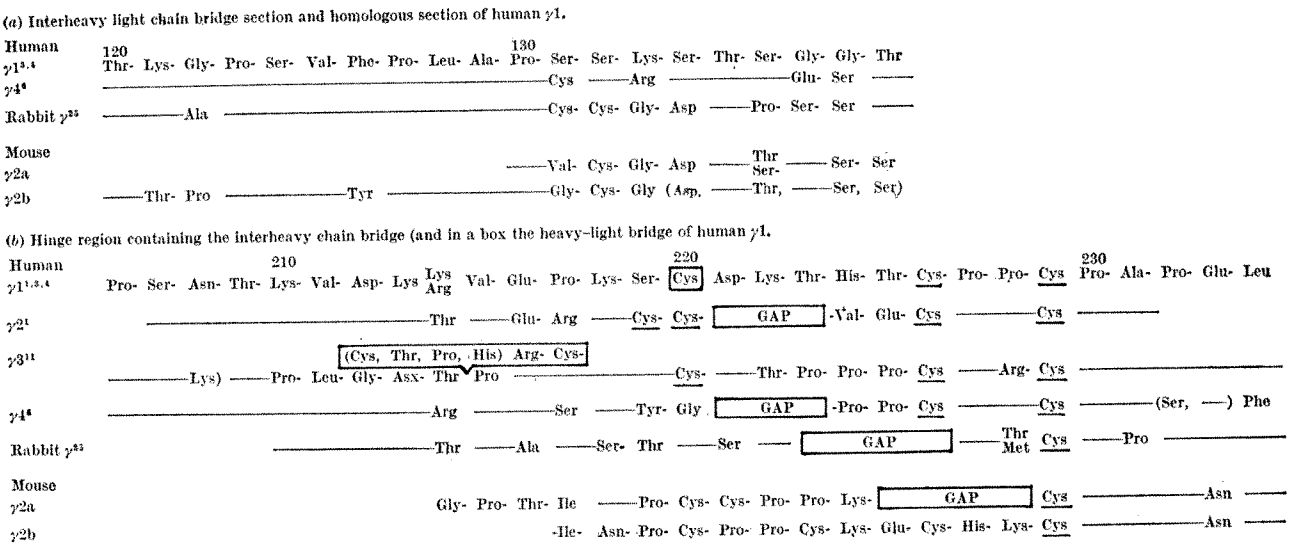


Fig. 3. Amino-acid sequences around interchain disulphide bridges or homologous regions in various immunoglobulins. Unbroken lines indicate identity to top line of sequence. Numbering is taken from ref. 4.

The disulphide bridging pattern of these peptides was established as follows. A peptic digest of the intact protein was fractionated by 'Sephadex G-75' chromatography and selected fractions were analysed by paper electrophoresis. The higher molecular weight material contained one major peptide which by amino-acid analysis and partial sequence was shown to be identical to R3. Its position on the 'Sephadex' column indicated that it occurred as a dimer. In addition a portion of the 'Sephadex' fraction was analysed without further paper fractionation giving an amino-acid composition very similar to the fractionated peptide. The experiment showed that no other peptide was disulphide bridged to any of the half cystine residues of R3.

From another fraction of the 'Sephadex' column a disulphide bridged peptide was identified by diagonal methods. This peptide contained the C-terminal end of light chains attached through a disulphide bridge to a peptide with an N-terminal sequence identical to R4. chain) in rabbit IgG[13]. The cystine residue in mouse IgG2a and IgG2b is in a position exactly homologous to the human IgD heavy–light bridge (see communication on page 934).

The biological importance of these differences in the hinge is unclear. The role of the disulphide bridges in complement fixation is still controversial, for Utsumi[14] and Kehoe and Fougereau[15] have isolated protein fragments containing the bridges but capable of fixing complement, whereas Schur and Christian[16] have destroyed complement fixing ability with reducing agents. The differences may allow different degrees of stretching of the Fab "arms" of the immunoglobulin molecule and thus influence antibody activity[17]. This could explain why certain antigens can apparently selectively stimulate production of particular IgG subclasses[18,19], although variable region sequences, and thus combining sites, seem to be capable of being shared by the different classes[20–24] and subclasses[6] of immunoglobulins.

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Interchain Bridges of Mouse IgG1

HERE we present a sequence containing all the interchain disulphide bridges of a mouse myeloma protein typed as γ1, κ(MOPC 21). As in human IgG1 the interheavy–light bridge occurs near the interheavy chain bridges in linear sequence.

The myeloma protein was partially reduced with dithiothreitol and alkylated with ^{14}C-iodoacetic acid (IAA). From a peptic digest of partially reduced protein, seven radioactive peptides were obtained. RP3 and RP4 were identified as the C-terminal sequence of κ light chains. RP1 and RP2, although present in low yield, were analysed and partially sequenced. The other three peptides (not included in Fig. 1) were digested with trypsin to yield two smaller peptides (RPHT1 and RPHT2) which were characterized as shown (Fig. 1). From a tryptic digest of the totally reduced, alkylated heavy chains, two radioactive peptides were selected: HT4 and HT4C (Fig. 1). HT4 was digested with pepsin to yield two major peptides: HT4PA (radioactive) and HT4PB (non-radioactive).

From a peptic digest of whole myeloma protein, a peptide (PH) was isolated and was unretarded on 'Sephadex G-50'. The peptide was digested with trypsin and the derivatives were fractionated by electrophoresis at *p*H 6·5. The fraction which had a mobility of 0·40 relative to aspartic acid gave rise, when oxidized by performic acid, to two peptides (PHTAO1 and PHTAO2). PHTAO1 was found to be the light chain C-terminal peptide. It was separated from PHTO2 only after destruction of the S–S bridges and therefore PHTAO2 must contain the half cystine residue of heavy chains which binds the light chains; but PHTAO2 contains four cysteic acid residues and it is not known which of the four is involved in this binding. PHTAO2 must also contain the interheavy chain bridge(s). This is so because it includes all the labelled residues of the heavy chain produced by partial reduction and ^{14}C-carboxymethylation in conditions leading to chain separation. In addition the rate of gel filtration through 'Sephadex' of the unoxidized peptide is significantly different from the oxidized derivative, indicating that it includes interchain bridge(s). It is likely that the three half cystine residues form interchain bridges, for they are very labile to reducing agents. The possibility of a labile intrachain bond between two of the half cystine residues has, however, not been excluded.

Confirmation for the sequence and also the location of the interchain bridges was obtained by isolating $F(ab')_2$ from a digest of the protein with pepsin at *p*H 4·0. The fragment was shown to have the Fab determinants by immunoelectrophoresis. By electrophoresis in sodium dodecylsulphate–polyacrylamide gels[1], the molecular weight before reduction was determined as 95,000. After partial reduction and alkylation with ^{14}C-IAA, two fragments were obtained of molecular weights 22,000 (light chain) and 25,000 (heavy chain fragment Fd′), both of which were shown by radio-

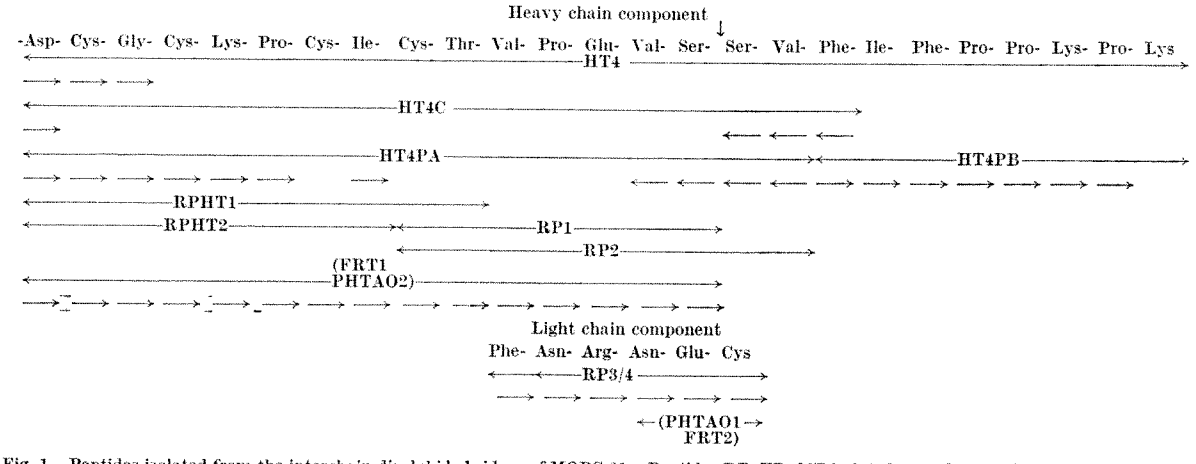
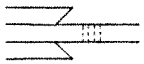
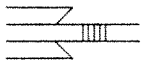
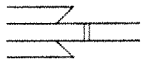
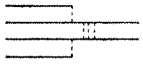
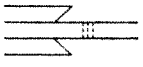
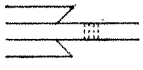


Fig. 1. Peptides isolated from the interchain disulphide bridges of MOPC 21. Peptides RP, FR, HT isolated as carboxymethycysteine derivatives, PH as cysteic acid derivatives. Arrows → indicate Dansyl–Edman results. Arrows ← show the sequential release of residues by carboxypeptidase A digestion. The vertical arrow shows the position of peptic split at *p*H 4·0 to yield the $F(ab')_2$ fragment (Fig. 2). Only those results considered essential for the sequence derivation are shown in this figure.

Table 1. INTERCHAIN DISULPHIDE BRIDGES AND SOME PROPERTIES OF THE SUBCLASSES OF HUMAN AND MOUSE IgG

Species	Subclass	Heavy chain	Electrophoretic mobility	Complement fixation	Skin fixation*	Suggested interchain disulphide bridge pattern
Human	IgG1	γ1	Slow	+	+	
	IgG2	γ2	Slow	+	−	
	IgG3	γ3	Slow	+	+	
	IgG4	γ4	Fast	−	+	
	IgG1	γ1 (φ)	Fast	−	−	
Mouse	IgG2a	γ2a (γ)	Slow	?	+	
	IgG2b	γ2b (η)	Slow	+	−	

* Results quoted are from tests on guinea-pigs.

Dotted lines for disulphide bridges indicate that evidence for interchain nature of bridges is incomplete.

References: for disulphide bridges see 2 (human), this and the preceding communication (mouse): for complement fixation and skin fixation see 3, 4 (human), 5, 6, 7 (mouse).

autography to be labelled (the 25,000 fragment being more strongly labelled). The $F(ab')_2$ molecule was partially reduced, alkylated with ^{14}C-IAA, then totally reduced and blocked with cold iodoacetate, and digested with trypsin. Two radioactive peptides were obtained—FRT1 and FRT2. The latter was the light chain C-terminal peptide and the former was at the C-terminus of the heavy chain fragment since it did not contain a basic residue in the C-terminal position and was a fragment of HT4 isolated from the intact heavy chain (Fig. 1). This established the position of the interchain bridges of MOPC 21 as being at the C-terminal end of the $F(ab')_2$ fragment and completed the sequence (Fig. 2).

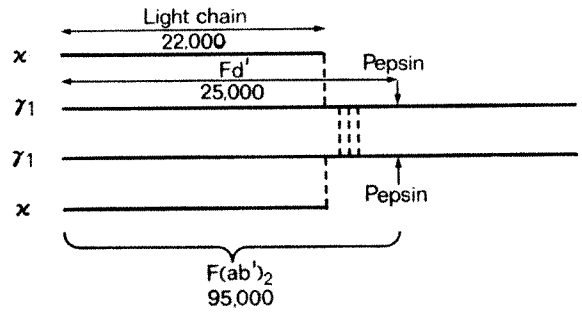


Fig. 2. Site of pepsin action at pH 4·0 on MOPC 21 and molecular weights of fragments.

Table 1 shows the arrangement of interchain disulphide bridges of the different subclasses of both human and mouse IgG together with some of their properties. Neither the proximity of the interheavy–light bridge nor the number of interheavy bridges is strictly correlated with some subclass properties such as the ability to fix complement or fix to skin.

In human IgG1[2] and mouse IgG1, the heavy–light disulphide bridge is very close to the heavy–heavy bridges, whereas in human IgG2, IgG3, IgG4[2] and mouse IgG2a, IgG2b (see preceding communication) they are probably separated by more than ninety residues. There are several possibilities as to how these may have evolved. It might be argued that there were two ancestral genes for the constant region of heavy chains before the divergence of mouse and humans. In one of the ancestral genes the interheavy–light bridge was close to, and in the other distant from, the heavy–heavy bridges in the linear sequence. With the divergence of the two species, one gene gave rise to the γ1 subclass in humans and mice, and the other to the γ2, γ3, γ4 subclasses in humans and the γ2a, γ2b subclasses in mice. In this case, the γ1 subclass in humans would be expected to differ from the γ2, γ3, γ4 subclasses more than γ2, γ3, γ4 differ themselves. But this is not the case—apart from the position of the heavy–light bridge γ1 and γ3 seem to be more similar than γ3 to γ2 or γ4. In addition γ1 of humans would resemble γ1 of mice more than it does other human γ chains, which seems not to be the case for the stretch of sequence shown in Fig. 3. It is more likely therefore[10] that these subclasses represent an example of parallel evolution in which the divergence of the γ1 from the γ2a, γ2b chains in mice is paralleled by the divergence of the γ1 from the γ2, γ3, γ4 chains in humans.

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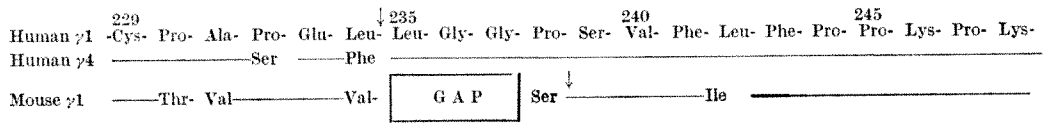


Fig. 3. Comparison of a sequence on the C-terminal side of the heavy–heavy bridges of human γ1 (ref. 8), γ4 (ref. 9) and mouse γ1. Numbering is as for human γ1. Unbroken lines indicate homology with top sequence. A deletion of three residues in mouse γ1 is necessary to preserve homology. Vertical arrows indicate positions of pepsin split to yield the $F(ab')_2$ fragment.

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Interchain Bridges of Human IgD

IMMUNOGLOBULIN D (IgD), found as a minor component of normal sera and as a rare myeloma protein, conforms to the general pattern of immunoglobulin structure, consisting of two heavy and two light chains. The heavy chains of IgD (δ chain) contain antigenic and have physicochemical characteristics that distinguish them from other heavy chains[1,2]. We report here studies of the interchain disulphide bridges of an IgD myeloma protein which have revealed a single interheavy to heavy (H–H) chain bridge and single heavy to light (H–L) chain bridge which has homology with the H–L chain bridges in IgD heavy chains.

IgD (Er 1) was isolated from human myeloma sera collected by Dr E. J. Ernisse and provided by Dr W. Hijmans (University Hospital, Leiden). It was purified in the presence of 5 mM ε-aminocaproic acid by Pevikon block electrophoresis in 0·05 M barbital *p*H 8·2, followed by column gel filtration on 'Sephadex G-200' using 0·05 M phosphate, *p*H 7·2, containing 0·2 M NaCl. Chemical typing of whole IgD using the procedure developed for the IgG subclasses[3] revealed well defined characteristics (Fig. 1) distinct from IgG subclasses, the radioactive peptides L1 and L2 being characteristic of the C-terminal peptide of λ light chains, while the peptides H1 and H2 were derived from the δ chain.

Table 1. CARBOHYDRATE COMPOSITION OF IgD AND IgD HEAVY AND LIGHT CHAINS*

	L-fucose	D-galactose	D-mannose	N-acetyl-D-glucosamine	N-acetyl-D-galactosamine	N-acetyl neuraminic acid
Whole IgD	0·95	15·1	20·3	16·5	11·1	19·0
Heavy chain IgD	0·40	8·1	9·9	8·1	4·8	8·0
Light chain IgD	0	0	0	0	0	0

* Moles of anhydroglycose/mole of protein using molecular weights of 184,000 for whole IgD and 70,000 for IgD heavy chain.

Partially reduced IgD was carboxymethylated with 2-^{14}C-iodoacetate[3] and was fractionated by gel filtration on 'Sephadex G-100' equilibrated with 5 per cent formic acid to give heavy and light chains which moved as single bands on sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis[4,5] and had indicated molecular weights of 70,000 and 22,000 respectively. Using these molecular weights, the molar specific radioactivity of the light to heavy chain was calculated to be 1 : 2·05 ± 0·2.

Carbohydrate analyses were made on IgD further purified by gel filtration on 'Biogel P6' equilibrated with 5 per cent formic acid. Gas chromatographic procedures were used to determine and identify the neutral[6] and basic[7,8] glycose components. The results are shown in Table 1.

The isolated heavy chain (60 mg) in 10 ml. of 5 per cent formic acid was digested with 2·5 mg of pepsin for 16 h at 37° C followed by digestion with 2 mg of trypsin in 1·5 per cent ammonium bicarbonate, *p*H 8·0, for 6 h at 37° C. Gel filtration of the product on 'Sephadex G-25' equilibrated with 5 per cent formic acid separated the peptides H1 and H2 which were purified by electrophoresis at *p*H 3·5 before and after performic acid vapour oxidation and by electrophoresis at *p*H 6·5. Sequence analysis of the peptides gave the results shown in Fig. 2. Papain digestion of H1 afforded valine and the peptides H1p1 and H1p2 (Fig. 2) which were isolated by electrophoresis at *p*H 6·5.

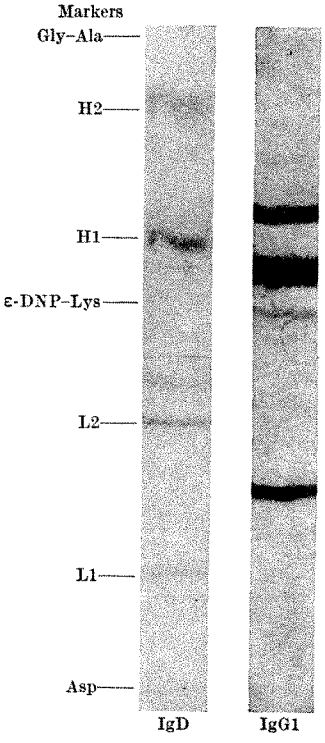


Fig. 1. Chemical typing of IgD (Er 1). Autoradiograph of the radioactive peptides from a peptictryptic digest of partially reduced and ^{14}C-iodacetate-labelled IgD (Er 1) compared by electrophoresis at *p*H 3·5 with peptides similarly obtained from an IgG1 type κ. L1 and L2 are two peptides derived from λ chains[10]: Pro-Thr-Glu-Cys-Ser (L2) and Cys-Ser (L1). Peptides H1 and H2 were also derived from isolated IgD σ chain. Electrophoretic mobilities at *p*H 3·5; L1, −1·03; L2, −0·44; H1, +0·21; H2, +0·62 (relative to glycylalanine = 1·0).

Peptides H1 and H2 were shown by the diagonal procedure to contain the half cystine residues involved in the H–H and H–L bridges respectively. A peptic–tryptic digest of whole IgD was fractionated on 'Sephadex G-50' in 5 per cent formic acid using ε-DNP-lysine marker. The pooled fraction containing peptides eluting before the marker was subjected to electrophoresis at *p*H 3·5. Two bands were selected (based on diagonal electrophoresis results) with mobilities of +1·05 and +0·78. (All mobilities at *p*H 3·5 are expressed relative to glycyl-alanyl marker = 1.) The band with the greater mobility contained a bridge peptide which, when oxidated by performic acid, gave rise to two derivatives having mobilities of +0·03 and −1·22 (*p*H 3·5 electrophoresis)

which were ninhydrin negative and isatin positive, indicative of N-terminal proline residues. Analysis showed that the purified peptides had the composition of the cysteic acid version of peptide H2 (Fig. 2) (mobility +0·03) while the other peptide (mobility −1·22) was identified as the C-terminal peptide of the λ chain. These define the H–L bridges.

Rabbits have chains containing both half cystine residues, but it is not known which of the two bonds to light chains. This raises the question of evolutionary pathways leading to the different locations of half cystine residues. One possibility is that the common precursor to Ig2b (mouse) and δ (human) was different from the precursor common to human γ chains. Other properties of δ chains,

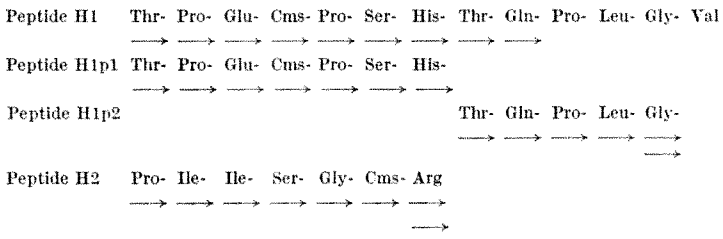


Fig. 2. Sequence of peptides H1 and H2 obtained from the σ chain of IgD myeloma protein. Cms, carboxymethylcystinesulphone. Arrows indicate Dansyl–Edman results. Double arrows indicate that the residue was obtained as free amino-acid at the end of the degradation procedure. Electrophoretic mobilities of peptides at *p*H 6.5 with respect to aspartic acid taken as 1·0–H1, 0·27; H1p1, 0·36; H1p2, 0; H2, 0·03.

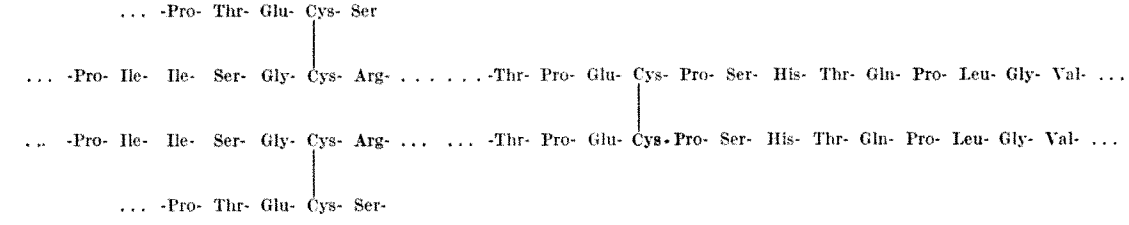


Fig. 3. Interchain bridges of IgD.

The second band (mobility +0·78) was subjected to electrophoresis at *p*H 6·5 (mobilities at *p*H 6·5 expressed relative to aspartic acid = 1) and a band (mobility + 0·03) which was yellow with cadmium-ninhydrin was cut out, oxidized with performic acid vapour, and subjected to electrophoresis at *p*H 6·5 to give a derivative (mobility +0·26) of altered mobility which like H1 was yellow with cadmium–ninhydrin and which had the same amino-acid composition as H1 (Fig. 2), thus locating the H–H disulphide bridge.

The results are summarized in the partial structure shown in Fig. 3.

Human γ2, γ3, γ4	-Pro-	Leu-	Ala-	Pro-	Cys-	Ser-	Arg-[13]
IgD (Er 1)	-Pro-	Ile-	Ile-	Ser-	Gly-	Cys-	Arg-
Mouse γ2b	-Pro-	Leu-	Ala-	Pro-	Gly-	Cys-	Gly-*
Rabbit	-Pro-	Leu-	Ala-	Pro-	Cys-	Cys-	Gly-[10]

* See communication on page 930.

Fig. 4. Sequence around the point of attachment of light chains to different heavy chains.

The presence of only one bridge between the heavy chains of IgD is unique in human immunoglobulins examined so far, but a single H–H bridge has been described in a rabbit IgG[9,10]. The presence of a single H–H bridge has been suggested in another IgD myeloma protein[11]. At this stage the interheavy chain bridge does not seem to have any convincing homology with known heavy chain sequences. The heavy to light bridge of IgD, on the other hand, is clearly homologous to the H–L bridges in other γ chains (Fig. 4). A comparison in fact shows that the half cystine of IgD occupies an identical position to the corresponding half cystine of mouse Ig2 chain, but one residue removed from the human heavy chains.

however, make this unlikely. The molecular weight of the δ chain determined by SDS-polyacrylamide gel electrophoresis was 70,000 which gives a value of 63,000 for the molecular weight of the peptide part of the heavy chain when the 12 per cent carbohydrate content is taken into account. This value is about 13,000 larger than the accepted value of 50,000 of γ chains and would represent an additional pseudo-subunit of about 120 amino-acid residues. SDS-polyacrylamide gel electrophoresis of glycoproteins with a similar carbohydrate content to IgD (Eril) gave determined molecular weights in close agreement with accepted values. More sequence data are clearly required for further clarification of the possible evolutionary origin of δ chains.

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Catalytic Mechanism of Cytochrome Oxidase

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Kinetic experiments in a stopped-flow apparatus provide evidence for multi-electron steps in the reduction of oxygen.

CYTOCHROME *c* oxidase and the "blue" copper-containing oxidases (ascorbic acid oxidase, laccase and caeruloplasmin) are the only enzymes that catalyse reactions in which both atoms of molecular oxygen are reduced to water[1]. A great mechanistic problem with these enzymes is therefore the way in which one-electron oxidation of substrate is coupled to a four-electron transfer to oxygen. As stressed by George[2], in particular, it is unlikely that this reduction can occur by way of four consecutive one-electron steps. The principal basis for this contention is the fact that the relatively large, positive free energy change for the addition of the first electron to oxygen, coupled with the high standard oxidation-reduction potentials for the enzymes, would make such a reaction sequence extremely slow. For this reason, several authors have discussed the possibility that oxygen reduction with these enzymes may instead involve multi-electron transfer (see refs. 1, 3 and 4 for reviews). This suggestion does, in fact, receive support from some simple considerations—for example, the chemical composition of the enzymes, which shows that they all contain at least four potential electron-accepting sites[1,3,4]. In spite of this, no kinetic experiments designed specifically to test this hypothesis seem to have been carried out with cytochrome oxidase. On the other hand, kinetic investigations[5,6] have provided strong evidence for such a mechanism in the case of laccase catalysed oxidations. The chief basis for this interpretation was the finding that, whereas all reducible centres are reoxidized very rapidly in the fully reduced enzyme, fast oxidation does not occur with partially reduced laccase. The purpose of this article is to show that a similar experimental situation may be created with cytochrome oxidase. Also, in this case, reoxidation of the partially reduced oxidase is slow, requiring several minutes, despite the fact that Greenwood and Gibson[7] found the slowest step in the reoxidation of the fully reduced enzyme to have a rate constant of 700 s^{-1} in conditions where the oxygen concentration does not limit the rate.

The cytochrome *c* oxidase used in this study was prepared from beef heart particles of Keilin and Hartree by the method of Yonetani[8]. The final fraction was dissolved in 0·1 M phosphate buffer (*p*H 7·4) containing 1 per cent 'Tween 80'. It had a ratio of absorbances at 444 and 424 nm of 2·35 for the reduced enzyme. Cytochrome *c* was type VI from Sigma, and was used without further purification. Reduced protein was prepared by the addition of a minute excess of sodium dithionite, followed by dialysis under argon against 0·1 M phosphate buffer (*p*H 7·4) containing 10^{-4} M EDTA.

Stopped-flow spectrophotometry was performed in a Durrum–Gibson apparatus having an optical path of 2 cm and a measured dead time of 4 ms. Cytochrome *c* oxidation was followed at 550 nm or 520 nm, depending on the concentration used; the reduction and reoxidation of cytochrome oxidase was monitored at 605 nm, at which wavelength the contribution from cytochrome *a* dominates. The following values were used for the absolute extinction coefficients and the difference coefficients (ΔE) between the reduced and oxidized proteins, all expressed in terms of 1 mM concentration : reduced cytochrome *c* at 550 nm, 27·6; ΔE for cytochrome *c* at 550 nm, 20; same at 520 nm (ref. 9), 6·4; reduced cytochrome oxidase at 605 nm, 21; and ΔE for the oxidase at 605 nm (ref. 10), 11.

Earlier investigations of cytochrome oxidase with rapid reaction techniques have, on the whole, been concerned with the anaerobic reduction of the oxidized enzyme or with the reoxidation by molecular oxygen of the fully reduced enzyme[3,4,7,11,12]. On the basis of experiments[5] with another oxidase, laccase (or *p*-diphenol

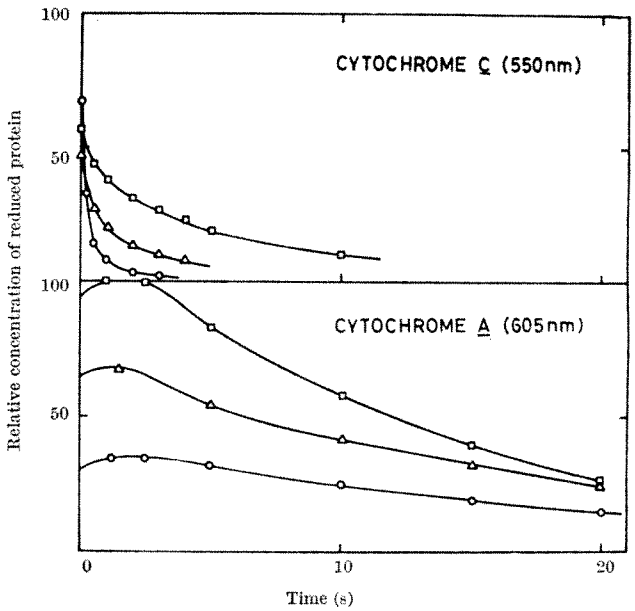


Fig. 1. The oxidation of ferrocytochrome *c* by cytochrome *c* oxidase in the presence of 280 μM oxygen. The reaction was carried out in 0·1 M phosphate buffer with 1 per cent 'Tween 80' at 20° C. Cytochrome *c* oxidation was followed at 550 nm and expressed as per cent of the original reduced cytochrome *c* remaining (top). The cytochrome *c* oxidase concentration (in terms of a unit containing one cytochrome *a* and one a_3) was 2·5 μM after mixing. The reduction and reoxidation was followed at 605 nm and expressed as per cent of the total cytochrome *a* reduced (bottom). Initial concentrations of ferrocytochrome *c* after mixing: ○, 0·9 μM; △, 1·9 μM; and □, 3·8 μM.

oxidase), we found it interesting to carry out transient kinetics measurements in the presence of both substrates, reduced cytochrome *c* and oxygen. In particular, we have worked with low cytochrome *c* concentrations to achieve a maximum concentration of partially reduced enzyme, the reoxidation of which by excess oxygen we could then follow.

Figs. 1 and 2 present some of our results. Those in Fig. 1 were obtained at constant oxidase concentration with varying concentrations of reduced cytochrome c (c^{2+}) close to and below stoichiometry with respect to cytochrome *a*. The curves in Fig. 2, on the other hand, were recorded with variation of both the c^{2+} and oxidase concentrations, which were, however, kept at a constant ratio with less c^{2+} than oxidized cytochrome *a* (a^{3+}). The fast phases in our progress curves agree well with the results from earlier kinetic studies[7,11,12]. Thus there is a rapid second-order reaction between c^{2+} and a^{3+}, as seen from the burst in product formation and the simultaneous reduction of the oxidase. The data in Fig. 2 at the two lowest concentrations of c^{2+} give a second-order rate constant for this step of about $3{\cdot}5 \times 10^6$ M^{-1} s^{-1} (see ref. 11). With higher concentrations of c^{2+} (data not shown) this initial phase was found to be followed by a steady-state period of c^{2+} oxidation. For example, a steady-state rate of 1·6 s^{-1} was found at 45 μM c^{2+}, in agreement with published turnover data[13] for Yonetani's preparations.

The most striking feature in our progress curves is the slow reoxidation of a^{2+}, which, in general, is not completed until several minutes after the exhaustion of substrate. This becomes most obvious at low concentrations of c^{2+}, which lead to the maximal formation of partially reduced enzyme (Fig. 2), but even at the highest cytochrome concentration used (45 μM) the reoxidation is considerably slower than what would be predicted on the basis of the published rate constant[7] for electron exchange between a_3^{3+} and a^{2+}. One way to interpret this by analogy with the situation for laccase[5] would be that rapid reoxidation only occurs with the fully reduced enzyme. But because the reduction of a^{3+} by c^{2+} is more rapid than subsequent internal redistribution, the reducing equivalents tend—towards the exhaustion of substrate—to be distributed on cytochrome *a* in different enzyme molecules. The formation of fully reduced enzyme therefore requires electron transfer between different oxidase molecules, and this limits the reaction rate.

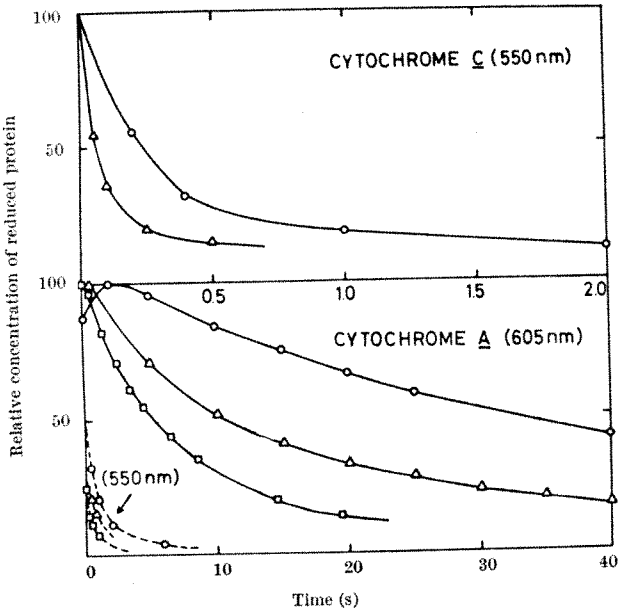


Fig. 2. The effect of the concentration of cytochrome *c* and cytochrome *c* oxidase on the reduction and reoxidation of the enzyme. The buffer, oxygen concentration, temperature and monitoring wavelengths were as in Fig. 1. The top part shows the fast phase in the oxidation of ferrocytochrome *c*, expressed as percentage of the original concentration of reduced protein (the data for the highest concentration are excluded, for too large a part of the fast change occurred within the dead time of the apparatus). The bottom part shows the same curves (dashed), including the one for the highest concentration, together with the reoxidation of cytochrome *a*, expressed as the percentage of the maximal concentration of reduced *a*, which corresponded closely to the initial concentration of ferrocytochrome *c* according to the total absorbance changes. Initial concentrations of ferrocytochrome *c* and oxidase (expressed as in Fig. 1), respectively: ○, 0·22 and 0·93 μM; △, 0·66 and 2·8 μM; and □, 2·0 and 8·4 μM.

In the kinetic experiments of Greenwood and Gibson[7], only trace amounts of cytochrome *c* and excess ascorbate were used to reduce cytochrome oxidase, and c^{3+} is an inhibitor of the enzyme[4], so that one might argue that the slow oxidation of a^{2+} is caused by the binding of c^{3+} to the oxidase. In such a case, however, the rate should increase with decreasing cytochrome *c* concentration, whereas the opposite is found (Fig. 2). As the concentration of oxidase is also decreased in the experiments of Fig. 2, the decrease in the rate of reoxidation in fact gives strong support for the suggestion that this is limited by intermolecular electron transfer. Calculations on this basis for the slow phases of reoxidation of a^{2+} all yield a second-order rate constant of $4{\cdot}4 \times 10^4$ $M^{-1}s^{-1}$, further strengthening this interpretation. This value is very similar to those obtained for electron exchange between some other redox proteins (unpublished results of B. G. M., A. Finazzi Agrò, C. G., E. A., M. B. and B. Mondovì).

Our experiments seem to require an abandonment of the concept that the reduction of oxygen by cytochrome oxidase occurs in four sequential one-electron steps. In the absence of more detailed information, we have assumed that only the fully reduced enzyme reacts rapidly with oxygen, but it would be sufficient for explaining our results to assume that rapid electron transfer from a^{2+} to a_3^{3+} requires two reducing equivalents. This would be analogous to present concepts about the "blue" oxidases[1], which assume that electron transfer from the "blue" type 1 copper ion only takes place if an additional reducing equivalent is available. This is thought to occur because the electron taken up by the initial acceptor (type 1 Cu^{2+}) is transferred to the two diamagnetic copper ions, which act as a cooperative two-electron acceptor.

Clearly, a more detailed picture concerning the steps by which oxygen is reduced to water in cytochrome oxidase requires more exhaustive kinetic experiments, utilizing the different spectroscopic and magnetic properties of four potential electron acceptors (cytochromes *a* and a_3, and the two copper ions). But we feel that our finding that the reoxidation of cytochrome *a* in partially reduced enzyme is slow, and limited by a bimolecular reaction between oxidase molecules—by analogy with the kinetic situation for laccase[5]—provides strong evidence that this process involves multi-electron steps.

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Reversion of Virus-transformed Cell Lines: Hyperploidy accompanies Retention of Viral Genes

by

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An increase in chromosome number accompanies reversion subtetraploid mouse cell lines from malignant transformed phenoty to growth-controlled normal phenotype. When growth control once again lost, the chromosome number drops back to the su tetraploid mode.

GROWTH control of a cell line is measured *in vitro* as a reversible cessation of cell division. Cell lines are maintained in culture by a periodic replacement of medium and serum. Populations with a degree of growth control will divide while sparse, but will cease to increase in number when they reach some cell density beyond confluence. Those cultures with maximum growth control cease dividing at the lowest cell density, that is at confluence. Populations with less growth control reach higher densities before stopping, and those with no growth control continue to divide until their medium is exhausted. Growth control can therefore be measured by assay of saturation density.

Permanent fibroblast cell lines derive from primary tissue cell populations by passage through three distinct phases of growth: an initial period of progressive rapid growth; a dormant period called crisis during which few cells are able to divide; and an extended period of rapid growth, as an established cell line[1,2].

Established cell lines are the descendants of the minority of cells able to divide through crisis. This defines the crisis as a period of selection, and in fact cell lines can be selected to have either high or low degrees of growth control[3,4]. Cell to cell contact seems to be the operative variable in this selection, for if cells are permitted to make contact during crisis, then a cell line will arise that has the ability to divide despite this contact, and such a line will retain little or no growth control[3-5]. Keeping cells from contact during crisis will yield a line with a very high degree of growth control, like the 3T3 mouse line[4].

In constant culture conditions, constant frequency of medium changes, and constant concentration of serum in the medium[6,7], saturation density is the parameter of a cell line that correlates best with its ability to grow into a tumour *in vivo*[3,8,9]. This point is shown clearly with the small polyoma and SV40 DNA viruses. These viruses induce a rapid loss of growth control in primary cells and in established lines of high growth control. Such cell lines, called transformed lines, are almost always more tumorigenic than their uninfected parent lines[3,9-12].

Reversion

The loss of growth control is reversible. By a choice of selective conditions, cell lines with high growth control (revertants) can be obtained from cloned populations with low growth control, including virus transformed cell lines. Revertants have been isolated by: (*a*) negative selection of transformed cells at high cell density with FUdR, a drug toxic to dividing cells[13]. Such cells are bigger than their transformed parents and look flat; (*b*) passage of transformed cells on cell layers fixed with glutaraldehyde[14], which presumably selects for cells with an altered surface that permits them to grow on this substratum; and (*c*) selection for hybrid hamster transformed lines with reduced chromosome complements[15] that have lost the chromosome(s) bearing the viral genome. Such cells are oriented and do not grow in agg Like the untransformed hamster line BHK21, howeve they grow to a high saturation density.

The viral genome, or part of it, is present in polyon and SV40 transformed cells[16-18]. Viral genes and the products must be responsible for the initial maligna transformation and viral genes are probably responsib for the maintenance of the transformed state in descen ants of the original transformant[19-21]. Thus the cel isolated by (*c*), which lack the viral antigens and a retransformable, arose as the viral genome was lost wi some chromosomes of the polyploid hybrid line[22]. Pr sumably, the genome was integrated into one of tho chromosomes. Similarly, hybrids between SV40-tran formed human cells and untransformed mouse cel originally retained the SV40 T-antigen, but lost it most human chromosomes were lost from the hybr cells[23]. By contrast, revertants of virus transforme cells isolated by methods (*a*) and (*b*) must retain at lea part of the genome of the transforming virus, becau they have viral T-antigen[13,14].

Revertant cell lines regain other phenotypic properti of untransformed cells in addition to increased growt control. Revertants isolated by methods (*a*) and (regain a completed cell surface glycoprotein, renderi them unagglutinable by a series of plant agglutinins th specifically agglutinate transformed cells and tumo cells[24,25]. Flat revertants of spontaneous[8] and polyon transformed[13,15] hamster cell lines isolated by (*a*) and (are less tumorigenic. Revertants of polyoma tran formed hamster cells isolated by method (*b*) are in son cases more tumorigenic than their parental transforme lines[26].

Stability of Reverted Cell Lines

Back reversion, or the reappearance of low growt control cells within a revertant population, occurs at low rate without experimental intervention in an population kept at maximum cell density for even a fe days. Only variant cells with diminished growth contr can divide at saturation, and in time their descenden overgrow the population[4]. Measured against this bac ground of spontaneous "transformation", stability the reverted phenotype varies with the selection systen Selections (*a*), (*b*) and (*c*) yield revertants as stable untransformed cells. That is, it is possible to sele dense variants from the revertant populations isolate by system (*a*) and these arise with about the same fr quency as such dense variants arise in untransforme populations.

Chromosomes of Transformed and Reverted Cell Line

Immediately on transfer from the animal to tissue cu ture, somatic mouse cells have forty acrocentric and tel centric and no metacentric chromosomes. They grad smoothly in size[27]. As the mouse cells in culture pas

(*Continued on page 96*

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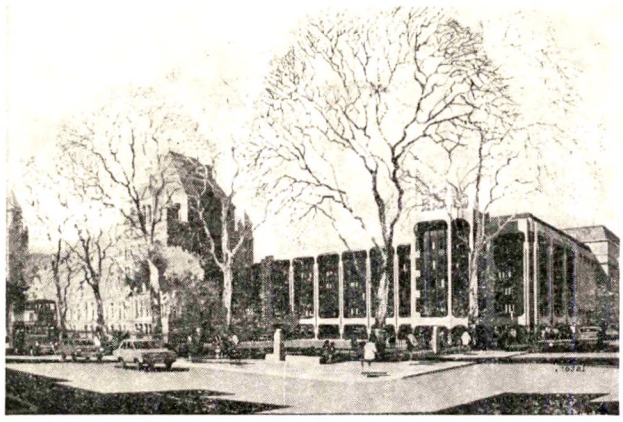
Museums and the Seventies

In Britain in the past few weeks, the national museums have been up in arms about the proposal by the new Conservative Government that visitors should in future pay a small charge. As on all these occasions, students and other deserving categories of the population are likely to be excepted, although this is not yet certain. As far as can be told from Mr Anthony Barber's collection of economies in public expenditure, the objective is chiefly to bring some extra revenue into the Treasury so as to help implement the government's proposal to take sixpence off the income tax next April. The notion that visitors should pay to visit national museums, foreign to Britain, is nevertheless not one to be rejected out of hand. Certainly the experience of museums elsewhere does not amount to proof that entrance fees serve to turn people away. The Louvre is, for example, always as crowded as the National Gallery. What matters is that there should be easy devices for allowing those who cannot afford to pay or who should not be expected to do so to go in free. But if doctrinaire protests against the imposition of charges are dismissed, it does not follow that everything the government now proposes on museums should be endorsed. Indeed, the chief case against the new proposals is that they are or seem to be simply a device for raising what will in the end be only a very modest sum of money. They are unhappily not a part of the public strategy for national museums on which the government should be working.

The first thing to be said about the national museums is that they are chronically impoverished. Altogether, the government is spending £9 million a year on the national museums. The British Museum, one of the great museums of the world, costs the government a mere £3 million a year. Institutions such as the Science Museum get by on less than £700,000 a year, which goes a long way to explain why a museum that ought to be able to take the lead not merely in the collection of antiquities but in historical research in science and technology finds itself having to rely on the generosity of industrial companies for much of the material which it exhibits. The British Museum (Natural History) (which should really find some other way of describing itself) is a little better off and is indeed able to support a vigorous and distinguished research programme as well as to look forward to an extension to its building, but even here there is room for useful development. To be sure, given the long history of parsimony, the government is likely to pretend that its commitment to spend close on £10 million on buildings for the national museums in the coming decade is sufficient for the time being, and even its meanest critics will welcome the decision (of the previous government) to develop a new site for the library of the British Museum in Bloomsbury. But the more immediate need is for more money to spend on acquisitions and research. But if the government does eventually succeed in persuading the museums to levy entrance charges, the least that it can do is to make sure that the extra funds find their way not into Mr Barber's bottomless pit but into the current budgets of the museums.

This, however, should be merely the beginning. In the coming decade, there is a serious danger that the national museums in Britain will fail to keep pace with their peers abroad for lack of funds for sensible development. The picture galleries are the hardest pressed. At present, the art market is much more bullish than the budgets of the galleries. The result is that important acquisitions frequently depend on the generosity of well-wishers rather than on the will of the government to see that these institutions flourish. The same troubles afflict the other insti-

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Artist's impression of the proposed extension to the British Museum (Natural History) in South Kensington. When completed in about 1973–4 the extension will provide storage space and research facilities for the museum's palaeontological department.

tutions on Mr Barber's payroll. Splendid collections are being developed less quickly than they should be. Libraries owe too much to the requirements of British copyright law that national libraries should receive a free copy of everything published in Britain, which in turn conceals the difficulty of keeping worthwhile collections of publications from abroad. Institutions such as the Science Museum cannot conceal their impoverishment in this respect. It is no wonder that Mr Barber's anxiety to collect some small change from those who visit these institutions should have served as a reminder that the museum service is seriously starved of working funds.

The truth is that in the decade ahead, these institutions should become even more widely recognized as centres of important research than they have been in the past. The Science Museum is a good illustration of this deficiency. Largely no doubt because of the straitened circumstances to which it has become accustomed, the museum has become a large and frequently overcrowded showcase with a small if distinguished appendage of historical research. Yet if the museum is to play a part in helping to develop the present stream of academic interest in the origins of the development of modern science, it will certainly wish for much more leverage than this. The ideal would be that the Science Museum should become as important a centre of academic research as the Natural History Museum has become. But the same principle applies to all the other institutions. The government must recognize that research is an indispensable part of the process of collecting objects of any kind, books, skulls or Grecian urns. In the long run, it may well be simpler to ensure that this doctrine is adequately recognized if responsibility for individual museums is transferred from the Department of Education and Science as such to the research councils and if, at the same time, the device of allowing responsibility for forward policy to rest with the body of trustees which has in the past given the British Museum (and the Natural History Museum) a delicious sense of independence could be extended to the other museum

None of this unfortunately will resolve the othe anomaly in the public posture of museums in th seventies—the question of how to present exhibitior to the public in terms that are meaningful to every body. The days have gone when scholars can b satisfied with the laconic collection of objects whic have become familiar aspects of museum exhibition The cases of stuffed birds serve no useful purpose fc research and scholarship, but also fail to excite th interest of the public. So should museums settle dow simply to be centres of research ? That would be t abandon one of their most important responsibilitie Indeed, as things have turned out, the need for devi ing ways in which museums can play a part in th developing public consciousness of important problem is more urgent than ever. Just as, towards the end c the nineteenth century, museums were able, fc example, to give Darwinism a fair wind, so now ther is a case for thinking that they have a part to play i drawing attention to the scientific foundations of great many important social problems. Why not, fc example, ask that museums should help to inforr people about the anthropological considerations whic play a part in contemporary racial problems ? Wh should not museums also help to provide a more vivi and objective appreciation of problems connecte with pollution ? It would, for example, be entirel proper to ask that every natural history museum shoul at this point in its history be concerned to demonstrat what ecology consists of and to prove that it is mor than merely "an attitude of mind". In short, ther is a strong case for asking that in the seventies museum should be increasingly concerned with matters of publ policy and that they should regard it as part of the public function to provide a continuing stream c comment on important public matters.

Evidently, important changes in the ways in whic museums are administered will be necessary if task like these are to be undertaken effectively and objec tively. There are, of course, great dangers in askin that museums should provide what may be considere a half-baked version of an analysis of contemporar problems. In the past few years, the inevitable tenc ency among those designing public exhibitions to len drama to their subjects has led sometimes to distortio and sometimes to exaggeration. The worthy effor made by museums in several nations to explain t visitors the role of space research in modern scienc have been wide of the mark. In an unexpected way museums are more prone to fall into the trap of over simplification than are popularizers of other kind: There are also dangers, unavoidable but not in them selves deterrents, in asking that museums should se themselves up to take a view one way or the other o some matter of public importance. This, however does not imply that museums should not try in th decade ahead to increase the value of their relatior ship with the outside world just as they must forg more durable links with the academic community On both counts, they need more money.

General Books

KUHN'S SECOND THOUGHTS

The Structure of Scientific Revolutions

By Thomas S. Kuhn. Second edition. (International Encyclopaedia of Unified Science. Vol. 2, No. 2.) Pp. xii + 210. (University of Chicago: Chicago and London, 1970.) 54*s* boards; 14*s* paper.

THE first edition of Professor Kuhn's well known book was issued in 1962. It immediately attracted attention and comment, as much for the novel questions it raised as for the particular doctrines that Kuhn was concerned to defend. For some forty years, the philosophy of science had been dominated by men trained in symbolic logic. Throughout this period, questions about the history and sociology of science had been set aside as devoid of serious philosophical implications. Kuhn's book had the great merit of compelling historians and philosophers, natural scientists and social scientists alike to pay more attention to the interconnexions between their respective specialities. In the subsequent eight years, his account of conceptual change in science as characterized by a sequence of "revolutionary" transitions from one "paradigm" to another—separating periods of "normal science", in which theoretical development proceeds cumulatively and without radical concept changes—has been the subject of much lively discussion.

The idea of theoretical "paradigms" was already familiar to philosophers of science and historians of ideas whose memories went back beyond Frege and Russell. It was originally introduced into the debate about the nature of physical explanation by Georg Christoph Lichtenberg, who was professor of physics at Göttingen in the mid-eighteenth century; it was revived by Ernst Mach and his successors in Vienna at the turn of the twentieth century, when Lichtenberg was for German-speaking philosophers the same kind of liberating influence that his British counterpart, David Hume, was in England; and it became the heart of the oral tradition of teaching about the subject in the later lectures of Ludwig Wittgenstein, as reflected, for example, in W. H. Watson's book, *On Understanding Physics*, and in due course introduced into the United States by Wittgensteinian philosophers in the 1950s.

In the discussions of men like Wittgenstein and Watson, there was no suggestion that the historical change from one paradigm to another was necessarily a "revolutionary" one; and it was this aspect of Kuhn's argument which—naturally enough—gave rise to most vigorous debate and criticism. What did his use of the term "revolution" imply? Was the transition from the pre-Copernican to the Newtonian world picture, for instance, a change so profound that a Ptolemy or a Buridan (say) could not even have talked intelligibly with a Laplace? At times, Kuhn's argument seemed to hint at rational discontinuities in the development of scientific thought so drastic that the very existence of natural science as a coherent rational enterprise seemed at stake.

Now, in this second edition, Kuhn gives us his second thoughts. For the most part, the book is reprinted unaltered. There are two brief textual changes in the body of the text, but the chief addition is an appendix (originally prepared for the Japanese translation) in which Kuhn discusses the objections and misunderstandings the book has provoked in the intervening years. The qualifications he now makes take much of the sting out of Kuhn's thesis; but at the same time they prompt the question, whether the original presentation of that thesis, in terms of an absolute contrast between "revolutionary" and "normal" phases in scientific change, was not a rhetorical exaggeration all along. At one point in the present appendix, for instance, Kuhn goes so far as to reinterpret this contrast as equivalent to the distinction between "propositional" changes within a given theory and "conceptual" changes in the basic terms of a theory; and this is a logical distinction, applicable to changes in a science going on at any stage, rather than a historical distinction between different kinds of phases in the growth of a science.

Looked at afresh, indeed, Kuhn's up to date account of scientific change turns out to be a much more orthodox empiricist affair than before—with successive scientific theories being designed to be more "predictive", more "specialized", more "elegant" and so forth—and the revolutionary aspect of the original thesis has largely faded into the background.

Whatever one's reservations about the original Kuhnian view, and whatever qualifications he now introduces into it, the virtues of the book remain; and many of the questions he raised in it will continue to occupy the centre of the discussion about conceptual change in the natural sciences. Instead of trying to turn the clock back, what philosophers and sociologists should now be doing is to take the argument further, and to analyse more exactly the detailed mechanisms of conceptual change within each theoretical change in a science whether propositional or conceptual or both, about which neither Kuhn's first nor his second thoughts have left us much the wiser. Meanwhile, it is a sufficient mark of the historical influence of the book that, in this respect, it has made an irreversible difference to the philosophical argument.

STEPHEN TOULMIN

A CLASH OF ARMS

March 4: Scientists, Students, and Society

Edited by Jonathan Allen. Pp. xxv + 177. (MIT: Cambridge, Massachusetts and London, September 1970.) 18*s* paper.

AT the Massachusetts Institute of Technology, on March 4, 1969, an open meeting took place. This little book is a record of the proceedings—the set speeches and some of the public discussion. What was it all about? The admirable "Historical Introduction" by Murray Eden explains how a heterogeneous group of students and faculty members managed to hold together long enough to appear on the same platform and talk on the same general theme—even if at cross purposes. In such a place as MIT, this was something of an achievement.

The theme is not, however, as broad as the title pretends. The key words are: USA, technology, academics—and, above all, war. Of their kind, the speeches are excellent; even W. G. McMillan manages by his utter sincerity to avoid sounding like a parody of the establishment patriot with a dirty job to be done. With each speaker in turn—Feigenbaum, the concerned humanist; Chomsky, the analytical critic; Weisskopf, the liberal "insider"; Rabinowitch, the internationalist; Mann, the radical "outsider" and so on—we nod our head and say "how true". All the parties to the issue are well represented; if you lack a philosophy, a programme and a leader, here is a supermarket where all the goods are clearly displayed for your choice.

But it is all so contradictory and confused. If this is the best that a bunch of academics can achieve on a serious topic, then we ought to look elsewhere for guidance. A mere confrontation of moral imperatives resolves nothing. War, as we have often been reminded, is too serious a business to be left to the generals; yet it cannot

be wished away by the expropriation of corporate capitalism, by international understanding, by political manipulation, by a strike of scientists, or by dodging the draft. If the academic community (does it exist?) is to exert any leverage for this vital reform of human society, then it must discuss and discuss, argue deeper and deeper, and arrive at a sufficient consensus to discover the crack into which to insert the crowbar. More little books like this, more position papers and cries from the heart, are not enough: we need long sustained thought; we need a sociology, a psychology, an economics—a whole science—of war. The appeal of direct action is that one knows the enemy; the simple emotions of loyalty to a cause, the very virtue of which "patriotism" is the norm, absolve us from scepticism, doubt and difficulties of thought. Nevertheless, the pen is the mightier weapon.

Writing as an Englishman, without personal involvement in the tragedy of modern America, I must sound altogether too detached. Perhaps we too are on the way to the same hell of technocracy, technolatry and technoclasm. But here again, careful analysis is needed; human behaviour is so extraordinarily non-linear that small differences of history and social structure may have entirely different consequences. We can learn some lessons about what not to do—for example, we must keep secret research off the campus—but will eventually commit our own brand of folly in our own immutable way.

In the end, I think Gar Alperovitz struck deep when he mentioned the insecurity that makes scientists frightened or competitive, rather than courageous against infamy. The moral trauma and social psychosis of which this book is a witness may perhaps toughen a new generation to stand beside the few—Bohr, Szilard, Franck, Rabinowitch—who neither turned aside nor gave way to despair, nor threw away their intellectual weapons in mad sorties, but fought on when they knew they could not win. The military metaphor, alas, comes all to easily to mind!

JOHN ZIMAN

INTERDISCIPLINARY CULTURE?

Science: The Center of Culture

By I. I. Rabi. (Perspectives in Humanism, Vol. 6.) Pp. xx + 155. (New American Library in association with World Publishing: New York and Cleveland, 1970.) $5.50.

PROFESSOR RABI has been aptly characterized as the *éminence grise* of the American scientific community. As an elder statesman of science, Rabi can look back on a distinguished and varied career. He was rewarded for his accomplishments as a physicist with a Nobel Prize in 1944. At the same time, he was emerging as an important military scientist through his work both at the MIT Radiation Laboratory and in the Manhattan Project. Rabi's political and professional stature continued to rise after the war. Not only was he the key figure in the development of the Brookhaven National Laboratory, but he held important posts in the government as well: for example, he was at different times chairman of both the Executive Office's Science Advisory Committee and the General Advisory Commission for the Atomic Energy Commission. Given such credentials, his book, *Science: the Center of Culture*, will undoubtedly attract a wide audience.

It is a slender volume, one in a series edited by Ruth Nanda Anshen under the rubric of "Perspectives in Humanism". The purpose of these works, according to the editor, is to proclaim the constancy of human values and the validity of metaphysical thinking. Only by operating on this level, we are told, will it be possible to transcend the "failures" of scientific humanism and socialism (which seek merely human ends) and to find the basis for a truly unified culture.

Within this framework, Rabi's book seems rather out of place. It is not really a book at all, but a collection of essays written either in the 1940s or during the past decade. Thus, not surprisingly, *Science: the Center of Culture* suffers from a sense of repetitiveness. It also can sound dated, as when Rabi hopes that students will one day get involved with political issues; the article which contains that statement first appeared in 1962. The author's reminiscences of his own career are, by contrast, fresh, engaging and, alas, all too infrequent. Yet in spite of these limitations, the reader is able to perceive how Professor Rabi's themes converge into a coherent thesis.

His principal concern is that modern society is bereft of "wisdom" largely because it has isolated science (and scientists) from the wider context of culture and politics. By cultural isolation, Rabi has in mind the divide which exists between natural science and the humanities. Although this is by now a well-worn theme, he is able to introduce some new illustrations of the phenomenon. (He likens, for instance, Bertrand de Jouvenel's attempt to speak about physics to that "of a Bronxville matron visiting an avant-garde Greenwich Village café—she is well meaning but not with it". But Rabi's most caustic remarks are reserved for American politicians, whom he describes time and time again as "scientifically ignorant" men.

For Rabi, scientific illiteracy is a tragic fact of modern life. He sees science as "the first of the liberating arts", one which can provide man with new knowledge, sound methods and a sense of freedom. His point about intellectual satisfaction is self-evident. As for methodology Rabi seems to be saying that important social and economic problems might be solved, if only we could view them with objective detachment. That the practice of science might give rise to a feeling of liberation is an argument related to the rate of technological change rather than to the attainment of personal fulfilment. Even at this moment, the author tells us, "we . . . have overcome the fear of innovation and experiment which seems to be innate in so many people". And, clearly, he believes that this attitudinal change represents an advance in human affairs.

The central problem thus becomes: how can science be integrated into essentially traditional patterns of culture and politics? On the cultural side, Rabi's proposals are too modest. He calls for greater interaction between specialists in humanities and the natural sciences, without indicating the motive forces or modes of communication necessary for the achievement of this programme. (He neglects the social scientists altogether.) His political reforms, on the other hand, are too ambitious and utopian. Here the elder statesman dreams of a Congress consisting of, in the main, freely elected (and scientifically literate) intellectuals.

The mention of politics in the United States is not wholly inappropriate here. Rabi's book bears the stamp of its national origins, not simply because it is addressed to an American audience, but because it reflects in two ways an ethos common to many American liberals educated in the interwar period. First, Rabi believes that all social and cultural problems, no matter how intractable, can be solved through sheer determination and hard work. ("We have the means; if only we had the will", and so on.) The second aspect—and it is closely related to the first—concerns Rabi's unquestioning faith in the durability and universality of social values. In the 1960s, however, the assumption of a politico-ethical consensus within America simply became untenable. One of the values most called into question, particularly by young radicals, was the very doctrine of *laissez innover* which Rabi himself has so ardently defended. Unlike the elder statesman, they (and I) are not prepared to accept the description of atomic and hydrogen bombs as the "natural consequences of the scientific knowledge at our disposal, and as such . . . inevitable".

PAUL GARY WERSKEY

PROGRESS AND ETHICS

The Sociology of Progress

By Leslie Sklair. (International Library of Sociology and Social Reconstruction.) Pp. xvi+272. (Routledge and Kegan Paul: London, November 1970.) 65*s*.

THIS book falls into two parts. The first, consisting of a brief history of the idea of progress, is competently enough done, but neither very original nor very profound. It serves chiefly as a setting for the more important second part, which is intended to establish, rather grandiloquently, "the" sociology of progress.

Here we start at once with the difficulty of definition. This is more than a verbal problem, for whereas for most other people working in the field progress means an immanent tendency for the system to "improve" in some sense to be defined, here it seems to be derived from an individualist basis, the desire of every human being for things to get better with him and to have his problems solved. This, of course, begs all the questions, such as the distinction between people's desire for progress and its actual historical experience, the success and effectiveness with which people pursue "progress" as against other aims, and the common situation in which the quest for "progress" by one group can be achieved only at the cost of regression by others. Indeed, because he maintains that all historical ideas are ideas of progress, and all history is the history of progress, certainly so in the case of knowledge and "probably" so in the case of social progress, it seems that the author has never considered the opposite, that is, regression, and the desire of powerful segments of all societies to turn back the clock. It is left to a footnote to make the proviso that "there have of course been periods in history when knowledge has stood still or has even been lost, but the general trend has been cumulative and progressive. I am speaking, of course, about the Western world", thus admitting that the sociology of progress is valid for only a small fraction of the historical experience, and not wholly valid even there.

The main claim to originality made in the book is the distinction between innovational and non-innovational progress. The discussion of this distinction which is intended to be central to the argument, makes heavy weather of it without advancing much beyond the Schumpeterian distinction between a new idea and its general acceptance, and ends up rather lamely with the admission that these two concepts are "ends of a continuum rather than twins in a dichotomy". The distinction between them is used for at least two major purposes. One is to show that non-innovational progress is progress of a kind, especially in the moral sphere, for example, the creation of a "better" society, nearer to an ideal that might well be an old-established one. The other is to claim that since the "institutionalization of science", which is here placed around the year 1800, the West has increasingly and mistakenly looked for, and worked for, the "innovational" kind of progress only.

Even if this concept is the most original, there is another which is more central to the argument. That is what is called the "sociological ethic", that is, the conversion of sociology into a normative science. One's sympathies are with anybody operating in this particular area where angels fear to tread; here the ethical criterion adopted is that of "meeting the needs" or "solving" the problem of individuals and societies: any society improving its performance in either of these two respects is, by definition, progressive. This, then, is the solution offered to all those who in the past have been sure of scientific and perhaps even economic progress, but never about moral progress. We can make statements about the moral progress of human society, and sociology can make value judgments, and the two are linked. Although the title of the book is about the former, one suspects that the author is more interested in the latter. Several value judgments are attempted on particular recent issues, such as the vast expenditure on the space programme at a time of widespread human poverty and disease, or the "biological time bomb", the tampering with genetic factors. On these, and other matters, the author has interesting, even if not original, things to say, but it is hard to see how "the" sociology of progress has helped him towards his views.

There are some glaring omissions in this approach, notably the a-historical attitude, in spite of some mention of relativism, and the failure to note the existence of classes and other opposed interest groups, whose "needs" and "problems" might conflict:—and then whose needs should have priority in the name of "progress"? There are also too many loose ends in the internal structure of the argument and the claims are consistently pitched too high, even in the title. But Mr Sklair is able to deal in an intelligent way with a number of the most urgent current problems, including the uses and abuses of science; and he deserves high marks for effort in introducing ethics into sociology.

SIDNEY POLLARD

HAPPY BIRTHDAY NRDC

Project X

The Exciting Story of British Invention. By Peter Fairley. Pp. 239+8 plates. (Mayflower: London, October 1970. 7*s* paper.

THE idea that Britain is uniquely bad at turning her inventions into new products is a persistent theme in Parliament and elsewhere. It is, in fact, a myth—fed by a lack of objective information and dependent on the Protestant view that inventiveness is a moral virtue not far short of godliness and cleanliness. In reality, almost all the important twentieth century technologies found their origins elsewhere; the motor car in France and Germany, the aeroplane in France and the United States, computers in the United States and Germany, electronics almost everywhere.

It is necessary to risk labouring this point because it was with the intention of remedying Britain's alleged weaknesses that the National Research Development Corporation was set up, and whose twenty-first birthday now provides a pretext for publishing this book. It opens with a wonderfully characteristic foreword by Mr Harold Wilson: "It is perhaps right that I should recall the opening words with which I commended the Second Reading of the Development of Inventions Bill to the House of Commons . . . the whole world will agree that this country has been second to none in the production of new ideas and of new inventions. But in the past we have sometimes been slow to put these ideas into full use, or even, occasionally, neglectful of them altogether, often with very grave results".

The history of NRDC since then should have laid such arguments to rest for ever. For all its success, it has handled very few inventions of the first rank. Only the hovercraft and the cephalosporin drugs qualify even for the middle rank in terms of economic impact or pecuniary return. Where, the curious might inquire, were all the major inventions which would otherwise have gone unexploited or abroad? The truth is that there weren't any. In spite of this, the illusions persist—proving once again that myth has a much greater hold on the public imagination than reality.

But what of the book? Peter Fairley is a science popularizer of the old school, somewhat overfond of a "gee-whizz" style of delivery. It is a style acceptable, even necessary, over the short course of half-column pieces in an evening newspaper, but it becomes trying over the long haul of a fattish paperback. Fairley does not shrink from quoting at length pieces originally published

elsewhere (usually in the *Evening Standard*) or great chunks in the rather different prose style of the NRDC itself. This contributes to a breathless impression, as if the book had been dictated during a bumpy taxi ride in between more important assignments. At one point several pages have been transposed, but one hardly notices.

For those able to pass over these defects, the book covers the ground adequately, and contains some scraps of new information. But I cannot help feeling that the NRDC deserved a better birthday present, both more measured and more thoughtful. This is a piece of public relations, and most people will recognize it as such.

NIGEL HAWKES

FOUNDATIONS OF SCIENCE

Nature and Nature's Laws

Documents of the Scientific Revolution. Edited by Marie Boas Hall. Pp. xii+381. (Macmillan: London, October 1970.) 100*s*.

THERE is much to be said for basing the study of the history of science firmly on documentary sources. Reading a learned analysis of a Shakespeare play is no substitute for the play itself and similarly it can be argued that it is better to read Newton directly than be satisfied with a secondary account of his work. Yet one cannot just sit down and read the *Principia Mathematica*; it might also be asking too much to expect a working scientist today to read right through Newton's *Opticks*. Although the carefully chosen experiments of Book I on the composition of white light may be numbered among the classics of the history of science, the most exciting (and influential) part of the *Opticks* is the "Queries" at the end, which are virtually independent of the preceding 300 pages. There is therefore a definite place for source books or selected readings in the history of science. Even quite a short excerpt here and there may provide an original flavour which would be sacrificed in the reported speech of a secondary account. Preferring a smaller number of fairly extensive excerpts, the editor of *Nature and Nature's Laws* has had to be more selective in the choice of texts, some translated especially for the book.

The object of the book is expressed in the sub-title: *Documents of the Scientific Revolution*. The author of any book on "the scientific revolution" is faced with a formidable task. Obviously something must be said of the contribution of Copernicus and also the powerful support given to his theory three generations later by Galileo; something too of the foundations of mechanics and the "Newtonian synthesis". Most historians of science would also agree on the importance of Francis Bacon and René Descartes and, if the science presented is not to be one-sided, there must be some physiology (William Harvey) and microscopy (Henry Power or Robert Hooke). Dr Hall has not only included all of these but also separate sections on pneumatics and optics for good measure. Adding texts on experimentalism and scientific organization, the editor gives a very good traditional picture of the seventeenth century. The trouble comes when she bravely tackles the problem of chemistry, the crucial period of which was unfolded a century later. It is presumably in order to be able to include Priestley and Lavoisier that Dr Hall defines the scientific revolution as a series of events occurring between about 1500 and 1790. Wishing to give full coverage to the seventeenth century without omitting the "chemical revolution" the editor leaves a large gap in the eighteenth century. Perhaps some of the earlier depth could have been sacrificed in order to include something by such diverse but important figures as Linnaeus or Buffon, Black or Hutton, Franklin or Boscovich. It is not true that after Newton's death in 1727 there was complete paralysis in science. A period of consolidation in mechanics and astronomy was accompanied by important advances on a broad front from electricity to geology. If these sciences are not included here it is because historians of science are not in complete agreement on the meaning and extent of the scientific revolution. The editor herself agrees that the term embraces a series of conceptual changes in different branches of science. A study of the circumstances in which conceptual change occurs in science is still one of the most fascinating of all problems facing historians of science.

The truth is that it is impossible to do full justice to the emergence of modern science within the covers of any one book and the editor is the first to admit this. Her introductions to the readings are authoritative and well written. The choice of texts shows some originality and fulfils admirably the aim of presenting a survey of

THE TOWER THAT FONTANA BUILT



This is the timber tower that the Italian engineer architect Domenico Fontana built on either side of the Vatican obelisk to move it in 1586 from its place in the Circus of Nero 275 yards to its new position in front of St Peter's in Rome. Fortunately for the history of engineering, Fontana made a meticulous record of the planning and execution of the colossal operation to move this giant monolith—the largest entire obelisk outside Egypt (it weighs 361 tons)—and his feat is told by Bern Dibner in *Moving the Obelisks*, a book which was first published by the Burndy Library in 1952 and which has been recently reissued as a paperback by MIT Press (Cambridge, Massachusetts and London, July 1970. 19s). The book has other fascinating contemporary engravings of this move, together with accounts of the different methods used later to transport the Paris, London and New York obelisks.

some important aspects of the emergence of modern science. If the book could be made available as an inexpensive paperback it could be very useful for students. In its present form it can be recommended as a valuable addition to the bookshelf of the scientist who recognizes the historical dimension of his profession or, through commendable curiosity, seeks some insight into the ideas of those who unlocked the gates to the modern world.

MAURICE CROSLAND

D'ALEMBERT'S ENLIGHTENMENT

Jean d'Alembert

Science and the Enlightenment. By Thomas L. Hankins. Pp. viii + 260. (Clarendon: Oxford; Oxford University: London, June 1970.) 70*s*.

THIS book is the first full-size study of one of the most influential personalities of the French enlightenment, who is also a major figure in the history of mathematical physics. There has been no lack of able investigations of particular aspects of this colourful period of French history, but nobody so far has had the pluck to attempt a synthetic account of the encyclopaedist's achievements in the field of science. For the successful accomplishment of this task we have to thank the flourishing American school of history of science, which combines rigour of method and soberness of judgment with unequalled efficiency in collecting and handling material evidence.

Jean d'Alembert is a revised version of a doctoral thesis, and it inevitably exhibits, together with the solid qualities of the school, the usual imperfections of the beginner's effort. The author has absorbed an incredible amount of information and not quite digested it: the presentation of his findings would gain by pruning and his style by more directness and a lighter touch. All his reading of the pithiest writings of which French literature can boast has not affected his own clumsy rendering of their contents; and disgraceful mistakes in unchecked French quotations reveal that French grammar may be as tricky as French wit is inimitable. Such minor blemishes, however, do not detract from the general excellence of the work. In the first place, the abundant and accurate bibliography, the wealth of well-ordered material (some of which is published here for the first time), the completeness and thoroughness of the discussion will give the book lasting value as a work of reference, not only about d'Alembert, but about his times and the history of the problems he investigated. Apart from this more technical aspect, the author's survey of his hero's thinking and achievements makes fascinating reading.

For all his weaknesses—compared with Euler's supreme virtuosity—d'Alembert ranks as one of the founders of the modern methods of mathematical physics, which were developed to exquisite perfection by his immediate disciples, Lagrange and Laplace. (Excellent reprints of d'Alembert's principal scientific works are available, incidentally, from Editions Culture et Civilisation, Bruxelles.) Still more important is the part d'Alembert played, together with Diderot and Condillac, in laying the epistemological foundations of science, its "metaphysics", as they called it then. On all these points, Hankins, even when he is not very original, has interesting views, always supported by first-hand evidence. Especially valuable is his analysis of d'Alembert's indebtedness to Descartes through the channel of Malebranche (and not, of course, through the diehard Cartesians of strict observance). He also throws light on the deep-seated difference between d'Alembert and Diderot on the question of the significance of mathematics for natural science—still a bone of contention today among theoretical physicists.

As regards the controversial problems raised by d'Alembert's *Traité de Dynamique*—his elimination of the concept of force, his summary dismissal of the *vis viva* dispute as a verbal quarrel, his way of handling infinitesimals—he does not shrink from the difficult task of retracing their history in order to establish d'Alembert's position with respect to the views of his predecessors; surprising as this may seem, he is here breaking new ground—so much is written, so little known about the course of Newton's or Leibniz's thinking on dynamics! Hankins should therefore not be blamed if his discussion at this point makes a somewhat sketchy impression: it gives at any rate a most instructive first orientation. Altogether, what the book lacks in brilliance is more than made up by the careful and intelligent treatment of its ambitious programme: it does draw a convincing portrait of d'Alembert.

L. ROSENFELD

A GENTLEMEN'S HOBBY

The Common Scientist in the Seventeenth Century

A Study of the Dublin Philosophical Society, 1683–1708. By Theodore K. Hoppen. Pp. xii + 297. (Routledge and Kegan Paul: London, October 1970.) 55*s*.

IT is useful to be reminded that great things start from small and obscure beginnings; and that this is as true of science as of anything else. It is by now well known to historians that the Royal Society itself was a fragile thing in its early days. Newton rescued it and made it a club fit for gentlemen, but the sort of Royal Society we would recognize now was, as the term "scientist" itself, a creation of Victorian times. In this work, T. K. Hoppen recounts the history of a society that never managed to strike roots. It was, in fact, a brave venture by William Molyneux and a few lesser *virtuosi* in Dublin. It died when Molyneux's interests were diverted, and flickered briefly into life again when his young nephew Samuel had a temporary interest in philosophy.

Whether there was then a "common scientist" is a question of some significance for the history of the period. It is true that there were no giants among the Dubliners. Their contributions are the common sort of gentlemen's amateur natural history, experimental philosophy, mechanical arts and medical experience, that filled all the "philosophical" journals of the time. But the failure of the ordinary members even to keep the society going is significant. It cannot be blamed on the colonial situation of Dublin, for the parallel society in Oxford fared no better. They were not "scientists" but gentlemen with a hobby; and the realization of Bacon's great dream was largely in the hands of such men, in Britain at least, until the industrial revolution.

J. R. RAVETZ

NEOLITHIC BURIAL MOUNDS

The Earthen Long Barrow in Britain

An Introduction to the Study of the Funerary Practice and Culture of the Neolithic People of the Third Millennium BC. By Paul Ashbee. Pp. xvi + 208 + 24 plates. (Dent: London, September 1970.) 126*s*.

EARTHEN long barrows were first studied as an archaeological category by John Thurnam in 1868, in his famous paper in *Archaeologia* entitled "On Ancient British Barrows, especially those of Wiltshire and the adjoining Counties. Part I: Long Barrows". Since then there have been many general studies such as that of Piggott, and regional studies such as those of Crawford, Grinsell, and Phillips; but it is not until this present book that we have been given a detailed and complete account. Mr Ashbee, who has already given us *The Bronze Age Round Barrow in Britain* (1960), and *The Fussell's Lodge Long Barrow* (1966), now puts us further in his debt with this

authoritative, careful, and clear survey of an important aspect of British prehistory in the fourth and third millennia BC.

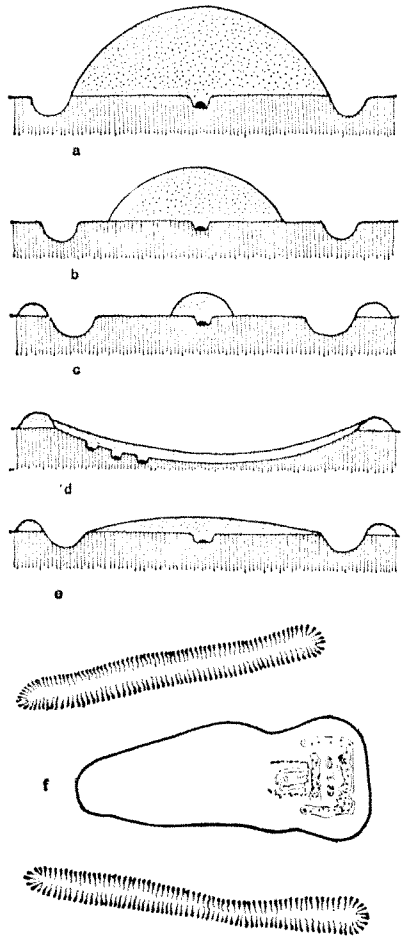
This is a well produced and expensive book, and is well illustrated with maps, plans and photographs. The use of upper-case lettering and the over-reduction of some maps and diagrams (for example, Figs. 1, 2, 15–18, 58) make them unattractive and difficult to read without a magnifying glass. Ashbee lists all the earthen long barrows known up to 1967 and many he mentions have not yet been published in full. The book has been a long time with the printers: two new earthen long barrows have recently been discovered in eastern Scotland, and in its first season of excavation of one of them, the Department of Archaeology of the University of Edinburgh, under Professor Piggott, discovered features very like those described by Ashbee from southern Britain. We are all caught up by the progress of archaeological discovery, and by the fluctuations in our assessments of carbon-14 dates. Twenty-five years ago few writers would have dated any of the British earthen long barrows as early as 3000 BC. Now Ashbee, from ^{14}C dates, gives the range as between 3500 and 2000 BC, but bristlecone pine recalibration suggests that the earliest long barrows date from before 4000 BC.

Ashbee believes that the earthen long barrows in Britain are part of a north European Neolithic heritage of long graves, long mounds, and mortuary houses, and he cites a wide variety of parallels from the long barrows of Brittany to the Kujavian tombs of Poland: he has been assiduous in his continental survey and very properly includes Bonnières-sur-Seine, Stein, Konjens Høj and the north German boulder-bounded long barrows. He must surely be right in this view and also in seeing the sepulchral earthen mounds as funerary versions of long houses such as were built and lived in from eastern and central Europe to the low countries in the fifth to third millennia BC. He is not dogmatic in the statement of his views, but he is persuasive. This is, anyhow, an over-cautious book: its subtitle is "An Introduction to the Study of the Funerary Practice and Culture of the Neolithic People of the Third Millennium BC"; but he deals only with earthen long barrows and eschews comment on their relation with the megalithic long barrows.

At one moment he throws caution to the winds in claiming that the great grave found by St Patrick at Dichuil was a long barrow. I suppose it just could have been but the texts are equivocal. But we are grateful to him for reminding us of this curious account, and also for publishing portraits of Thurnam and Greenwell, his early predecessors in the study of non-megalithic long barrows in Britain.

GLYN DANIEL

ARCHAEOLOGY FROM A–Z



Types of barrows (round or elongated mounds covering one or more burials) found in Britain drawn by Judith Newcombe for *A Dictionary of Archaeology* by Warwick Bray and David Trump (Allen Lane (The Penguin Press): London, November 1970, 45*s*). The sections show (*a*) bowl barrow; (*b*) bell barrow; (*c*) disk barrow; (*d*) pond barrow; (e) saucer barrow; and (*f*) plan of Nutbane long barrow.

MAN AND THE EARTH

The Dominion of Man

The Search for Ecological Responsibility. By John Black. Pp. vi+169. (Edinburgh University: Edinburgh, July 1970.) 30*s*.

AT the beginning of this thoughtful and unusual book Professor Black quotes a definition of progress by a twentieth-century geographer: "increasing ability to dominate the forces of nature". He identifies the convictions that man's role on Earth is to exploit the rest of nature to his own advantage and that human populations will continue to increase as those ingredients of the western world-view primarily responsible for the ecological crisis about which so much is being talked and written at the present time. These convictions belong to the class of basic presuppositions which usually remain unquestioned although they underlie a large proportion of our thoughts and actions. Black sets himself the interesting task of tracing these particular presuppositions to their origins, and his search leads him back to the Judeo-Christian element in our western world-view and to the biblical accounts of the creation.

There are two such accounts in the Book of *Genesis*, reflecting alternative ways in which "the original mythical material was hammered into theological shape". The Priestly version of the first chapter states: "so God created man in his own image . . . and God said unto them, be fruitful and multiply, and replenish the earth, and subdue it; and have dominion over the fish of the sea, and over the fowl of the air, and over every living thing that moveth upon the earth". Black finds here the essential clues to the development of the western attitude towards man's relationship to nature. Increasing scientific knowledge may have made it impossible to accept this account of creation as literally true, but its effect on our world-view has nevertheless persisted.

The earlier Jahvist version of the creation myth appears in the second chapter of *Genesis*. In it the sequence of creation is quite different from that in the Priestly version in which man arrives last, after plants and all other animals. In the Jahvist version God made the Earth, then man, then a garden for man to live in. Only subsequently were animals and birds created as companions for man. They proved not entirely adequate for this

purpose and God eventually made woman. In this version man is nowhere given dominion over the animals.

Black suggests that the existence of these opposing views of man's relationship to animals may be evidence of an ambivalence extending at least as far back as the compilation of *Genesis* and persisting today. It manifests itself as a dualism in attitude towards the totality of other living organisms: the drive to control and dominate against a sense of the majesty and mystery of nature.

Subjugation of nature may have seemed to the Hebrew tribes the only means of permanent survival in their hard semi-arid environment. It is not surprising that God's injunction to them to control and dominate became an emphatically recurrent theme in their literature and part of their enduring contribution to the western world-view. But unrestrained exploitation of nature leads to disaster, especially in environments as fragile as that of the Hebrews. There was need therefore for an effective system of checks on over-exploitation. This was provided among the Hebrews by the concept of man's responsibility to God for the proper management of the Earth so as to avoid any diminution of its productivity. It may be supposed that the idea of stewardship would have found favour amongst those holding that other view of man's relationship to nature implicit in the Jahvist version of the creation story. And, in so far as the opposing views reflect a persisting dualism in human reactions to nature, it is intelligible that the compromise-concept of stewardship and accountability to God should have retained a place in orthodox Christian thought. "The end of man's creation," wrote Chief Justice Sir Mathew Hale in 1677, "was that he should be the vice-roy of the great God of Heaven and Earth; his Steward, Villicus, bayliff or farmer of this goodly farm . . . and hereby Man was invested with power, authority, right, dominion, trust and care . . . to preserve the face of the Earth in beauty, usefulness, and fruitfulness."

Man's present situation is that he has retained the basic presupposition that his role is to dominate and exploit the world of nature but is rapidly losing any effective sense of accountability to God for its proper management. At the same time, to be fruitful and multiply continues to be thought by many a right if no longer a duty. The result is that the ancient moral checks on over exploitation are becoming lamentably ineffective. They might by now have been replaced by new checks securely based on increased ecological understanding and on rational population planning, but short-term economic and nationalistic considerations too often gain the upper hand in any conflict over planning the utilization of natural resources. Black can offer us no certain prospect of reaching a stable relationship between man and nature within the framework of our existing society. So far, western civilization has failed because we have been unable to "engender a feeling of responsibility for the use to which we put our control over nature". This book should make all its readers ponder on the likely consequences of continued failure.

A. R. CLAPHAM

GEOGRAPHICAL ENQUIRY

Explanation in Geography

By David Harvey. Pp. xx+521. (Arnold: London, November 1969.) 65*s*.

DR HARVEY's book appears thirty years after Hartshorne's *The Nature of Geography* sought to define the purposes and scope of geographical enquiry and the place of geography among the sciences. There has been growing dissatisfaction in recent years with Hartshorne's analysis, derived from the writings of Kant and Hettner, of geography as the study of the areal differentiation of the Earth's surface. At least in part this dissatisfaction has resulted from the growing popularity of quantitative methods in a subject in which their use had previously been uncommon: William Bunge's *Theoretical Geography* (1962) was the first, rather erratic, indication of the changes in purposes and methods resulting from the quantitative revolution.

Explanation in Geography is thus a timely and important book. Harvey seeks to bring order out of the diversity of both traditional and recent geographical work by concentrating on the logical structure of explanation. In so doing he looks for guidance not to the work of geographers themselves, as did Hartshorne, so much as to the work of philosophers of science and of students in neighbouring disciplines. He takes the deductive model of Hempel and Nagel as his point of departure, and in a wide-ranging survey considers the nature of theories, laws and models; the use in theory-construction of mathematical, geometric and probability languages; problems of observation, measurement, classification and data collection; and, finally, alternative models of explanation, including causal, temporal, functional and systemic models.

Unlike *The Nature of Geography*, this book is not directly concerned either with the objects or objectives of geographic study, nor with the place of geography among the sciences. Harvey draws a sharp distinction between the philosophy and the methodology of the subject, and confines his treatment to the latter. The resulting lack of a philosophical framework leads to difficulties: the concluding discussion of alternative modes of explanation, for example, seems eclectic and undiscriminating, and no clear prescription for geographical enquiry emerges. Harvey is more concerned with exploring alternatives and their implications, rather than with defining the structure and bounds of the subject itself. The absence of such a structure is most apparent when he discusses the possibility of indigenous theory in geography. He suggests that such indigenous theory may be largely geometrical in origin and to do with spatial form, in contrast to the process or temporal theories derived from other disciplines such as economics or geology. But his own terms of reference prevent him from adequately discussing how far such indigenous theory is intrinsically geographical, and to what extent it must be further defined in terms of object and scale.

This book is, without doubt, one of the two or three most important to have appeared in geography since Hartshorne's in 1939. It does not claim to be definitive, but it clarifies the problems which have developed in recent years and suggests a variety of solutions. New problems also emerge. Kuhn's views on paradigm change, with their implications for the history of geography itself and for the way in which geography as a discipline functions, are perhaps too lightly accepted, by Harvey and by others. Harvey's book is, in fact, important because the problems it raises extend to all fields of geographical enquiry. Harvey himself likens his aim to establishing the rules of cartography before beginning to create a map: he has now made it possible to go back and reconsider the problem which Hartshorne hoped to solve, that of the extent and nature of the territory to be mapped.

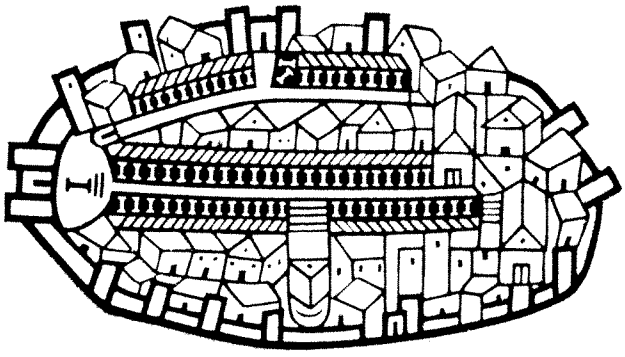
D. R. STODDART

PALESTINE AND ITS PEOPLE

Atlas of Israel

Cartography, Physical Geography, Human and Economic Geography, History. Pp. 296. (Survey of Israel, Ministry of Labour: Jerusalem; Elsevier: Amsterdam, 1970.) 1,092*s*.

HAD it consisted merely of a set of maps, this book, for all the splendour of its production, could hardly have qualified for review in a scientific journal. But the Elsevier definition of an atlas has always been generous



Jerusalem fragment of Medeba map of about AD 560.

and this imposing volume, originally published in a Hebrew edition, contains much of direct scientific interest. No country with a terrain as varied as that of Palestine and no people with an ancestry and history as rich as that of Israel can fail to command the attention at least of geologists, archaeologists and sociologists, but botanists and zoologists too will find a fund of information far exceeding that available in a traditional Bible atlas. There is a map of the Mediterranean breeding areas and migration routes of the locust; another giving the distribution of plants on the verge of extinction—but no comment on the presence of lions in the Sephelah in the days of Samson.

For the ethnologist, Israel contains material for a thousand PhD theses, but among a welter of data on the settlement of the land during the past century and comparative demographic figures for the various population groups, only scant information is apparently available on the countries of origin of Jewish immigrants: there is simply a breakdown into two groups—those born in Europe, America or Australia, and those from Asia or Africa. On the other hand, the documentation of world Jewry is extensive and one is confronted with the fact that there are many Jews (2·5 million) in the Soviet Union as in Israel, and twice this number in the United States. (The world total is listed as 13·6 million for 1967.)

Having scanned through the remaining sections on local agriculture, industry, social services, culture and so on, most readers, and especially those interested in the history and religious significance of Israel, will want to go back to the collection of old maps—the Medeba map, for example, in a magnificent two-page spread. Archaeology is given meagre coverage, but there are good historical maps from the times of the patriarchs up to the six-day war. (The standard map used throughout the book has the new *de facto* borders of June 1967.)

At 52 guineas, the book might have been graced with a rather more luxurious binding. Yet essentially this is not a prestige or presentation work, and those who will buy it will be readers likely to press it into hard service.

J. H. MORRIS

STUDIES OF ORGANIZATIONS

Organizational Analysis

A Sociological View. By Charles Perrow. (Behavioural Science in Industry Series.) Pp. xiii+192. (Tavistock: London, August 1970.) 30*s*.

THIS book contains one basic idea about the study of organizations, many interesting case studies and even more argumentative and, in my view, superficial statements about the division of labour in the social sciences.

The basic idea is good, simple and constructive: "organizations differ in their tasks, and thus in the way they are run" (p. 49); "once this is realized, it becomes possible selectively to utilize the many techniques offered for solving organizational problems" (p. 91). In elaboration of this apparent truism Professor Perrow suggests a typology of organizations based on their technology and predicts organizational trouble if there exists a mismatch between technology and structure on the one hand, and control mechanisms or personnel policy on the other. In the development of these ideas the author presents a spirited defence of bureaucracy once technological problems are solved, for "no alternative way has been found to cope with the problem of organizing large numbers of people to produce goods and services efficiently" (p. 90).

This basic idea is, of course, not new and has been applied in this country by the Tavistock group in what they call socio-technical system analysis (which receives a somewhat condescending acknowledgment) and by Joan Woodward's studies which are fully and adequately reported. Still, a good idea gains from repetition and elaboration, particularly when presented in a readable, if somewhat breezy, style, as in the case here.

The book's most distinctive flavour stems from the skilful use of case studies when discussing the environmental context within which organizations function and their diverse organizational goals. Some of the material comes from *Fortune* magazine which publishes regularly profiles of business organizations, but non-profit organizations are also included (hospitals, charities, and the like) to support the interesting conclusion that business apparently suspends competition by legal and extra-legal means more often than one would expect, whereas non-profit organizations engage in more competition than a naïve observer might anticipate.

The book is marred by a sociological imperialism—structure is "in", psychology is "out"—which cannot, of course, be maintained consistently. Not only does the foreword by Professor Vroom advocate an interdisciplinary approach, whereas the author's first paragraph claims the supremacy of sociology, the author himself is forced to inconsistencies, particularly in the interpretation of case studies where individual attitude and behaviour seem to count for more than his general stance would permit. The resistance to unorthodox labour-saving devices—Skinner pigeons as quality inspectors of pills—cannot be understood in structural terms, and he does not even try to do so. Whether the study of industrial organizations should begin or end with the study of the behaviour and attitudes to work of individuals is a debatable point; to exclude it from investigation is surely not possible.

MARIE JAHODA

STRESSES OF DAILY LIFE

Environment and Human Efficiency

By E. C. Poulton. (A Monograph in the Bannerstone Division of American Lectures in Living Chemistry.) Pp. xxi+328. (Thomas: Springfield, Illinois, April 1970.) $15.50.

THE purpose of this book is to summarize the knowledge that has emerged from experiments on human performance as affected by particular stressors. Each stressor is given only about ten pages so there is space to cover a large range including isolation, sleep disturbance, heat, cold, compression, vibration, noise, radiation and drugs.

The treatment is intended to be clear and unequivocal; each chapter begins with a summary and ends with some recipes on how to predict, reduce or avoid the effects noticed. Unfortunately, the state of the art is such that being unequivocal about human behaviour usually means being trite. For example, "radiant heat can produce burns. So also can contact with hot material" (p. 143), "decompression increases with height"

(p. 153), "vibration interferes with accurate vision" (p. 216), "glare makes seeing more difficult" (p. 246), "people who are not addicted to smoking would be well advised not to start" (p. 277). Some of these deceptively simple statements are probably not true: for example, "constant vigilance is required in driving along crowded highways" (p. 68), "a cart vibrates when it is pushed over rough ground, the faster the cart goes the greater the amplitude of the vibration" (p. 209), "drugs can be classified as stimulants or depressants according to the effect which they have upon the brain" (p. 265). It is only fair to add that many of these findings which now seem obvious do so only with the benefit of hindsight; for example, in studies of motion sickness it is important to control or record head motion, the importance of directional lighting to facilitate fault identification, stimulants may increase efficiency at dull work but might be dangerous in case of emergencies which lead to over-arousal and errors.

The book illustrates well the assets and liabilities of the school of human performance experimental psychologists which has flourished since the Second World War. Dr Poulton has been a member of the Medical Research Council's Applied Psychology Research Unit at Cambridge for more than twenty years. He is the complete exponent of this kind of psychology. His chapters in the first part on technical problems of experimentation are a model of erudition; he quotes dozens of experiments in the second part and in each case he carefully describes what the results were and mentions the limitation of the particular experimental design, number of subjects and so on. There is a tendency to be parochial. It is claimed in the introduction that the two hundred and fifty references represent only the most relevant ten per cent of possible references. In fact, if four or more references from one author are listed, that author is likely to be a fellow member of the Cambridge unit. The school adheres to the philosophy that the understanding of behaviour is confused by too many people having too many opinions based on inadequate evidence. Therefore, the thing to do is to stick to the evidence, trust only the controlled laboratory experiment and build up a body of knowledge by carrying out experiments suggested by the findings of earlier experiments. This is fine, providing it is not assumed that what emerges is a comprehensive picture of human behaviour. Confine presentation of information to that which has no history, no context and no real consequences, confine also the possibility of outputs to a small range of simple responses which are easily recorded, confine the human subjects to servicemen and students acting on simple instructions and after twenty years what you get is a picture of human behaviour which is totally unrecognizable to anybody not playing this particular psychological game.

In part one, two models of human performance are described. The first one sees the human being as an information processor with inputs, outputs, selectivity, long and short term stores and, in the middle, a slow "computer" of limited capacity. It is at least recognized that this is inadequate and so we have a second model entirely disconnected from the first, which centres on the concept of arousal. There are discrepancies in measurement, but arousal is seen as a unidimensional factor and

FELL AND DALE



These craters are not on the Moon but are corrie basins in the heart of the English Lake District. The Coniston fells are shown in the foreground and the Langdale area is beyond. This photograph, taken by C. H. Wood (Bradford) Ltd, is one of several excellent plates in a new addition to the series "The Regions of Britain"—*The Lake District* by Roy Millward and Adrian Robinson (Eyre and Spottiswoode: London, October 1970, 75s). *The Lake District* follows the format of the earlier *The Pennine Dales* and *The Upper Thames* in introducing the reader to the geology, scenery, prehistory, and the history up to modern times of this small region, but there is, as well, enough detail for the specialist to enjoy reading sections of the book. Like its predecessors in the series, *The Lake District* is very readable and the text is helped along with 40 plates and many useful text figures and maps.

so, for the present, we have performance arousal, physiological arousal, subjective arousal and finally "actual" arousal. Individual differences are a tiresome source of error; in discussing experimental design it is stated that ". . . there is a certain amount of variability between people which cannot be removed" (p. 44).

Given these self imposed limitations of approach, however, Poulton has done his job. Anybody interested in brief summaries of what controlled experiments have revealed about any of twenty-odd separate human stressors will find it in his book. The last chapter describes what little is known about pairs of stressors acting together. What happens in the normal human condition of different individuals pursuing different goals with different drives under a range of stressors is still anybody's guess.

W. T. SINGLETON

KNOTTY PROBLEMS

Knots

By R. D. Laing. (World of Man: a Library of Theory and Research in the Human Sciences.) Pp. 90. (Tavistock: London, September 1970.) 30*s*.

IT is strange to find what appears at first sight to be a collection of poems among a series entitled "World of Man: A Library of Theory and Research in the Human Sciences", but then R. D. Laing is noted for his pursuit of novel forms of inquiry into the human psyche. The poems displayed in this book are for the most part descriptions of the bizarreries and intricacies of attitude that are commonly expressed in the course of human interaction. The result is a series of snapshots which capture, in the precise form of bold type on white paper, the strangled, semiconscious, less-than-verbalized arguments we trap ourselves in.

The poems are word games of occasionally vital personal importance, during which statement and answer are related by a thread of confused yet justifiable personal logic.

> Mother loves me
> because she is good
> I am bad, to think she is bad
> therefore if I am good
> she is good
> and loves me
> because I am good
> to know she is good.
>
> I am bad
> to doubt she punishes me for doubting
> she loves me by punishing me
> for doubting she loves me,

and so on.

We all experience the urgent need to express highly complex feelings and situations when relating to another person. This need is often inadequately fulfilled, the words becoming bogged down in an impasse, a full circle, a knot. How often this has turned a conversation into an escalating conflict. We recognize the familiar truth that the representations possess with a sense of uncomfortable amusement:

> She is devoured, by him being devoured by
> her devouring desire to be devoured
> He is devoured by her being devoured
> by him not devouring her.

The episodes recreated here are like a picture of a hand finishing the outlines of another hand which is finishing the outlines of the first. Laing's drawings have this type of paradoxicality, but they are not *trompe l'oeil* fantasies but reproductions of actual situations. What is impossible in physical space is not only possible but common fare in intrapersonal space. We constantly find ourselves portraying another's act of portraying us. It is in terms of our notions of the other's portrait of us that we have to constitute our own self-portrait. But the other's portrait of us is itself based on the other's estimate of our own self-portrait, and so on. Thus, in order to provide ourselves with a self-image and an other-image which will enable us to determine the way we are going to act towards them, we have to go through operations of enormous logical complexity. It is not surprising that sometimes the fuses blow and we end up enmeshed in self-deception and circularity. This idea, explored in *The Politics of Experience*, is displayed in *Knots* in a more sophisticated form, stimulating the reader to an extensive logical and metaphorical introspection.

For this reason the book is entertaining to read, and in proportion as the passages impress upon us their familiarity, it is revealing. There are places, however where the mode of presentation and the presenter seem to be more in focus than what is being presented. But then it is very difficult for somebody else to provide us with our mental belongings without putting their finger prints all over them. It may be philistine to wonder whether certain parts of the book are conceived with slightly more levity than would seem consistent with the book's apparent general aim, but again, some will find that heartening.

STEVE FULDER

SOCIOLOGY OF CRIME

Crime and Its Treatment

By John Barron Mays. (Aspects of Modern Sociology The Social Structure of Modern Britain.) Pp. 164. (Longman: London, October 1970.) 30*s* boards; 15*s* paper.

THIS elementary but well informed text summarizes current criminological ideas in a concise and stimulating manner that should have a wide appeal to students. The realistic emphasis on the factual content of criminological data is helpful. It is pointed out very early on that ninety per cent of criminal convictions are for offences against property, which are predominantly committed by working class males, many of them schoolboys. The complementary quality of sociological and psychological theories is also clearly demonstrated. On the one hand, property crime may be said to be part and parcel of the conventional way of life in the economically poor urban neighbourhoods. On the other hand, whether any particular child in the working-class ghetto becomes an official delinquent is largely decided by the quality of his home life and upbringing.

Most of the adult habitual offenders known to the prison authorities are men whose personality deficiencies render them vulnerable to temptation. They are exploited as "jackals of the trade" by the small but troublesome minority of more competent, organized, professional criminals who are responsible for an increasing number of well planned and profitable coups. These organized criminals pose a threat to the welfare of the community and present a problem altogether different from that of the rank and file of more or less pathetic cases that make up the clientele of social workers and prison administrators.

The relative immunity of the affluent classes from criminal convictions is discussed sceptically. Embezzling tax evasion and business fraud, when found out, are often dealt with as if they were not really crimes at all. The offenders themselves and their associates would never put themselves in the same category as the common burglar although the amounts taken may be incomparably greater.

Professor Mays brings an experienced and humane approach to bear on these emotional topics. In the section on penal treatments he discusses frankly the absence of convincing evidence of the effectiveness of any particular panacea. At the same time he wisely points out "What we do does not seem to make very much difference either way, but to do nothing is to resign ourselves to despair and

possibly to cynicism" (p. 123). He also makes the point, which may seem obvious but cannot be too often repeated, that it is during the transition from institution to community living that the ex-inmate stands in greatest need of help and guidance. He regrets that the new parole system, by selecting the deserving cases, may serve to push to the end of the line the homeless and helpless who most need assistance.

This short, forthright book, which draws on the best of academic thinking and research, has the effect, possibly not intended, of revealing the extent to which criminological ideas reflect statistical trends and generalizations about offenders which conceal the complexity and variety of the problems involved. On almost every fundamental issue, from the true distribution of criminal behaviour in the community to the question of what treatment is best suited to different types of offender, firm answers will not be forthcoming without further research. The kinds of inquiry most needed may be difficult and embarrassing, but without much more and bolder research political decisions will continue to be made on the basis of prejudice rather than knowledge.

D. J. WEST

Physical Sciences

ASTRONOMY 1850-1939

The Royal Institution Library of Science
Astronomy

Edited by Sir Bernard Lovell. (Being the Friday Evening Discourses in Physical Sciences held at the Royal Institution, 1851–1939.) Vol. 1: Pp. xvi + 416. Vol. 2: Pp. x + 397. (Elsevier: Barking and Amsterdam, 1970.) 200*s* the set.

A SPECTATOR of the London scientific scene during the 1870s remarked on the unique character of the Royal Institution Friday evening discourses. They were, he said, "a wonderful combination of science and society, of physics and fashion"; yet, at the same time, they were, "of sufficiently high class to please all but a purely scientific audience". These two volumes, reprinting Royal Institution lectures on astronomy, confirm the truth of his appreciation. Most of the great names in British astronomy between 1851 and 1939 appear here, together with some, well enough known in their own day, who are either now generally forgotten or, at least, are not remembered as being interested in astronomy.

The period covered by these volumes is, of course, determined by the Royal Institution's history, but it also happens to span very nicely that most important astronomical event—the origin of astrophysics as a fully



The Board of Visitors of the Royal Observatory, Greenwich, photographed at the Observatory on Visitation Day, June 5, 1897. Among the party are several astronomers who delivered discourses at the Royal Institution. From left to right : R. B. Clifton (Royal Astronomical Society), A. W. Rücker (Royal Society), H. H. Turner (Savilian Professor of Astronomy, Oxford), Sir George Stokes (Royal Society), W. D. Barber (Secretary), Lord Rayleigh (Royal Society), J. W. L. Glaisher (Royal Astronomical Society), G. H. Darwin (Plumian Professor of Astronomy, Cambridge), Earl of Rosse (Royal Society), A. A. Common (Royal Astronomical Society), W. Huggins (Royal Astronomical Society), and F. W. Dyson (Chief Assistant, Royal Observatory). (Photo: Royal Greenwich Observatory.)

fledged science. At the beginning of this period, astrophysics was almost non-existent. Thus the first lecture printed here is Airy's discourse on the forthcoming solar eclipse of 1851. This was the first to be observed in detail by astronomers, and it led to the long sequence of nineteenth century eclipse expeditions, which raised so many questions concerning the nature of the solar atmosphere —and answered a few of them. At the other end of the time sequence, the last discourse is by another Astronomer Royal, Spencer Jones, on the projected 200-inch telescope: the instrument which better than any other symbolizes modern astrophysics.

These volumes allow us to follow the often complicated development of astrophysical ideas during the past century. Many of the conceptual changes have been remarkable: none more so than in the timescale allotted to the universe. In 1887, Sir William Thomson explained to his audience: "Five or ten million years ago he (the Sun) may have been about double his present diameter and an eighth of his present mean density . . . but we cannot, with any probability of argument or speculation, go on continuously much beyond that". Just about fifty years later Sir James Jeans told his audience: ". . . we find that 5 million million years ago, the Sun was probably many times as massive as now and many times as bright". The current value for the age of the Sun is more or less the geometric mean of these two estimates.

The timescale has been a central question in astronomy throughout the nineteenth and twentieth centuries; other areas, less subject to intense scrutiny, have experienced fluctuations in fashion. Dewar's experiments on the gaseous constituents of meteorites—reported by him to the Royal Institution in 1886—were for many years the best of their kind: it is only in the past two decades that there has been a renewed interest in this type of investigation. Similarly, there have been varying fashions in ideas: Balfour Stewart's discourse (1867) on the Sun as a variable star refers to the part played by the planets in influencing the behaviour of sunspots. This suggestion was revived, albeit in modified form, just a century later, in the 1960s. In view of the current British plans for the development of infrared astronomy, Piazzi Smyth's preliminary site-testing in Teneriffe (reported to the Royal Institution in 1858) may also come in useful after a lapse of a century. Smyth, happily, was enthusiastic about the site. "Then how truly serene and quiet, and transparent, too, was the air above our 8,900 foot elevation; for, on erecting our telescopes, not only was each star, high or low, seen with an exquisite little disc and nearly perfect rings, but the space-penetrating power was extended with the same instrument and same eye from the 10th magnitude at the sea level, to the 14th."

These two volumes are fascinating, if rather expensive, and can be recommended both for serious and for bedtime reading. Misprints in the body of the text cannot, of course, be blamed on the publishers, because these are photographic reprints. But the blurb describes the editor, Sir Bernard Lovell, as having been president of the Royal Astronomical Society in 1964; he is in fact the current president.

A. J. MEADOWS

RADIO ASTROPHYSICS

Radio Astrophysics

Nonthermal Processes in Galactic and Extragalactic Sources. By A. G. Pacholczyk. (Series of Books in Astronomy and Astrophysics.) Pp. xxi + 269. (Freeman: San Francisco and Folkestone, September 1970.) 122*s*.

A LARGE body of observational data is available for the 10,000 or so radio sources now known. These include, among other parameters, measures of source spectra, energy flux, size and polarization. Before this material can be interpreted in terms of the physical conditions within the sources, it is necessary to have a well understood theory of the emission mechanism. Indeed, the synchrotron mechanism in which radiation is generated by relativistic electrons gyrating in a magnetic field is believed to be an adequate theory for the majority, if not all, of the galactic and extragalactic continuum radio sources.

Dr Pacholczyk has written a useful text on the emission processes in radio sources. He has concentrated on the synchrotron process for which he has provided a thorough introduction. The effects on the emission by the surrounding plasma are included as are the effects of electron scattering on the emitted synchrotron radiation. A well chosen bibliography is included at the end of each chapter.

A new range of non-thermal processes have recently been recognized in spectral line sources. The most studied of these are the regions of anomalous emission from OH and H_2O molecules in interstellar space. Brightness temperatures of up to 10^{13} K have been measured which are enormously in excess of the kinetic temperatures which exist in these regions and are interpreted as originating in maser action where a weak background signal is amplified in the cloud of interstellar molecules to these high intensities. Line-narrowing, temporal variability and a high degree of circular polarization are trade marks of this type of emission.

It is probably a sign of the rate of advance technically and the associated rate of new discoveries in radio-astronomy that this subject is scarcely touched on in Pacholczyk's treatment. A major branch of non-thermal radio emission mechanisms has been deliberately excluded from this work, namely, the various radio emission mechanisms known to operate in the atmosphere of the Sun. The reason for this latter omission in not so clear, because these mechanisms provide excellent examples of emission mechanisms other than the synchrotron process.

In all, I find this a welcome book bridging the gap between the extensive theoretical treatments and the wide range of observational data. As an aside, it is interesting to find Pacholczyk, a theoretical astrophysicist, falling prey to the observational astronomers' and space-scientists' nightmare—confusing the senses of polarization which an antenna receives and transmits. An antenna receives and transmits the same sense of circular polarization as is adequately demonstrated in Kraus's *Radioastronomy*.

R. D. DAVIES

THE FACE OF THE MOON

Geology of the Moon

A Stratigraphic View. By Thomas A. Mutch. Pp. 324. (Princeton University: Princeton, New Jersey; Oxford University: London, August 1970.) 165*s*.

PROFESSOR MUTCH, a stratigrapher who has worked at the US Geological Survey's Center of Astrogeology at Flagstaff, is well qualified to discuss lunar stratigraphy. His book is not, however, as specialized as the subtitle might indicate. The author appeals to all the important aspects of lunar geology, albeit on a superficial level. The text is relatively free from prejudice and, together with the general lack of great depth of treatment, combines with the well-chosen illustrations to provide an excellent students' introduction to the geology of the Moon.

Two introductory chapters on aspects of history of the subject and the Moon's orbit and figure are brief and more adequate treatments will be found elsewhere. The third introductory chapter deals, again briefly, with photographic documentation of the Moon and the results drawn from some of the physical methods (such as optical and thermal) used to examine the lunar surface. Key charts identify the locations of the Lunar Orbiters' photographic coverage (but neither cardinal points nor coordinates are

given on the full Moon charts). It is useful to be reminded how the Orbiter photographic strips were assembled from scans of the 70 mm film carried aboard the spacecraft.

Lunar photographs are used liberally—and are among the best ever taken. They are pleasingly reproduced, with a matt finish; but one could wish for better because many of them tend to be rather "muddy".

With the onset of lunar mapping by the US Geological Survey, several years ago, lunar stratigraphy emerged as an interpretative tool of central importance in understanding the history of the Moon's surface. It is by no means the only effective means of access to an understanding of lunar geology, but it is one of the essential paths that can be investigated in a reasonably short time only by a team of lunar workers larger than is commonly encountered in, say, the universities. Dr E. Shoemaker led this mapping programme and directed much attention to a study of Mare Imbrium and the characteristic facies (some would say "deposits") that are associated with it and are found over a large part of the Moon's surface. Mutch expounds on the Imbrium stratigraphy and proceeds to describe the stratigraphies of other lunar maria and craters, and then of volcanic flows and deposits on both Earth and Moon. He avoids one important problem concerned with the flow in Mare Imbrium (Fig. VIII–21), in which the number density of craters is appreciably higher on the most recent flow than on stratigraphically older units (one might expect the converse) by stating, in the caption, "Crater densities on both sides of the flow are similar . . .".

Interpretation of reported facts is minimal and is coloured by prevailing US Geological Survey views. Most of the viewpoints expressed will find general support; some of these ideas may change, but Mutch does not stick his neck out, and one of the values of his book is that it will remain a useful reference work. The chapter on highland stratigraphy, and the crisp impressions of lunar rocks in the photographs taken during and following the Apollo XI mission, highlight the terminal parts of the book: it should be read by students of the Moon.

G. FIELDER

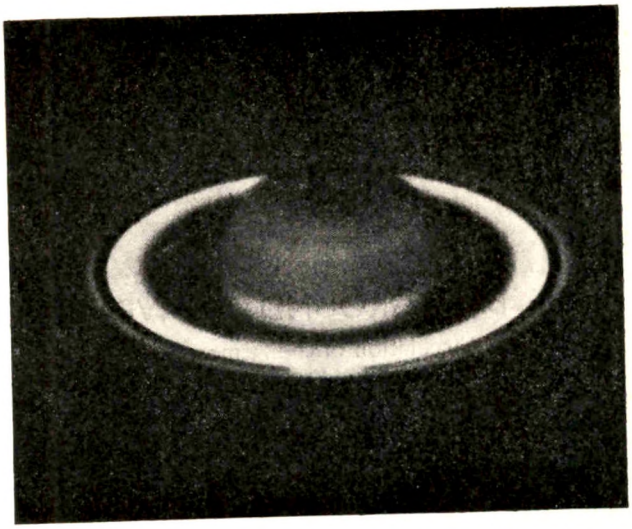
MAN AND THE SOLAR SYSTEM

The Atlas of the Universe

By Patrick Moore. Pp. 272. (Mitchell Beazley with George Philip: London, September 1970.) 215*s*.

THIS is definitely a book for the coffee table rather than for the professional library, but the many excellent colour pictures of the Earth from various NASA surveys are alone worth the cost of the volume, and may perhaps ensure that it will be found on the coffee tables of professional institutions as well as those of the private individual. The rest of the book leaves much to be desired in view of its all-embracing title. It is not an atlas of the universe (let alone the definitive work suggested by the use of the definite article), any more than a book of the same length could claim to be a history of the world; it is only on page 188 that discussion of the stars is introduced (along with more excellent illustrations), and less than 10 per cent of the volume is devoted to discussing external galaxies.

It is unfortunate that the author has overreached himself in this way, because his product is much more impressive when considered as an atlas of the Solar System. The text adds nothing to the information available in other books with less extravagant titles, but the illustrations (both photographic and diagrammatic) are so profuse and of such a high standard that they are informative in themselves, and may encourage some readers to delve further into a study of astronomy and space science. Because of this, the atlas seems particularly suitable for schools.



Saturn's rings, photographed by the Lowell Observatory, Flagstaff, Arizona (from *The Atlas of the Universe*).

Although I cannot agree with the packaging of the book, its contents are of undoubted worth, and would be even more commendable presented in the context of man's exploration of the Solar System, from which its inspiration and much of its content are clearly drawn. The apparently belated recognition of this by the producers of the book makes the discrepancy between package and contents still more irritating—if the epilogue by NASA's Dr Thomas Paine had been used as a prologue, and the title of the book suitably altered, it would have been wholly admirable.

JOHN GRIBBIN

IONOSPHERIC PHYSICS

Propagation of ELF and VLF Waves near the Earth

By Yakov L. Al'pert and Dora S. Fligel'. Translated from the Russian by James S. Wood. Translation edited by James R. Wait. Pp. v+171. (Consultants Bureau: New York and London, 1970.) $22.50.

THIS book represents a concise summary of several of the theoretical problems encountered in the study of waves in the frequency range $0{\cdot}001 < f < 30$ kHz propagating in a bounded medium. The authors, in their introduction, suggest that they wish to give an insight on this branch of radiophysics to the inexperienced reader. In this they have been unsuccessful because many of the problems discussed are presented assuming that the reader has a strong background. As a reference text for the expert, however, this book contains a wealth of information pertaining to certain boundary value problems encountered in the propagation of electromagnetic waves in the ionosphere. In particular the authors give an excellent account of the evaluation of the roots of the pole equation, which does much to enhance the readability of the mathematical development. To acquire an adequate background before attacking this book, the reader should first study Al'pert's original book *Radio Wave Propagation and the Ionosphere* of which this new book is a natural offshoot. This would give the reader a chance to familiarize himself with Al'pert's style and notation, as well as giving him a feeling for the transition in thinking from the pre-satellite days of Al'pert's first book to the present time when satellites are capable of making *in situ* measurements of the wave phenomena which Al'pert studies theoretically. The reader benefits from a large number of comments by the editor (J. R. Wait). As the editor

is himself an expert in electromagnetic boundary value problems, this comes as a welcome addition to the text.

In summary, this book cannot be considered as a useful text for the inexperienced reader, but it stands as an excellent reference work for the expert wishing a concise summary of certain of the boundary value problems encountered in ionospheric physics.

GORDON ROSTOKER

THE BACKGROUND TO WEATHER

The Global Circulation of the Atmosphere

Edited by G. A. Corby. Pp. 257. (Royal Meteorological Society: London, 1970.) n.p.

FOLLOWING a well tried pattern for scientific symposia, the joint conference of August 1969 between the Royal Meteorological Society and the American Meteorological Society consisted of a number of invited review papers by leading workers, together with a comparatively large number of additional contributions submitted and presented by their authors. The pattern usually ensures successful sessions, but publication then presents a problem, because the invited papers are rarely "original" and the submitted papers are inevitably of uneven quality.

The decision, in this case, to publish only the principal invited survey papers in a conference report, leaving the remaining thirty-five papers to take their chance in the scientific journals, may be regretted by many participants, but certainly provides a handy volume of permanent value. It must surely stand as a 1969 landmark in the progress of understanding of the circulation of the Earth's atmosphere on the global scale.

It is a remarkable fact that, in spite of all the complications and variability of weather, the climate of the planet Earth has a rather simple background structure—equatorial rains, subtropical deserts and so forth—evidently related with prevailing wind systems, the doldrums, the trades, the middle latitude westerlies, the monsoons. The description and explanation of this atmospheric structure in four dimensions, the exploration and the theory of world climate, have exercised meteorologists at all times, but not until very recent years could we claim to have the makings of a dynamical theory. By expressing the basic laws of fluid mechanics in standard forms and by making gross but plausible assumptions to overcome the barriers of ignorance on boundary conditions and on the statistical effects of small scale phenomena, it has, in the past decade, become possible with high speed computers to calculate mean world circulations which are recognizably realistic. This success encourages the belief that with sufficient application almost any degree of verisimilitude is attainable, and the large cooperative world programmes of World Weather Watch (WWW), and Global Atmospheric Research Programme (GARP), are an earnest of faith in progress.

The first paper is by E. N. Lorenz and nobody is more competent than he to describe "The Nature of the Global Circulation", or to recognize the difficulties which, unsuspected by some optimists, may lie ahead. His recent (1967) monograph on this subject, published by the World Meteorological Organization (WMO), is a masterpiece. Then there is a paper by J. Smagorinsky, also from the United States, who has done perhaps more than any other person in the world to advance numerical simulation of large scale atmospheric circulations. Other authors are P. A. Sheppard, D. H. Johnson, C. W. Newton, R. J. Murgatroyd, R. Hide, V. E. Suomi and B. R. Bolin, an impressive list of experts presenting articles on the boundary layer, the role of the tropics, of extratropical disturbances, on the stratosphere, on laboratory experiments, satellite techniques and GARP. In addition to this a group of four from the Massachusetts Institute of Technology, R. E. Newell, D. G. Vincent, T. G. Dopplick and D. Ferruzza, assisted by J. W. Kidson from New Zealand, provide a remarkable 50-page summary on the global energy balance—a work of reference in itself.

A practical benefit of these studies could ultimately be found in scientifically objective long range weather forecasts based on a sound knowledge of the global circulation and the associated climatic factors, first of their average state, then of their variability, from year to year. An alternative approach, an extension of short range forecasting by mathematical modelling, is to predict the variability with time as far ahead as possible as an initial-value problem, using data for the whole planet. The mean global circulation and world climate are then the averages of the predicted variable fields, and one check on the prediction model is provided by the realism of the outcome. Thus theoreticians will ask whether the model gives a true picture of energy fluxes, of momentum fluxes, of vorticity balance, of moisture balance and so forth. This conference introduces all these thoughts as well as more practical considerations of observational needs for the continuous surveillance of the global atmosphere. The impression is gained of highly complicated science and costly operations which must continue for many years before the goal of long range forecasts is likely to be reached by this arduous path. But given the present awareness of the problems and their formulation in convincing scientific terms, continuing efforts must be supported. Another Anglo-American conference in ten years' time will have much progress to report if the pace of the last decade is no more than maintained.

R. C. SUTCLIFFE

STUDENT GEOPHYSICS

Global Geophysics

By R. H. Tucker, A. H. Cook, H. M. Iyer and F. D. Stacey. Pp. vii+199. (English Universities: London, September 1970.) 45*s*.

IT took Chapman and Bartels more than a decade to write their famous definitive work on geomagnetism. If that sounds like the result of the so-called literature explosion, it is perhaps pertinent to recall that the treatise in question was first published in 1940, well before the post-war expansion in science. The most recent "standard" work on geomagnetism was the product of multiple authorship; and it does not need a prophet to foretell that no single author, or pair of authors, will ever again write a book on geomagnetism which has any pretensions to completeness. And in so far as geomagnetism is but a small part of geophysics, it is obvious that a single-author treatise on the latter is completely out of the question.

Multiple authorship of advanced texts is thus dictated by the exigencies of modern science and the shortness of life. The case for multiple authorship of a short, introductory book on geophysics is less obvious; but if it exists, it should be made if only because there is a clear tendency nowadays to use multiple authorship where it is not dictated by the subject simply to produce "instant" commercial properties at the expense of science and the scientific budget. The valid case can, of course, only rest on the advantages of combining the expertise and authority of writers in diverse fields. I am able to report that this book is one happy proof of this validity.

The book comprises four chapters. Mr R. H. Tucker first sets the scene for the later chapters on the solid Earth by considering the Earth as a body in space—the part it plays in the solar system and the consequences of its intrinsic rotation. Professor A. H. Cook then follows in a somewhat longer section on the Earth as a rotating body with a gravitational field. This is the least successful

chapter purely in the sense that mathematics is unavoidable in what is intended as an essentially non-mathematical text. Next, Dr H. M. Iyer deals with seismology and earthquakes. This is a remarkable chapter by any standards, from the points of view of content, style, level and organization. Dr Iyer has succeeded admirably in writing an introduction to the principles and results of seismology without becoming hopelessly mathematical and has, at the same time, managed to include recent topics such as earthquake prediction, man-made earthquakes and nuclear seismology. Finally, Dr F. D. Stacey discusses geomagnetism, palaeomagnetism, the magnetosphere and the Earth's radiation belts.

Although this volume is limited in scope, it is nevertheless the best introduction to geophysics I have yet seen, combining, as it does, basic ideas with exciting recent developments in eminently readable form. Its merits are such that it has already been recommended as collateral reading for first year science students at the Open University.

PETER J. SMITH

SALTS IN SEDIMENTS

Principles of Lithogenesis

Translated by J. Paul Fitzsimmons. Edited by S. I. Tomkeieff and J. E. Hemingway. Vol. 3. By N. M. Strakhov. Pp. xii+577. (Oliver and Boyd: Edinburgh; Plenum: New York, August 1970.) 210*s*.

THE third volume of Academician N. M. Strakhov's monumental work *Principles of Lithogenesis* was published in Moscow in 1962. Its appearance in English translation completes the wide introduction of this important study to the scientific community in the West.

This volume is about the way in which metallic salts become concentrated in sedimentary deposits by the operation of processes essentially contemporaneous with or directly responsible for sedimentation. The term halogensis is used to cover the genesis of such concentrates, whether Strakhov is concerned with the production of sedimentary ores of copper, lead and zinc, or the formation of the more familiar potash and halite deposits. The discussion of these processes and their expression through time is divided between the three parts of the volume.

The first part deals with the earliest stages of halogensis, as expressed at the present time and in the past. There is a long account of sedimentary ores of copper, zinc and lead, and some treatment of the way in which calcium and magnesium carbonates become concentrated under different geological circumstances, from shallow seas to river floodplains.

The second part of the book describes the present-day occurrence of salts and the physico-chemical processes that are responsible. The deposits in the well known Kara-Bogaz Gulf are described on the basis of the latest Russian studies, but many other salt lakes, chiefly in Asia, are given detailed description and analysis.

In the third and longest part of the book, Strakhov outlines the geological occurrence and facies structure of ancient deposits of salts. The deposits are described in terms of a broad environmental classification: continental, lagoonal, isolated gulfs, and epicontinental seas. The concluding chapter summarizes the geological occurrence of salt deposits and discusses the development of halogensis throughout Earth history.

This book is an invaluable source of detailed information and ideas about salts deposits in Russia and Asia generally. Its publication opens up for Western geologists an important new world of data and ideas. The production is, on the whole, very good, with line drawings that are a substantial improvement on those in the previous two volumes. The omission of an index is, however, a serious defect.

J. R. L. ALLEN

STATISTICS IN GEOLOGY

Statistical Analysis of Geological Data

By George S. Koch, jun., and Richard F. Link. Pp. xi+375. (Wiley: New York and London, September 1970.) 125*s*.

A NUMBER of books dealing with statistics in geology have been published in the past eight years. Although covering much the same ground as standard statistics texts, they seem in general to have been well received by geologists. Examples are set in a geological context, methods are evaluated from a geological standpoint, and stress is placed on procedures, such as trend-surface analysis, which are of particular significance in Earth science. Although many of the earlier books have been biased towards stratigraphy, sedimentology or petroleum geology, this new volume by Koch and Link is oriented towards the mining geologist.

Although this is not indicated on the title pages, this book is intended as the first of two volumes, and regression, correlation and multivariate methods are not included. The longest section of the volume deals with frequency distributions and population parameters, confidence intervals, analysis of variance and transformations. A subsequent section considers sources of variability and problems of sampling, largely in a petrological and geochemical context. Much of the second section could be read without reference to the first. Although there is no extensive bibliography, an adequate number of key references is given.

The book is intended for readers with some geological training but little or no statistical knowledge. One hopes that such readers will not use it as their only source of statistical information. The well-trodden ground of elementary statistical theory is presented in a manner that is inconsistent and is in places difficult to follow. The geological significance of the theory is not always clearly established and examples are frequently either not geological or based on fictitious data. Many, perhaps most, of the statistical methods of importance in geology are not even mentioned. This book on its own is thus of limited value and volume two must be awaited to determine whether it forms part of an integrated whole. There, the authors intend to place geology first, statistics second, and introduce real examples in which the statistical principles described in volume one are put to work, extended and refined as necessary. No publication date for the second volume is mentioned.

T. V. LOUDON

COMPENDIUM OF INEQUALITIES

Analytic Inequalities

By D. S. Mitrinovic in cooperation with P. M. Vasic. (Die Grundlehren der mathematischen Wissenschaften in Einzeldarstellungen mit besonderer Berücksichtigung der Anwendungsgebiete, Band 165.) Pp. xii+400. (Springer-Verlag: Berlin and New York, 1970.) 88 DM; $26.

THE publication in 1934 of the classic *Inequalities* of Hardy, Littlewood and Polya, established the topic as a field of study in its own right, a field since then intensively cultivated. Dr Mitrinovic's compendium will be of great value to the worker who feels sure that a desired inequality must exist if only he could find it. It will also delight those who relish "tricks to show the stretch of human brain" for their elegance or quaintness.

A short introductory chapter deals largely with convex functions, from whose properties many of the fundamental inequalities can be easily inferred. Then there are two principal sections, on general and on particular inequalities. The dichotomy is not entirely clear, but, roughly,

the general inequalities are widely used with applications in a variety of places and the particular inequalities are likely to be of interest purely for their own sakes. In the general section, proofs are usually supplied although they are usually omitted in the other part, but throughout the work the documentation is extremely thorough and the author has ransacked the minor as well as the major mathematical journals for references. He has also been able to include a number of hitherto unpublished results.

To keep the volume within reasonable limits, much has been excluded: deliberately, there is nothing about geometrical and isoperimetrical inequalities, or those connected with number theory or with special functions. Moreover, the author avoids too much overlap with Dieudonné's excellent discussion in his *Calcul Infinitésimal* (1968) and with the 1961 book by Beckenbach and Bellman.

Many items will suggest further research to complete or extend known results. For instance, there is a full account of work on the curious cyclic inequality for positive x's,

$$\frac{x_1}{x_2+x_3}+\frac{x_2}{x_3+x_4}+\ldots+\frac{x_{n-1}}{x_n+x_1}+\frac{x_n}{x_1+x_2}\geq \tfrac{1}{2}n.$$

Mitrinovic has traced the earliest occurrence in the *Educational Times* for 1903 (in the simplest case $n=3$), but remarks that this may not be its first appearance; it would not be surprising to find it somewhere in those Victorian repositories of perverse ingenuity, the Cambridge college and tripos examination papers, though I cannot find it in Wolstenholme or Carr. Interest in it began after the last war; in 1956 it was proved for $n=3,4,5$, whereas Lighthill showed that it is false for $n=20$. In 1958 Rankin proved that it is false for all sufficiently large n, but though much has since been added, there are still nine values of n for which the question remains undecided.

T. A. A. BROADBENT

GEOMETRICAL OPTICS

Geometrical Optics

By Hans-Georg Zimmer. (Applied Physics and Engineering: an International Series, Vol. 9.) Pp. vii+172. (Springer-Verlag: Berlin and New York, 1970.) 34 DM; $9.40.

THIS book is fairly conventional in scope, dealing with gaussian optics and third order aberration theory. The approach used is, however, most unorthodox, in that as far as possible the author avoids discussion of ray paths and the shape of wavefronts. He takes, instead, the standpoint that the function of an optical system is to transfer energy by means of tubes of light, and builds up his analysis in a manner allegedly derived from the principle of energy conservation. In effect he takes as his basic axiom that a quantity called the "linear conductivity" of a light tube is invariant in a perfect imaging process. This is stated (but not proved) to be consistent with energy conservation. The procedure is essentially equivalent to using the Lagrange invariant as the prime means of specifying an optical system. The theory of geometrical optics is then reformulated in terms of the quantity "linear conductivity".

Regrettably this approach serves only to make simple issues seem rather complicated and renders the book most unsuitable for students. Other readers may be put off by a turgid text, a stilted translation and a notation of eccentric obscurity. This is a pity, because the book contains a few interesting original ideas which deserve a better presentation.

M. E. BARNETT

SPIN DENSITY MATRICES

Spins in Chemistry

By R. McWeeny. (Current Chemical Concepts: A Series of Monographs. A Polytechnic Press of the Polytechnical Institute of Brooklyn Book.) Pp. xi+153. (Academic: New York and London, June 1970.) 79*s*.

CHEMICAL physicists with interests in magnetic resonance or valence theory will be familiar with Professor McWeeny's important work on spin distributions and electron density matrices in molecules. This compact and tightly argued series of lectures is particularly welcome, because it links together a variety of useful concepts in theoretical chemistry and brings out the relations between them in new ways. The theme of the book is the theoretical description of electron distributions in molecules which possess unpaired spin or orbital angular momentum, and the origin of the various spin Hamiltonians which are used to describe their energy levels in a magnetic field.

The first lecture on spin and magnetism provides a gentle introduction, and leads naturally into an interesting discussion of the principles which underlie the valence bond theory. The third lecture shows how the Dirac equation leads to magnetic interactions in the molecule, and gives an example of an effective crystal field Hamiltonian with fictitious spin and anisotropic g tensor.

The second half of the book is much more advanced and more formal in its approach. The object is to develop a very general description of electron distributions in terms of spin populations, or one and two-particle distribution matrices, which holds for any kind of molecular wave function with a definite spin and symmetry. The advantage of this approach is that the results are not tied to a particular form of wave function, such as a molecular orbital approximation. A drawback is that one has to learn a lot of difficult notation and master some deep ideas before being able to deal with even simple examples. But McWeeny should easily convince his readers that the effort is worthwhile, because of the very general and powerful methods which he devises. For example, his anisotropic spin-correlation function for triplet states and his spin-orbit coupling density are most elegant concepts. Another point which he brings out well, during a discussion of electron-nuclear hyperfine interactions, is the power of the Wigner–Eckart theorem in reducing the complexity of interactions between different electronic states. The lecture on g tensors and nuclear spin-spin couplings again shows up the virtues of the general methods. This generality should make the book of permanent value to theoretical chemists. There is an interesting appendix on the wave functions of two overlapping molecules with spin, which describes a new and elegant generalization of the Heisenberg spin-coupling idea and the Heiter–London method.

The book is clearly written in a lively style, and those who are willing to work hard should find it very useful.

A. D. MCLACHLAN

SYMMETRIES AND QUARKS

Symmetries and Quark Models

Edited by Ramesh Chand. Pp. xii+406. (Gordon and Breach: New York and London, September 1970.) $33.50; 280*s*.

THE last few years have seen an enormous increase in the number of summer schools, conferences and symposia in many fields, including elementary particle physics. This, combined with the aggressiveness of certain publishers, has led the conference organizers to believe that their meeting is a failure unless proceedings are issued, with a consequent flooding of the market. There is a real

need for such books, possibly roughly produced, but cheap enough for research students and, because of the transient nature of much of the material, brought out quickly. This volume is exceptional in that it is well printed (in East Germany), but otherwise its year delay and its price mean that it is one more book aimed for library consumption.

The style of the contributions is very varied. In the mid-1960s the symmetry game advanced very fast; people speculated and were rewarded. Those days seem to have passed, and the contributions of the people who try to carry on the revolution are not in general very inspiring.

If there were not such strong prejudices against the basis of the Naive Quark Model, the mass of evidence in the review of Dalitz, complemented by that of Mitra, would make the theory universally acceptable. It would be convincing if somebody were to find quarks, but as Jones regretfully concludes, "I suspect that most experimentalists feel that physical quarks are either unobservable or do not exist". The discussion by Lipkin of the quark model as a guide to hadron dynamics is interesting, as are the various comparisons of symmetry predictions with experiment, for example, by Meshkov, Okubo and Overseth. There are also papers on current algebras, partially conserved axial vector current (PCAC), infinite multiplets, and the like.

There is a very thorough article by Yodh on the experimental situation concerning Ξ^* resonances which are predicted by the symmetry schemes. But one has the feeling that more up to date information could be obtained by looking up the latest publication of the Particle Data Group (free of charge). COLIN WILKIN

MAGNETS

Superconductivity and its Applications

By J. E. C. Williams. (Applied Physics Series.) Pp. 213. (Pion: London, 1970.) 60*s* boards; 46*s* paper.

THIS book divides into three parts, unequal in length and quality: first, sixty introductory pages; second, nearly one hundred pages on hard superconductors and their applications; and third, nearly forty pages of other applications.

The middle section is by far the best part. It begins with an introduction to the critical state model. There follows a very full discussion of magnets, including stabilization, considerable detail on materials and examples of specific magnets. Then comes a good chapter on a.c. power devices. The calculations for stabilization, carried out chiefly for the model geometry of a thin plate in a parallel field, are presented in detail. The whole middle section forms a complete and lucid introduction to magnet design, and should be of value to anybody moving into this field.

The introductory part is not so good. It is turgid in places, and it contains several erroneous statements which may not matter for this book, but which are irritating to see. For example, the decrease of the energy gap with temperature has nothing to do with the increase in the number of real phonons present. The vortex core radius is not equal to the free path in the dirty limit.

The miscellaneous applications (magnetometer, Josephson junctions, computer elements and some oddments) are dealt with satisfactorily, except that in my view the Josephson effect is too lightly dismissed. The measurement of e/h (voltage standard) is not even mentioned. The rather promising use of weak links as infrared detectors is not discussed either, although it finds a place in Kimmitt's companion volume on far infrared.

There is a good index, and a bibliography which is thorough for magnets. The paperback version costs nearly as much as the hardback. D. R. TILLEY

NEUTRON SCATTERING

Magnetic Neutron Diffraction

By Yurii A. Izyumov and Ruslan P. Ozerov. Translated from the Russian. Pp. xviii + 598. (Plenum: New York; Heydon: London, June 1970.) 350*s*; $37.50; 150 DM.

THE neutron possesses a magnetic moment and it therefore interacts with the magnetic field produced by unpaired electrons in an atom or ion. The study of the scattering of slow neutrons by magnetic substances is a rich source of information on their magnetic properties. These properties may be divided into two categories, static and dynamic. By the former is meant the magnetic structure or ordering, that is to say, the pattern made by the directions of the magnetic moments (or spins) of successive atoms in the crystal. Originally the only known patterns were the ferromagnetic, in which all the moments point in the same direction, and the antiferromagnetic, in which alternate moments point in opposite directions. Neutron diffraction, however, has elucidated not only these, but several others such as an "umbrella" type in which successive moments lie along the surface of a cone the axis of which remains constant.

Information on these static properties comes from a study of the elastic scattering of slow neutrons. Further valuable information on the dynamics of the moments comes from the inelastic scattering. Here the neutron is scattered by a process in which it causes excitations in the arrangements of the spins. The energy of the latter changes, and this manifests itself as a change in the kinetic energy of the neutron. The knowledge obtained is valuable not only for the magnetic properties themselves, but also for the light it sheds on critical phenomena in general—a poorly understood branch of physics.

This book by Izyumov and Ozerov is a translation of an original version published in Russia in 1966. The title is somewhat misleading in that the word diffraction is usually restricted to elastic scattering, whereas the book also discusses inelastic scattering. The authors discuss the magnetic structures of rare earth metals and their compounds, double metallic oxides, and magnetic alloys. There are chapters on the distribution of magnetic moments, spin dynamics, and the behaviour of ferromagnetic crystals containing impurity atoms. The theory of neutron scattering is developed from first principles, and in spite of the formal nature of much of the analysis, the arguments are presented very clearly. There is much emphasis on the symmetry concepts developed by Shubnikov and his school.

The book will be extremely useful to research workers—both experimental and theoretical—in the field of neutron scattering by magnetic materials, although the price will limit sales. G. L. SQUIRES

INORGANIC RINGS

The Chemistry of Inorganic Ring Systems

By Ionel Haiduc. Part 1: Pp. vi + 1–622. Part 2: Pp. 623–1197. (Wiley (Interscience): London and New York, August 1970.) 500*s* the set.

THE ring symbolizes power, authority, betrothal and marriage, eternity, and perfect form; "in my beginning is my end". Rings have been used in medicine (to ward off the cramp), and poison rings for murder or (as by Hannibal) for suicide. The serpent biting its own tail, an alchemical sign for the unity (or perfect form) of matter, and for circulatory processes, was the image that came to Kekulé when he thought of the ring structure for benzene.

Inorganic rings were made, unwittingly, by Liebig and Wöhler, and by Rose, in 1834 (*cyclo*phosphazenes), and by Gregory in 1835 (*cyclo*tetrathiazene, S_4N_4). But demon-

stration of their ring structures took 60 and 110 years respectively, in spite of the stimulus to find ring systems that followed Kekulé's benzene ring formula in 1865. Many were formulated, and most were wrong. Butlerow in the 1870s and 1880s predicted that rings and polymers should be common in inorganic as well as organic chemistry; but Werner's coordination theory of valency in 1893 tended to discourage the quest for the ring. The *cyclo* tri and tetraphosphates were correctly described in 1875–80, Stokes's *cyclo*phosphazenes in 1895, and siloxanes by Dilthey in 1905. Borazine was discovered by Stock in 1925 and its structure proved in 1931. For *cyclo*tetrathiazene a ring formula was discussed (among others) in 1936, and demonstrated (by electron diffraction) in 1944. Now the rate of advance is exponential.

The study of inorganic rings is rich and rewarding, and Ionel Haiduc's book in two volumes is worthy of its subject. As a Romanian who has worked during the past ten years both in the Soviet Union and in the United States he spans the international literature admirably. Nevertheless, he cuts a clear path through the jungle of proliferating derivatives. Introductory chapters deal with systematic nomenclature, structure and bonding, and ring equilibria. He then covers homocycles and heterocycles (of which 230 are listed in his ring index) of group III to VI elements; and polycycles, and heterocycles containing titanium, or vanadium. A useful chapter describes coordination heterocycles containing non-transition metals and also the recent chemistry of transition–metal heterocycles, which form a link between the metal complexes, and ring compounds of non-metals and semi-metals. A further achievement, of author and publisher, is to add, in proof, literature references up to mid-1969.

I have few criticisms. In view of the author's subject, one may perhaps condone some cyclic logic (under "Hybridization" it is stated that a (cyclic) arrangement, involving only atoms in their characteristic hybridization states, will be preferred to structures involving a non-characteristic hybridization. This is said to explain the instabilities of such groupings as —B=O, >Si=O and the like). The implications of spectroscopic and theoretical work are not usually discussed, nor are mechanistic implications generalized, although the references are well covered. But critical acumen, which is so often lacking in books that contain this amount of information, is evident here, and the organization is good.

It is a pity that the price will prevent chemists from buying their own copy of this very useful book.

JOAN MASON

REACTORS AND RANDOM NOISE

Random Noise Techniques in Nuclear Reactor Systems

By Robert E. Uhrig. (Prepared under the auspices of the US Atomic Energy Commission.) Pp. x+490. (Ronald: New York, 1970.) $20.

ALTHOUGH this book is primarily designed for engineers and scientists interested in applying random noise techniques to nuclear reactor systems, it is not limited to that field. Only three of the twelve chapters deal specifically with nuclear processes. One describes the statistical techniques for dealing with the detection of individual neutrons in a nuclear assembly, one treats this detection process as continuous and examines the analysis of the fluctuations of the detection current, and one is made up of several examples of experimental noise measurements carried out on nuclear reactor systems in recent years, which presents a well balanced and wide ranging selection from the wealth of such work. These chapters require a working knowledge of nuclear reactor theory to be understandable.

Other chapters develop the theory of random noise from elementary probability theory through special probability densities and distributions to correlation and autocorrelation functions, power and cross spectral densities and coherence functions. In these mathematical chapters no attempt is made to be rigorous, the main definitions and results are stated or made plausible and numerous examples and explanatory diagrams are given. There are a number of missing or misplaced indices and suffices which cause momentary confusion in places.

The remaining chapters are concerned with the instrumentation and measurement techniques for the acquisition, transmission, recording and processing of data from random noise experiments, and here the pages are shot through with first-hand knowledge. Practical measurements of correlation functions, spectral densities and impulse-response functions, analog and digital techniques, multiplexing and the use of pseudorandom variables, all these and more receive a clear and concise treatment. These chapters along with the mathematical ones could be equally applied to a wide variety of physical and engineering systems. Herein lies the book's usefulness.

The author has to a large extent succeeded in his aim of making the book self-contained, although to be told after equation (3–1) that "all symbols have the definitions commonly accepted in reactor theory" suggests that perhaps an additional chapter or appendix on basic reactor theory would have made it even more so. Each chapter has a good selection of references, and the index is fully comprehensive and adds much to the overall utility of the book.

DON FIRTH

POLICY IN MATERIALS SCIENCE

Materials Science and Engineering in the United States

Edited by Rustum Roy. (Proceedings of the National Colloquy on the Field of Materials held at the Pennsylvania State University, University Park, Pennsylvania, April 14–16, 1969.) Pp. xiv+177. (Pennsylvania State University: University Park and London, October 1970.) 90*s*.

SOME ten years ago metallurgy and solid state physics married and materials science was born. The way in which this new discipline has developed since then, the role of government, industry and the university, the future trends, are all aspects which formed the basis of the colloquy which is reported in this volume. The substance of the principal addresses given at the colloquy, together with summaries by rapporteurs of the discussions, are included. The contributors are all scientists and engineers who have made important contributions to the non-scientific development of materials science and engineering in the United States.

The colloquy was the first of its kind, which makes the content of this book very special and timely. It is a pity that the title is so specialized, because apart from the obvious significance to those involved in materials research, some of the subject matter is relevant to people engaged in research policy making in all branches of applied sciences, including the life and Earth sciences. The reason for this is not obvious but should be apparent when it is understood that materials science is a classic example of an interdisciplinary subject, which has now emerged as a discipline in its own right. Currently many of the important growing areas of research have factors common with materials science and there are lessons to be learned.

In assessing the development of materials research in the past decade, a significant step was the sponsorship of the Advanced Research Projects Agency Interdisciplinary University Materials Laboratory Program (ARPA-IDL) in 1960. The products of this scheme are discussed

in this volume along with the problems involved. Particular problems of university teaching and research, university–industry–government interaction, and future trends are also discussed.

In the book there is a distinct trend of growing social awareness in all the discussion on future research. Six national civilian goals have been identified by the Co-ordination Committee on Materials Research of the Federal Council for Science and Technology: (1) low cost housing; (2) high speed ground transportation; (3) air pollution control; (4) water pollution control; (5) biomedical materials; and (6) energy generation and transmission. The potential problems of tackling such mission-oriented projects are not obvious and the opinions presented here are extremely perceptive.

There are also accounts of materials research in Britain and France, together with some surveys of university materials programmes in the United States and some information from questionnaires on industrial research. These aspects are all treated too briefly to be of much value.

The book tends to lack structure and continuity which is to be expected when there are so many contributors. I find it disappointing, however, that such an important topic which involved many important people for several days is reported in what is relatively a short book. Nevertheless, there is some extremely important information in the book which makes it worth reading for all who are in any way concerned with research policy decisions in the applied sciences.

T. R. WILSHAW

Biological Sciences

BABOON ECOLOGY IN KENYA

Baboon Ecology

African Field Research. By Stuart Altmann and Jeanne Altmann. (Bibliotheca Primatologica No. 12.) Pp. vii + 220. (Karger: Basel; University of Chicago Press: Chicago and London, 1970.) SFr.45; 108*s*.

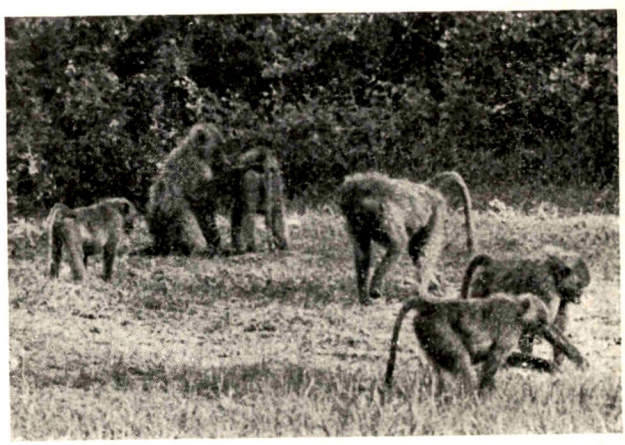
THE realization of the theoretical importance of baboon ethology and ecology for the inferential analysis of the early history of the human species has been accompanied by an enthusiasm for theory and speculation which, although often of undoubted heuristic value, has lacked adequate foundation in hard fact. Although good hypotheses provide guide lines for research only data, often brutally won by physical effort in an uncompromising habitat, can yield the information with which they can be tested. This monograph by the Altmanns does much to improve the factual side of the baboon saga and it is an important continuation of the field studies carried out in Africa during the past decade by, among others, Sherwood Washburn, K. R. L. Hall and Irven DeVore.

After a widespread survey, the Altmanns chose the Amboseli Game Reserve in Kenya as their principal field study area. Here they settled to complete a study that lasted from June 1963 to August 1964. Although the duration of their field study was not exceptional the volume of data collected demonstrates the authors' intense devotion to field work and a well organized method of data acquisition. This monograph is concerned with the ecological aspects of the investigation—reports on behaviour and social organization are in preparation.

The story begins with a detailed account of the local baboon groups (*Papio cynocephalus*), their population dynamics, group size, birth, death, and emigration rates and problems of group composition, sex-ratio, group fission and the inter-troop movements of individuals. Observations on sleeping behaviour follow and the daily ranging is described. The progression rate of foraging animals on daily ranges, patterns of aggregation and dispersal, home range size, overlap and exploitation are mentioned together with an account of seasonal contrasts. Dietary and water requirements and acquisition are examined together with social aspects of feeding in a section that slightly disappoints because so far there is no detailed analysis of social behaviour in relation to precisely quantified variation in the seasonal availability and nutritional quality of food. As the authors indicate, this indeed must be the next step in field primatology. Predation on the baboons of Amboseli, especially by leopards, is not uncommon and the Altmanns provide observations of a largely anecdotal kind. The monograph ends with general observations on the baboon's relations with other animals and a speculative chapter. At the end of most chapters there is a comparative section relating the Amboseli data to studies on other populations of the genus—sometimes accompanied by overlengthy quotations from other writers.

Some highlights of the Altmanns' survey are as follows. In contrast to most other studies the Amboseli male baboons show considerable evidence of movement between groups. Some males, furthermore, evidently remain isolated for a period which may be associated with the timing of a change in group membership. This isolation occurs in spite of the demonstrable dangers from predation in the Amboseli environment. Migrations of individuals between groups may be an important factor affecting sex ratios in different groups. Only adult males are known to show such migration and this seems to be associated with a higher mortality. The Altmanns state that there were a calculated $1{\cdot}916 \times 10^{-3}$ deaths per monkey-day for males but only $0{\cdot}256 \times 10^{-3}$ for females.

Amboseli baboon groups sometimes congregate in certain groves for sleeping, indicating a pattern of inter-group behaviour hitherto reported only for the congeneric hamadryas baboon in Ethiopia. When foraging, members of a group often show far less cohesion than earlier reports have suggested. At times, groups become spread out in long lines or are widely dispersed, but members congregate during encounters with predators,



Baboons (*Papio anubis*) in arid country in the Awash Valley, Ethiopia. Although a different species these baboons are similar in appearance to the animals studied by the Altmanns.

when approached by another group, when traversing unfamiliar terrain or when in heavy undergrowth. In open familiar terrain individuals often become much dispersed. In view of the often grossly exaggerated popular accounts of the significance of animal territory for human behaviour it is worth stressing that the Altmanns' baboons showed "no convincing evidence for territoriality, in the sense of a defended area". This seems to be true generally for open country primates most similar in many behavioural respects to man.

The Altmanns noted that their baboons prey on insects, worms, reptiles, birds' eggs and chicks, hares, a fossorial rodent, a young Grant's gazelle, vervet monkeys and bushbabies. True hunting and chasing were recorded, particularly from certain individuals who appeared to be adepts. After the capture of meat there were often agonistic encounters over its possession which were settled apparently according to the social status of the disputants. Rarely do baboon watchers observe actual attacks by predators on their animals nor the defensive behaviour that may sometimes be shown. The Altmanns recorded cases in which males and sometimes also females lunged at or chased a predator, and they also observed a new response to the appearance of a predator—crouching cryptically and motionlessly in the grass. The records of a degree of cooperative defence are important for their occurrence has sometimes been denied. Such events have also been seen in the Patas monkey in a comparable African habitat.

This study confirms the impression that baboon social life is rich and that the relation between social dynamics, population dynamics and ecology comprise for these animals a complex system that will require long-term quantitative investigations of known marked populations over several years, supplemented by experiments on separate test populations. Until primate ecology reaches the standard of comparable work with birds much will remain mysterious. But monkeys are not birds and we can glimpse already the elements of the socio-ecological system which, on a parallel track, was to lead eventually to man.

This study is essential reading for primatologists, social ethologists and anthropologists and can be read with profit at any stage of an individual career.

JOHN H. CROOK

CHIMPANZEE TOPICS

Physiology, Behavior, Serology, and Diseases of Chimpanzees

Edited by G. H. Bourne. (The Chimpanzee, Vol. 2.) Pp. x+417. (Karger: Basel and New York, 1970.) 115 francs; 230*s*.

THE chimpanzee has, for long, attracted scientific attention, principally because of the well documented view that, along with the gorilla and orang-utan, it is structurally and functionally most closely related to man. Morphological study of the chimpanzee dates from Tyson's publication in 1699 of the *Anatomy of a Pygmie*, but the foundation, on an appreciable scale, of pioneering studies of the physiology and behaviour of this genus, dates only from the 1930s. Because the animal is relatively big and presents other difficulties in its husbandry, research into chimpanzee physiology and behaviour is still limited although, in common with work on other primate groups, its study has received impetus during the past decade following the establishment in the United States of America of centres for primate research, and with the elaboration of the space research programmes.

This volume is the second of a planned series designed to form ultimately "a comprehensive study of this species". As a matter of editorial policy, subjects are dispersed, each volume having "a selection of chapters dealing with a variety of aspects of the study of the chimpanzee". The result is that this volume, like its predecessor, covers a big and seemingly disconnected variety of topics ranging from studies in visual physiology, through analyses of renal function, energy metabolism and body temperature, to an examination of the chimpanzee's taste modality. Morphological studies comprise a significant section of the work and include a formal description of the spinal cord of the chimpanzee. This is followed by an exemplary analysis of the limbs, carried out in the now established tradition of inquiring quantitatively into correlations between, on the one hand, form and structure and, on the other, locomotor and other functional uses.

The chromosomes of the animal are next considered and this study is followed by chapters dealing with social and other types of chimpanzee behaviour. The now well known modern work on chimpanzee serology is summarized, and the volume concludes with two chapters aimed towards an advancement of the husbandry of the species, and dealing first with the viruses pathogenic to chimpanzees and second with the isolation of mycoplasmas.

Comparison with other primate genera—even those for which reasonable amounts of information are available—is, of necessity, limited. Nevertheless, comparison with man and sometimes with the other apes understandably permeates most contributions, and here two contrasting groups of findings emerge. In some fields, for example adaptation in the limbs, the chimpanzee is very different from man. In many other fields—including some as fundamental as studies of the chromosomes—there is, in spite of some contrasts, a great similarity between this ape and man. In the case of the "genetically informative macromolecules" the similarity is such as to depict (in this one feature) "a closer genealogical relationship of the African apes to man than to the Asiatic apes".

The synthesis of such contrasting information to provide a basis for assessing evolutionary relationships is a problem now developing in both concept and methodology. Its solution could well be the responsibility of a future generation.

E. H. ASHTON

ATLAS OF AFRICAN BIRDS

An Atlas of Speciation in African Passerine Birds

By B. P. Hall and R. E. Moreau. Pp. xv+423 (439 maps). (British Museum (Natural History): London, 1970.) 300*s*.

To quote the foreword by Professor Ernst Mayr, this is "a superb achievement". It represents the gigantic task of plotting, for 962 species, the localities recorded in the extensive literature and in data of principal museum collections. The information is very clearly presented by symbols on a series of identical maps; further, it is arranged in a meaningful way, so that the atlas is very much more than an assembly of study material. The theme is the superspecies, or in some cases the wider species group, and all the species belonging to any one such category are shown together on a single map. The grouping of species in this way is of course a matter of taxonomic judgment, but the authors' reasons are duly given in the text. Of the total, 176 species are ranked as standing alone and are separately mapped.

Each map, with its explanatory text, occupies a page; and, where practicable, closely related groups are allotted facing pages. The species included are the members of the Passeriformes breeding in continental Africa south of 20° N latitude. (Non-passerine birds were found, in general, to require treatment on a world rather than a regional basis and have therefore been wisely excluded from the scope of the work.) Species peculiar to Mada-

gascar and other islands have been omitted, except in the small minority of instances where they represent superspecies of which other forms are truly African. In the case of superspecies of wide distribution, inset maps of the world are provided to show the extra-limital representatives. The production of the large volume is excellent.

Nothing of the kind on this scale has been done for birds before. The earlier studies of evolution and speciation, from those of Darwin and Wallace onwards, were made on island populations, for which boundaries were predetermined and tended to remain stable, so that the outcome of segregation was often diagrammatic in its clarity. In a continental area the situation is apt to be much more complicated; and secondary changes in the environment produce secondary effects in the distribution of the avifauna. Keast had lately shown, however, that similar methods of study could be applied to Australian birds; and Mrs Hall herself had done it for the francolins of Africa. Here she has given an exposition on a grand scale, and with most impressive results.

She received much help and advice from the late R. E. Moreau, whose own work had documented the history of climate and vegetation in Africa in their relevance to the avifauna. The contraction and expansion of vegetation zones fracture the originally continuous distribution of avian (and other) species, and the resulting segregations lead to fresh speciation. Later, further changes in the environment bring the isolates into secondary contact, and various patterns emerge. The study of these last offers rich scope for further research, particularly by the field worker. Here is a firm basis for it; and case after case pose an intriguing problem.

A. Landsborough Thomson

ENCOUNTERS WITH SHARKS

The Shark

Splendid Savage of the Sea. By Jacques-Yves Cousteau and Philippe Cousteau. (The Undersea Discoveries of Jacques-Yves Cousteau.) Pp. 277. (124 photographs in colour.) (Cassell: London, October 1970.) 50*s*.

In February 1967 the Calypso sailed from Monaco to collect material for a series of television films on the sea. This book is the first of a series of six commissioned by Cassell, each of which will deal with a particular aspect of the work undertaken on this voyage: coral reefs, shipwrecks, whales and archaeology are the subjects of the next four volumes.

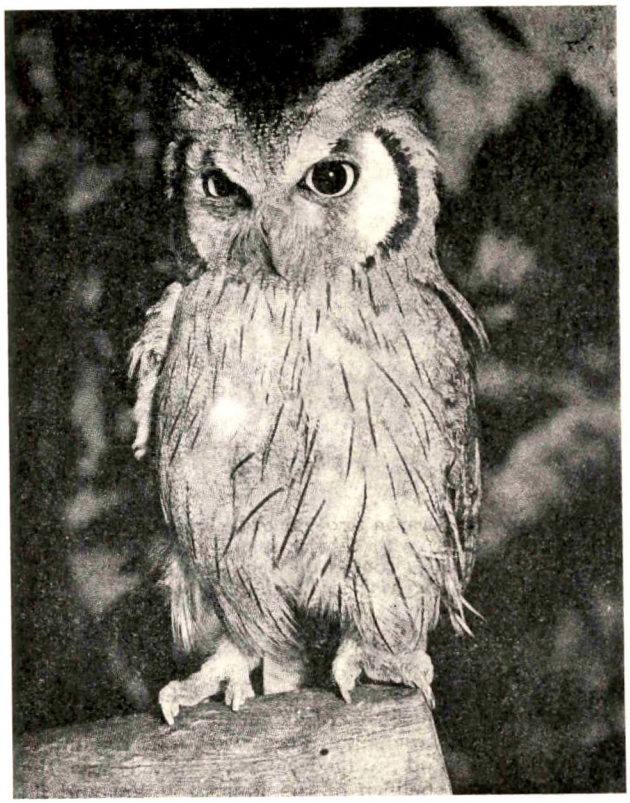
Many people who have enjoyed Cousteau's films may want to know more about them. Cousteau's introduction makes the point that the films tell little of the technical and personal problems, difficulties, and triumphs involved: these books are to complete the picture. This book is not a scientific account of sharks and this can never have been the authors' intention: it is not set at a lower level but rather in a different direction. The two Cousteaus—father and son—adopt a narrative style to highlight some of the expedition's encounters with sharks in the Red Sea and Indian Ocean and they do it rather well. I was impressed by a simple account of a submarine dive near Socotra (pp. 181–8); and there can be few readers who will not be astounded by the description and photographs of the whale sharks. Not unexpectedly, much of the book is centred on or returns to the shark as a predator: there is a lot of biting and a lot of blood. Equipment, ship and crew are described and figured. I noted, with envy, that the Calypso carried a first-class cook and a maître d'hôtel!

The text is translated from the French and generally reads well. But here and there dates and places jump. There seems to be a confusing break in continuity on p. 180. The principal part of the text is printed on glazed paper and the layout is pleasant. But the production standards are not maintained in the last 40 pages which are unglazed and include an adequate index and twenty-two line figures. In the figure section the pages are confusingly see-through and are not numbered. The colour photographs vary in quality and some show a surprising amount of red which may result from using artificial illumination of a spectral composition inappropriate to the depth of water. But as a whole the book is good value for money.

This book, and the others to follow, should interest a wide public and libraries may expect a waiting list for their copy. The biologist will find some interesting observations: attacks on sharks by porpoises; evidence for territoriality in reef sharks; and the presence and behaviour of mice, hawks, and hermit crabs on the island of Derraka. But after reading the book I had the unhappy feeling of having been cheated: so much spectacle, so little measured. For example, the Cousteaus must have hundreds of metres of film on the movements of sharks. But has there been any attempt to relate fish length and swimming speed to tail-beat frequency? What I really want is another sort of book. Calypso sailed from Monaco, a town which I associate, not with the Grand Prix, but with Prince Albert's *Résultats des Campagnes Scientifique accomplis sur son Yacht*. May we yet look forward to a similar series, albeit less munificent, from Cousteau and his Calypso?

F. R. Harden Jones

A PARLIAMENT OF OWLS



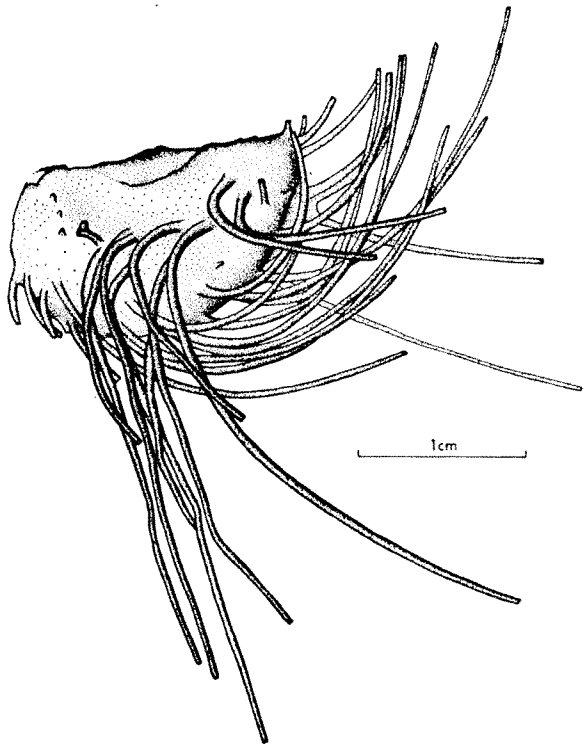
A white faced scops owl (*Otus leucotis*), one of more than 130 species of owl, some very rare, which are to be found around the world. The natural history of these curious birds of prey is told effectively by John Sparks and Tony Soper in *Owls* (David and Charles: Newton Abbot, October 1970, 50s), a book which should appeal to the naturalist and the general reader alike.

BRACHIOPOD EVOLUTION

Living and Fossil Brachiopods

By M. J. S. Rudwick. (Hutchinson University Library: Biological Sciences.) Pp. 199. (Hutchinson: London, October 1970.) 38*s* boards; 16*s* paper.

IT is surely a gamble to publish a general account of a phylum within five years of having collaborated with several other specialists in an exhaustive study of the same group. On the one hand the book might turn out to be an indigestible if deferential summary of authoritative fact and opinion written with an eye on omission rather than content. (How many of us judge the soundness of a paper by the number of honourable mentions we receive in the bibliography?) On the other hand it might be a defiant declaration of cherished beliefs embarrassingly free of the constraints of other researchers' observations. Occasionally, however, when an author like Dr Rudwick skilfully redeploys well known facts and assembles new information to illustrate novel aspects of phyletic evolution, the gamble comes off and the rewards are rich.



Lateral view of a Permian strophomenide brachiopod (*Marginifera* Productacea) with tubular spines on ventral valve: these spines probably rooted the shell in a soft substrate (Fig. 46, in *Living and Fossil Brachiopods*).

The originality of Rudwick's approach is his exploration of the functional implications of changes in shell morphology that arose during brachiopod evolution. The phylum is modestly represented in modern seas, but it was the dominant marine shellfish of the Palaeozoic era and its fossil record is remarkably complete back to the early Cambrian. This unrivalled range of living and extinct species has been well exploited in this book in chapters devoted to each of the basic functions that can be related to exoskeletal features like "muscles and hinges", "senses and protection", "lophophore and feeding", and so on. In these chapters of clearly written text and well designed illustrations, Rudwick shows how feasible living and working models may be derived for the shells of even the most bizarre of extinct species. There are abundant new data here, such as the ontogeny and disposition of setal grilles and the correlation of columnar muscle with the development of accommodating skeletal platforms. Indeed, every chapter contains an instructive phyletic chart based on brachiopod superfamilies indicating the range of features associated with a particular function. The sections on the "classification of brachiopods" and "history of the phylum" are less effective because parts of his classification, like the assignment of the Thecideacea and certain spire-bearing genera to the Strophomenida, are contrived and disputable. Yet they do serve to emphasize the personal flavour of the text and in that respect do not detract from the merits of a book that can be unreservedly recommended for its scholarship, originality and presentation.

ALWYN WILLIAMS

TENTACLED FLOATERS

The Medusae of the British Isles

Vol. 2: Pelagic Scyphozoa, with a Supplement to the first volume on Hydromedusae. By F. S. Russell. Pp. xiii + 281 + 15 plates. (Cambridge University: London, October 1970.) 140*s*.

EVER since the eighteenth century British marine biologists have found the coelenterates most rewarding objects of investigation. John Ellis was the first to demonstrate that zoophytes were ramifying animals, and John Graham Dalyell could have forestalled Michael Sars as the discoverer of alternating generations had not publication of his observations been delayed. The young T. H. Huxley, studying tropical zooplankton during the voyage of the Rattlesnake, first revealed the two-layered structure of these members of the "Radiata". Later workers were no less fascinated by the wealth of form and beauty and the range of adaptive radiation exhibited by these simply constituted animals. The beautifully illustrated memoirs of Forbes, Hincks, Gosse, Allman and Alan Stephenson come readily to mind.

F. S. Russell joined this distinguished company in 1953 with the publication of the first volume of his *Medusae of the British Isles* which dealt with the Hydromedusae. His second volume, here reviewed, deals with the pelagic Scyphozoa with a supplement which brings the subject matter of the first up to date, largely by the association of more medusae with the hydroid generation.

This is a model of what a taxonomic memoir should be, gaining undoubtedly by the few species demanding study. Out of about one hundred and sixty known species of these larger jellyfish, only thirteen are British. Six of these occur inshore, the remainder in oceanic waters off the continental shelf and often at considerable depths. All are subject to exact description in words aided by explicit text-figures and by both coloured and black-and-white plates. Apart from taxonomic distinctions, synonymy and full anatomical description including variations, there is a wide survey covering habits, physiology, life history and distribution.

Few animals are better known to the shore naturalist or more certain of comment by all who encounter them than the inshore species, the pale saucer-like *Aurelia aurita* occurring widely in many seas, the brown bell-like *Chrysaora hysoscella*, the two species of *Cyanea*, the brown *C. capillata* and the blue *C. lamarckii* with bunched tentacles and widely spreading, frilled manubria. The first of these, the lion's mane, has a formidable sting and mention is here made of its encounter by Sherlock Holmes although—probably the sole omission in the comprehensive bibliography—not of the more sinister de-

ductions from the evidence suggested by J. W. Hedgpeth[1].

The more solid *Rhizostoma octopus*, normally confined to southern and western coasts, has the manubrial opening divided into a complex series of minute openings along the ramifying oral arms admirably fitting the needs of a jellyfish feeding on the smaller members of the zooplankton. The hemispherical, thick jellied and luminescent *Pelagia noctiluca* is less common inshore but occurs occasionally in swarms.

The oceanic species of *Nausithoë*, *Atolla*, *Paraphyllina* and *Periphylla* are fully described and illustrated although, inevitably, far less is known about their biology, notably their life histories. A most significant section of the British marine fauna receives definitive treatment in this handsomely produced volume and all marine biologists are in debt to Sir Frederick Russell. C. M. YONGE

[1] Hedgpeth, J. W., *Scient. Mon.*, **60**, 227 (1945).

PLANT ULTRASTRUCTURE

Electron Microscopy and Plant Ultrastructure

By A. W. Robards. (European Plant Biology Series.) Pp. 298. (McGraw-Hill: Maidenhead and New York, September 1970.) 72*s*.

PLANT ultrastructure is at last becoming quite well covered by textbooks of a variety of levels. This book aims at the level of the undergraduate course, and sets out to describe both plant ultrastructure and the methods by which it is studied.

The first part of the book deals with the electron microscope and its ancillary techniques. The electron microscope is covered clearly and straightforwardly, though with some unnecessary passages about historical development and constructional details. The micrographs showing the effects of over and under focus and astigmatism are particularly useful. The chapter on techniques describes adequately most of what it covers, but has some serious omissions. Negative staining is dismissed in a few lines, differential centrifugation is not described at all, and the section on freeze-etching makes no mention of antifreeze agents or prefixation. This is particularly regrettable because the results of these techniques are well dealt with in the rest of the book.

The following chapters are each devoted to a single organelle or group of organelles. In his introduction to each topic, Dr Robards tends to forget his readers. A botany undergraduate is not likely to need a table showing him the principal groups of plants and microorganisms, nor the information that heterotrophic angiosperms exist. This will tend to put off potential readers, but it should not, for Robards's ultrastructural descriptions are excellent. He details the structure, as elucidated by various means, of each organelle, and describes what is known of its function. He does not gloss over controversy, but discusses both sides fairly. The only fault is a slight shortage of photographs, presumably on grounds of economy, even though lithographic reproduction is used. The photographs are, however, well chosen, and there is an abundance of line diagrams.

There follows a chapter giving a comprehensive but cursory survey of the specialized structure of different cells. As the author rightly points out, it would be impossible to do more in a work of this scale. The remaining chapters, on algae, fungi, prokaryotes and viruses, have been contributed by J. D. Dodge, R. Marchant and D. G. Smith. Each is an excellently clear account of the chief ultrastructural features of the group concerned. Although microorganisms have been extensively used in investigating specific problems, their general ultrastructure tends to be inadequately covered in textbooks.

The book as a whole is very readable: it is also useful as a reference book because of its comprehensive index, a detailed contents list and a straightforward layout. I hope the tendency to talk down to the reader on general topics does not antagonize potential purchasers, for the important part of the book, the description of ultrastructure, is an excellent advanced and up to date text.

GUY COX

INSIDE THE FLOWERING PLANT

The Physiology of Flowering Plants

Their Growth and Development. By H. E. Street and Helgi Öpik. Pp. viii+263. (Arnold: London, August 1970.) 70*s* boards; 35*s* paper.

THERE is currently almost an embarrassment of simple and attractive volumes available to introduce the absolute novice to the flowering plant and its problems. The advanced student and the specialist are similarly well catered for, but what Professor Street and Dr Öpik have done so well is to combine the best features of each of these extremes into an excellent intermediate text; a guide to plant physiology for those who already have some knowledge of plant anatomy, biochemistry and biophysics. Indeed such prior knowledge is essential, for unlike so many other plant physiology texts, the authors have confined themselves strictly to physiology. Little space is spared to introduce, for example, basic plant biochemistry, or to develop the concept of metabolic pathways.

The authors have obviously tried hard to make their book easy to read, and they have succeeded. It is rare to find an introductory textbook which is quite so enjoyable, either to study for information or for merely browsing through for interest. In addition to chapters on the more usual subjects such as germination, water relations, mineral nutrition and growth, due emphasis is given to such neglected topics as frost and drought hardiness and energy economy. Other chapters cover transport of metabolites, growth, cell differentiation, growth movements and morphogenesis. Each subject is developed in sufficient detail to satisfy the needs of the first or second year undergraduate, and a short list of the more important references and a guide to further reading are given at the end of each chapter.

The book has been nicely laid out and the figures are clear, but it is a great pity that the volume could not have been more attractively bound. If it sells as well as it deserves to, it will owe nothing of its success to the drab olive green colour of the cover (why must botanical texts be bound in green ?) or to the uninspiring design with which it is ornamented. ALAN CANE

REVIEW OF GENETICS

Advances in Human Genetics

No. 1. Edited by Harry Harris and Kurt Hirschhorn. Pp. xi+339. (Plenum: New York and London, 1970.) $19.50.

As the editors say in their preface to this volume, original papers dealing with different aspects of human genetics are appearing so frequently and in so many journals, and result from investigations originating in so many different disciplines, that there is a real need for critical review articles which summarize the positions reached in different areas. This first volume of a new series contains five such articles.

As might be expected with editors of this distinction and experience, the authors of the five chapters are well chosen and briefed, and each chapter is a mine of infor-

mation and references. The first, by J. H. Edwards, is on the analysis of pedigree data. It is less a review of work in this field than a formal analysis of the scope and limitations of the method. It is stimulating, and will repay reading by anybody concerned with pedigree data; but they should not expect a practical manual on the techniques available.

The second chapter, by Orlando J. Miller, on autoradiography in human cytogenetics, is a thorough and wide-ranging review, covering almost any conceivable aspect of the application of autoradiography to the study of chromosome identification, structure and replication. Cytogeneticists will find it invaluable.

The third chapter, on the genetics of immunoglobulins, by H. Hugh Fudenberg and Noel E. Warner, is likewise a thorough and highly technical review of the immunoglobulins, and of their chemical, structural and immunological features in relation to their genetics.

The fourth and fifth chapters overlap and complement each other. The fourth, by Charles R. Scriver and Peter Hechtman, on human genetics of membrane transport with emphasis on amino-acids, describes the mechanisms and specificity of such transport, together with a consideration of the genetic loci involved, and includes a section on treatment and counselling. The fifth, by Jean Frézal and Jean Rey, on the genetics of disorders of intestinal digestion and absorption, is a description of the clinical and chemical-pathological findings in genetically determined disorders of this sort; there is little formal genetics, but much of the ground has already been covered in the previous article.

The quality of production of this book justifies its price. There are few typographical errors considering the technical nature of the material. If subsequent volumes maintain the standard of this one the series will be a memorable one.

E. H. R. FORD

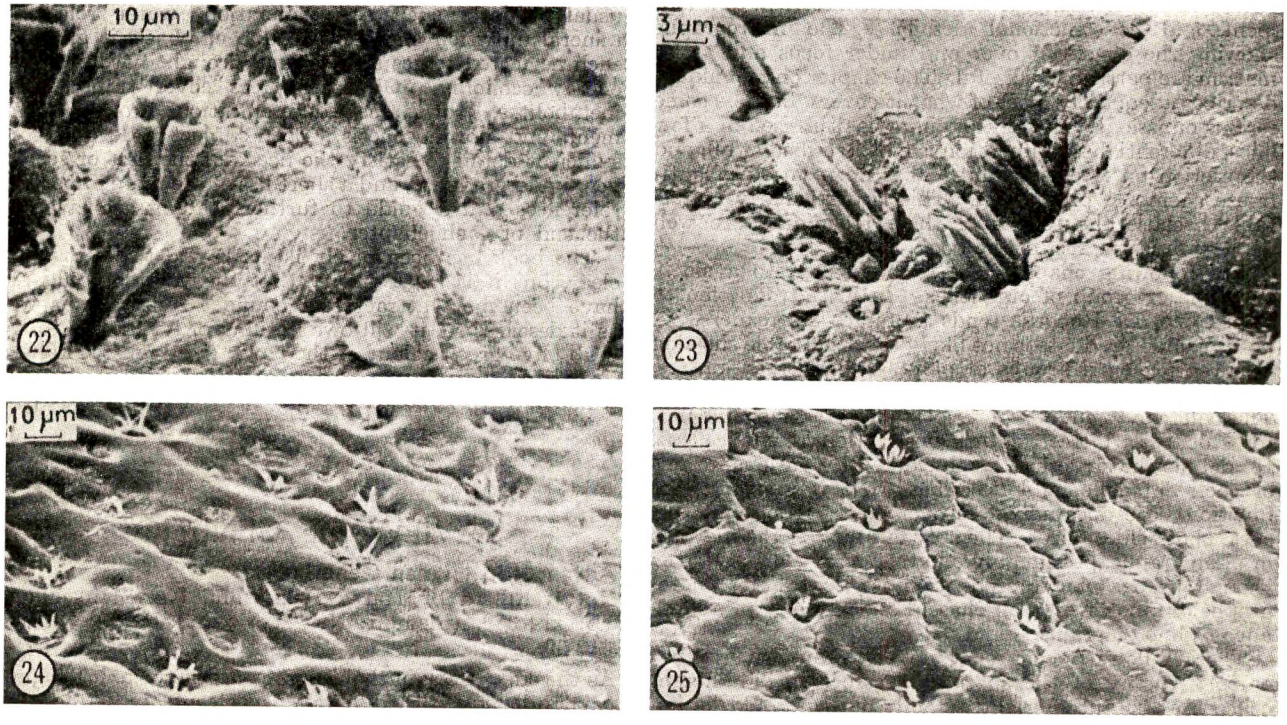
MEANINGFUL MESSAGES

The Molecular Basis of Mutation

By John W. Drake. Pp. 273. (Holden-Day: San Francisco, February 1970.) $15.00.

"ONE of the most potent mutagens described to date for microorganisms is Adelberg, Mandel and Chen, 1965." This excellent, unintentional example of a deletion mutation resulting in a different but still meaningful message is taken from p. 155 of this book and is rather better than the deliberate examples given on p. 83. Mutations are, in fact, largely rearrangements within an encoded message and consideration of their molecular basis is the author's aim. The time is ripe for such a book, and this one will go a long way to fill the gap which

INSECTS UNDER THE MICROSCOPE



There is strong evidence that the setal receptors found between the ommatidia in the compound eyes of many adult insects are extensions of the receptor system of the head on to the eyes. These scanning electron micrographs, taken from a paper "Some Little Known Surface Structures" by H. E. Hinton in *Insect Ultrastructure* (edited by A. C. Neville for the Royal Entomological Society of London, Blackwell (Scientific): Oxford and Edinburgh, 1970, 70s), show some of the setae found on the head and between the ommatidia of some species of aquatic bugs of the family Naucoridae. In this family different genera have quite different kinds of cephalic sensilla and in each genus the kind of sensillum on the head is also present between the ommatidia. In *Cryphocricos mexicanus*, the cephalic setae are trumpet shaped (Fig. 22) and those between the ommatidia are also trumpet shaped but are smaller. In *Heleocoris mexicanus*, the inter-ommatidial sensilla (Fig. 23) consist of tufts of closely appressed setae that are precisely like those of the head. In *Aphelocheirus aestivalis*, the cephalic setae (Fig. 24) are also tufts of setae but the setae in each tuft are widely divergent. The inter-ommatidial setae are almost similar in form (Fig. 25).

exists. It is aimed at graduate students and professional investigators, especially those entering the field of molecular biology from other disciplines. The first part is indeed a joy, with lucid expositions of basic facts and ideas of molecular biology which surpass those in more explicitly educational books. The professional investigator, however, demands more than this, particularly where current problems and research are concerned, and it is here that the book's limitations are seen. Dr Drake's reputation as a geneticist rests primarily on his work with bacteriophage T4. The fact that he has naturally chosen to concentrate on this organism is both the strength and weakness of his book. The importance of T4 is obvious in those sections dealing with the principles of molecular genetics. T4 is, however, an organism largely independent of the cellular functions of its host and the ways in which mutations arise in it are often different from those of bacteria and even most other bacteriophages, particularly with regard to the involvement of repair and recombination processes. The connexion between recombination genes and mutation in bacteria has been a profitable field for several years and the scant attention which it receives exemplifies the book's relative neglect of the involvement of cellular processes in mutagenesis.

There are several errors (for example, in the report of the work of Kada and Marcovich on p. 161) and omissions (for example, the discoverers of the potent mutagenic action of nitrosoguanidine, Mandell and Greenberg in 1961). Nevertheless, it is a book to be commended as a reasonably elementary guide to mutation, particularly for first degree students in their final year and predoctoral workers. An interest in mutation is not out of place in a world which man is making more mutagenic than at any time since the ozone layer began to shield it from the worst effects of the Sun's radiation.

BRYN BRIDGES

MACROMOLECULES AND GENES

The Molecular Basis of Gene Expression

By Benjamin M. Lewin. Pp. x+446. (Wiley (Interscience): London and New York, August 1970.) 130*s* boards; 70*s* paper.

WITHIN the past decade important advances in microbial genetics, protein biosynthesis and nucleic acid biochemistry have greatly enhanced our understanding of the functioning of the genetic material, particularly in relation to control mechanisms in prokaryotic cells. As those involved in teaching or research in this field will be well aware, the rate of progress has been so rapid that it is difficult to keep fully abreast of new discoveries and their significance. The task of integrating recent developments into a coherent body of knowledge is therefore both formidable and important.

This account by Dr Lewin of the interrelationships of macromolecules involved in gene function is a timely and welcome addition to the comparatively few books in this important field. The scope of the book has been deliberately restricted chiefly to bacterial systems, which is reasonable because relatively less is known about the expression of genetic information in higher organisms. It is a pity, however, that the discussion of the control of the synthesis of multi-chain proteins such as haemoglobin and immunoglobulin is not a little more detailed.

Many of the recent developments discussed in this book are based on experimental work which is often highly sophisticated both conceptually and technically. On the whole, the presentation of intrinsically complicated material is clear and intelligible, but a good background knowledge is an advantage if not essential at times. For this reason, the book is most suitable for postgraduate and advanced undergraduate students, and for research workers active in this area. A glossary of technical terms would be of help to the reader, and perhaps this can be added in a future edition. Another aspect which could with advantage be covered more fully is the molecular basis of mutations.

There are few major errors, but the description on p. 93 of the procedure used by Dintzis for investigating the sequential synthesis of haemoglobin peptides is not entirely accurate; ribosomes in eukaryotic cells are not always attached to endoplasmic reticulum as suggested on p. 96; and the mechanism of action of puromycin is rather different from that which appears to be implied on p. 135. Several minor mistakes, for example the numbering of the pyrimidine ring (p. 25 and Fig. 2. 1), the spelling of several authors' names (pp. 171–173), the concentration of magnesium at which ribosomes dissociate into subparticles, which is much lower than that given on p. 96, will, I hope, also be corrected on a future occasion.

On the whole, however, this is a well written and worthwhile book which will serve a useful purpose and it can be highly recommended.

H. R. V. ARNSTEIN

CONJUGATION MECHANISMS

Metabolic Conjugation and Metabolic Hydrolysis

Vol. 1. Edited by William H. Fishman. Pp. xv+461. (Academic: New York and London, August 1970.) 261*s*.

MANY organic compounds administered to animals are excreted in the urine or bile in conjugated form and it was long thought that this is largely a process of detoxication. There is now evidence, however, that hormones and other bodily constituents are likewise coupled with glucuronic acid, sulphate or other groups, so that the term "metabolic conjugation" has been introduced. The view has developed that a balance between conjugation in certain cells and cellular compartments and hydrolysis in others has important effects on the physiological and pharmacological activity of hormones and drugs. This is the first of three volumes devoted to an authoritative account of these reactions.

R. L. Smith and R. T. Williams describe the history of the discovery of conjugation mechanisms. Although this information has been available elsewhere, notably in Williams's book, *Detoxication Mechanisms* (Chapman and Hall), it is here conveniently summarized. D. S. Lane describes new metabolic conjugates of steroids, including coupling with N-acetylglucosamine and glucose, acetylation, phosphorylation, methylation and combination with glutathione. Variant forms of glucuronic and sulphuric acid conjugation also remind us that biological reactions are seldom simple. C. C. Irving discusses conjugation of N-hydroxy compounds which are of special interest in cancer induction. It is often thought that conjugated hormones are biologically inactive, but M. Mason summarizes work showing that conjugated steroids can bind with and affect the function of enzymes and other proteins. A comprehensive review of the glucuronic pathway of conjugation is given by T. A. Miettinen and E. Leskinen, and sulphoconjugation and sulphohydrolysis are described by K. S. Dodgson and F. A. Rose. The lysosomal localization of many intracellular enzymes that can hydrolyse mucopolysaccharides is reviewed by E. A. Davidson. Although much useful information has accumulated, there are unsolved problems such as the nature of the enzymes breaking down dermatan or keratan sulphates and the exact defects in storage diseases such as Hurler's and Hunter's syndromes. The final chapter by J. L. van

Lancker on hydrolases and cellular death emphasizes how little is known about cell damage and death: whether release of hydrolases is primary or secondary in many instances, and what part the hydrolases play in normal and increased protein turnover.

In general, this book provides a useful account of the current state of knowledge about metabolic conjugation, although much of the ground is already familiar. The three volumes taken together will doubtless provide a standard reference, though the chapters on topics in which ideas are most rapidly changing, for example the role of lysosomes in cell damage, are likely to become dated within a few years. A. C. ALLISON

STUDENT IMMUNOLOGY

Basic Immunology

By E. R. Gold and D. B. Peacock, with chapters by J. Verrier Jones, T. E. Blecher, M. O. Symes and W. J. Harrison. Pp. xii+416. (Wright: Bristol, June 1970.) 90*s*.

IMMUNOLOGY continues to recruit a steady stream of students at various levels and from various sources, many of whom, in spite of avowed special interests, sooner or later begin to feel the need for a wider grasp of immunological concepts. A textbook of "basic" immunology might be judged, then, at least in part, by how far it succeeds in meeting this need. The textbooks that have appeared so far (with one or two deservedly well known exceptions) tend to suffer from a plethora induced by the unselective inclusion of too many facts and observations, squeezing out the important consideration of how we are to make the best sense of them in relation to each other, and of how the many questions that follow may best be framed.

This book's success with this problem is less than complete—perhaps because coming from six different pens it forfeits a unified approach. From the students' standpoint, the best chapters are undoubtedly those on the production of antibody and on cell-mediated immunity and delayed hypersensitivity, which describe some of the most telling experimental and clinical observations, and by critical discussion and pertinent questioning build up a clear picture of present trends in thinking about these two basic immune activities. In another chapter the *in vitro* correlations of cell mediated immunity are carefully enumerated, but less critically evaluated, so that the "exciting vistas" this work foreshadows show up less clearly than they should.

The chapter on immunoglobulins gives a straightforward account of modern knowledge of their molecular structure and the significance of its variations, but it perhaps assumes too much knowledge or insight in the reader, who, to give one example, might well have some difficulty in grasping the meaning of the Valentine diagram for IgG, given only the background of the text. On the other hand, few students would require the detailed chemical and isomeric analysis of antigenic determinants provided here.

So far as immunopathology goes, there is a useful summary in the section on autoimmunity, which is largely clinical in context. It is in this section that one of the two paragraphs in the book on the biological significance of immune complexes occurs—a surprising lack, because this is a part of immunopathology about which we know something, and which seems now to play an even more important part in disease than was once thought. It is in this section, too, that the subject of immunological tolerance appropriately, though scantily, receives attention. Other aspects of immediate hypersensitivity are discussed elsewhere in the book, and there are chapters also on complement, transplantation immunity and tumour immunology. The book is perhaps likely to appeal most to the reader seeking a ready aid in refreshing rather than extending his basic knowledge of immunology. E. J. HOLBOROW

24 HOUR CLOCKS

Human Circadian Rhythms

By R. T. W. L. Conroy and J. N. Mills. Pp. ix + 236. (Churchill: London, 1970.) 60*s*.

THE more than exponential increase in scientific information causes such bewilderment that many reviews of a field are no more than catalogues of possibly related items. Papers can easily be referred to by the dozen and yet not studied critically for their content and limitations. *Human Circadian Rhythms* by Conroy and Mills, however, is refreshingly different. The authors have limited their aspirations to what they can reasonably achieve. Their style is direct and clear. It will be only apparent to those familiar with some of the field that the authors have carefully studied the tables and graphs of other authors. In doing so they have been able to assess the significance of the findings and they have wisely erred on the side of demanding real evidence for conclusions.

The book is largely a development of Mills's classical article in *Physiological Reviews* in 1966. It is at best in the chapter on the kidney. This chapter contains a lucid account of the real difficulties in establishing that, for example, the adrenal cortical rhythms control sodium and potassium excretion. It is a model for scientific writers.

The work is, in effect, in three parts. After an initial discussion on methods and a critical consideration of the inevitable limits of studies on humans there is an account of the twenty-four hour rhythms established in man and their persistence without time clues and on non-twenty-four hour schedules. This second part is the most detailed and is followed by a concluding and shorter attempt to assess the practical significance of the work which has been carried out.

The writers at every turn occupy themselves with the question of how far the rhythms of the variable discussed are endogenous. They consider the synchronization which occurs during development and the interesting desynchronization which can arise from shifts of phase and activity and lightning schedules. There is no evangelism in the whole work. One is left with the findings and the various claims of other writers plus apt comments on the possibility of alternative and more parsimonious views.

The ubiquitous nature of approximately twenty-four hour measuring systems in living organisms, the presumably fundamental nature of the clock-like processes at the molecular level, and the observations dealt with in this book show how far we have already entered a field which will illuminate much in biology. It already seems likely that related processes affect eclosion in *Drosophila*, the opening and closing of some flowers and the human renal excretion of potassium, and so on. This is relevant to night watchmen's efficiency, to diagnosing Cushing's syndrome, to designs for space flight, and the laying of eggs by chickens.

Conroy and Mills mention very briefly work on the possibility that processes in the production of RNA are the central timing device, and that the hypothalamus in man contains the master clock. The book is, however, chiefly concerned with what has been shown in studies on man himself; the wilder flights of speculation are left for the reader. These he can more aptly pursue after reading an excellent book. F. A. JENNER

(*Continued from 938*)

through crisis and cell lines are established, the population ceases to have a single chromosome number, and the median number of chromosomes/cell increases to slightly less than twice the diploid number, that is subtetraploidy. In addition to the loss of diploidy, cell populations cease to reject modified chromosomes on transition to cell lines, and chromosomes appear that are not found in preparations made from primary cells. Metacentric chromosomes, for example, are a common morphological indication of chromosomal abnormality in mouse cells, which normally have only acro and telocentrics.

An increase in chromosome number accompanies establishment and transformation of diploid primary cells because the subtetraploid mode is characteristic of most transformed cell lines[11,28–30]. For example, when normal cells go through crisis to become established lines, they do so with an increase in chromosome number. Also, although transformation of already subtetraploid lines is not accompanied by any change in chromosome number, chromosome number increases when subtetraploid transformed lines are recovered from primary diploid cells after viral infection. Thus, we would have predicted that revertants should have either fewer chromosomes or the same number of chromosomes as transformants. But neither result was found. We report now that a consistent, anomalous increase in chromosome number above subtetraploidy accompanied reversion of spontaneous and virus-transformed cell lines from the malignant transformed phenotype to the growth-controlled normal phenotype, and that subtetraploidy returned with back reversion.

The histogram (Fig. 1) shows the heteroploid spread of 100 selected cells from each cell line, while the median range data (Table 2) give the actual frequency of cells in the median range for each cell line.

Of the four established cell lines, 3T3 had growth control and the other three lines did not. The chromosomes of 3T3, 3T6, SV3T3 and Py3T3 were very similar. All showed a major subtetraploid peak with a minor hyperploid component (Fig. 1*a*, *b*, *d*, *g*).

SV3T3 had the most homogeneous chromosome distribution. 97 per cent of the nuclei scanned at low power fell in the range of the major peak (Table 2). 3T3, 3T6 and Py3T3 had about the same hyperploid component. The subtetraploid peak was still the major chromosome pattern, even in Py3T3, the line with the most hyperploid cells (Table 2). In both transformation of 3T3 and establishment of the low growth control line 3T6, sub-

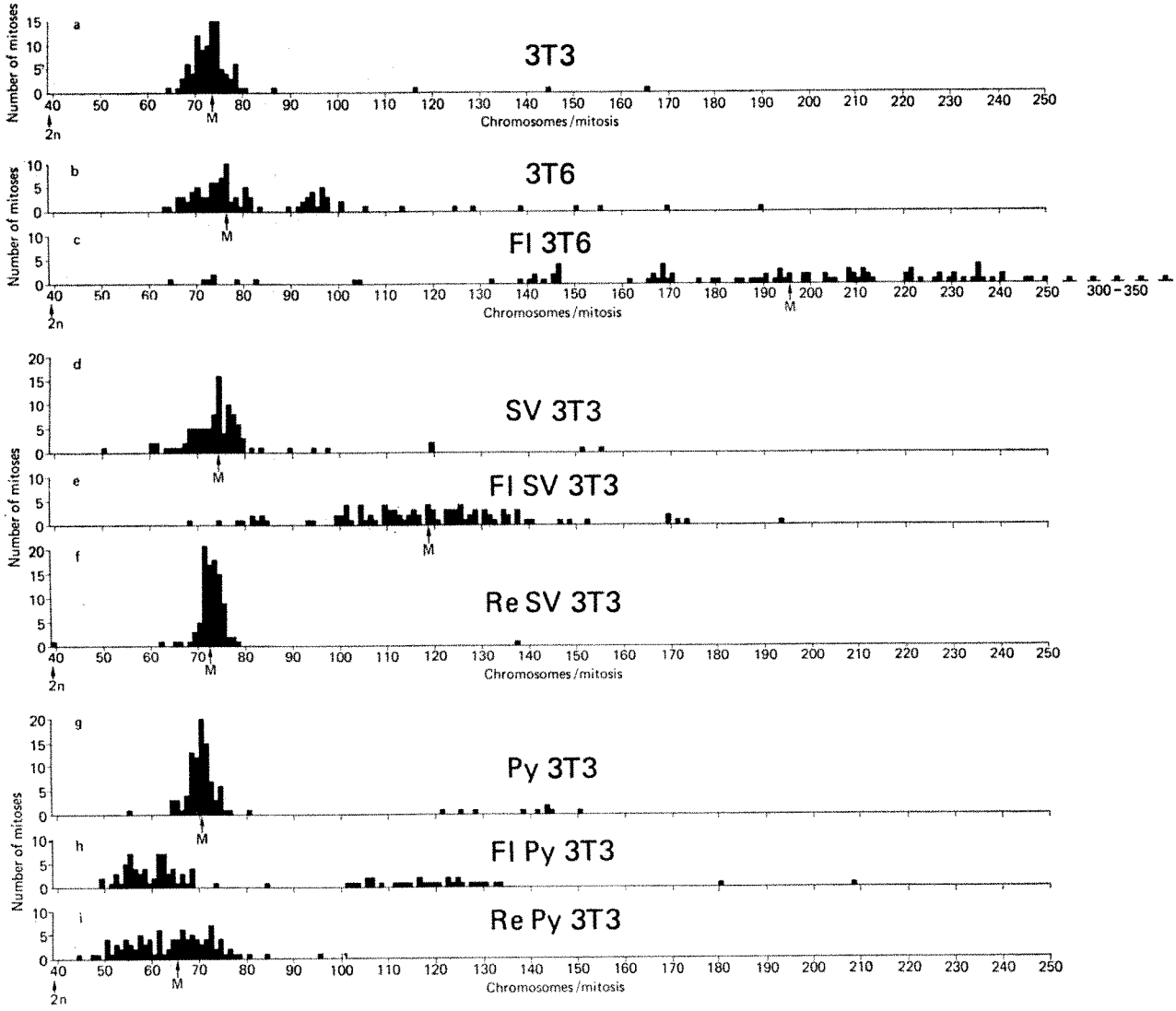


Fig. 1. Histograms of chromosomes/cell against number of cells. For each mouse cell line 100 metaphase preparations were counted at 1,000× magnification. The diploid number (2 n) of the mouse is 40. The median number of chromosomes for each line is indicated (M).

tetraploidy remained the dominant mode as the population lost growth control.

Polyoma and SV40 transformation of primary cells is accompanied by heteroploidy, with the appearance of many abnormal chromosomes[11,30]. 3T3 was, however, already heteroploid and about 11 per cent of 3T3 cells contained metacentrics (Table 2). There were 13 metacentrics per hundred cells for 3T3, 19 for 3T6, 32 for SV3T3, and 10 for Py3T3 (Table 2). Thus, no consistent increase in metacentrics accompanied transformation in these cell lines.

The median chromosome number of 3T6 went from 76 to 195 on selection of variant F13T6 cells. 10 per cent of the F13T6 cells had more than 240 (=12 n) chromosomes. One cell of the 100 counted exactly had 340 chromosomes (~17 n). The overlap of distributions between 3T6 and F13T6 was slight: 10 per cent of 3T6 cells had more than 100 chromosomes, and 8 per cent of F13T6 cells had fewer than 100 chromosomes (Fig. 1*b*, *c*). As expected, F13T6 cells are bigger than 3T6 cells.

The fraction of cells lacking metacentrics declined slightly, from 84 per cent to 63 per cent, but the majority of F13T6 cells still lacked metacentrics (Table 2), and the total number increased slightly, from 19 to 46/100 cells.

The subtetraploid 3T6 population, once committed to the state of low growth control as a cell line, reverted to a state of high growth control (F13T6) with massive additional hyperploidy. This was unexpected, because 3T3, a subtetraploid cell line with growth control, was obtained with no more hyperploidy than the dense cell line 3T6 (Fig. 1*a*, *b*) by selection from the diploid population before establishment.

Revertants selected from the two virus transformed 3T3 lines also had a marked increase in chromosome number and in addition an increased number of metacentrics per cell. The median chromosome number of SV3T3 increased from 75 to 118 with reversion. The F1SV3T3 population had a much broader histogram than SV3T3 (Fig. 1*d*, *e*), but overlap between F1SV3T3 and SV3T3 chromosome number was limited: 6 per cent of SV3T3 cells had more than 90 chromosomes, and 10 per cent of F1SV3T3 had fewer than 90 chromosomes. Five per cent of F1SV3T3 cells counted had more than 160 (=8 n) chromosomes. In contrast to 3T6 and F13T6, F1SV3T3 had many more metacentrics/100 cells than SV3T3 (196 vs 32, Table 2). Only 9 per cent of F1SV3T3 cells were free of metacentrics, as compared with 74 per cent of SV3T3 cells (Table 2).

F1Py3T3, selected from Py3T3, did not shift entirely to higher chromosome number. F1Py3T3 showed a bimodal distribution of counted chromosomes/cell (Fig. 1*g*, *h*). Low power scans showed that F1Py3T3 cells were evenly distributed between the two ranges (median =60,120). No overlap occurred between the two peaks (Fig. 1*g*, *h*). The higher peak is very near to the peak of F1SV3T3. The lower peak clearly is not identical to the major peak of Py3T3; it is about half the higher peak.

Metacentric abnormal chromosomes increased most strikingly in the selection of F1Py3T3 from Py3T3. The percentage of cells with metacentrics increased from 4 per cent to 91 per cent. The number of metacentrics/100 cells went up almost forty-fold, from 10 to 353 (Table 2).

The absolute number of metacentrics/100 cells increased in all three flat revertants. In the two virus-transformed revertants, the ratio of metacentrics to total chromosomes increased in addition (Table 2), whereas in F13T6 the increase in metacentrics just paralleled the increase in total chromosomes per 100 cells.

Both F1SV3T3 and F1Py3T3 grew as rapidly in sparse culture as their parent lines, and both flat lines contained viral T-antigen (Table 2). Thus, the increase in chromosome number accompanying flat reversion did not eliminate the entire viral genome, and did not produce slow-growing lines.

Table 1. ORIGIN AND PROPERTIES OF MOUSE CELL CULTURES

Cell	Growth control	Established line	Transforming virus: Name	Transforming virus: T-ag	Original selection: From	Original selection: By
MEF	+	No	None	None	—	—
3T3	+	Yes	None	None	MEF	—
3T3	−	Yes	None	None	MEF	—
F13T6	+	Yes	—	None	3T6	FUdR
SV3T3	−	Yes	SV40	SV40	3T3	Focus
F1SV3T3	+	Yes	—	SV40	SV3T3	FUdR
ReSV3T3	−	Yes	—	SV40	F1SV3T3	Focus
Py3T3	−	Yes	Polyoma	Polyoma	3T3	Focus
F1Py3T3	+	Yes	—	Polyoma	Py3T3	FUdR
RePy3T3	−	Yes	—	Not done	F1Py3T3	Focus

Some years ago[4] two permanent cell lines were established from mouse embryo fibroblasts. One line, 3T3, retained maximal growth control, while the other, 3T6, did not. 3T3 was transformed by SV40 and by polyoma in 1963 (ref. 31). Transformed sublines were isolated and cloned twice. These lines were stored in liquid nitrogen. 3T3, 3T6, SV3T3 and Py3T3 were thawed and cloned twice in 1967 (ref. 13). They have been kept in culture since. Phenotypic revertants, designed as flat cell lines (F1) because of their low saturation density and their large, flat morphology in culture, were isolated by FUdR negative selection from 3T6, giving F13T6, and from the two virus transformed 3T3 lines, giving F1Py3T3 and F1SV3T3 (ref. 13). Recent spontaneously arising sublines with low growth control were isolated as dense foci from monolayers of F1Py3T3 and F1SV3T3. These were cloned twice and are designated in these experiments as RePy3T3 and ReSV3T3. All cell lines were maintained at 36·5° C in 20 cm² plastic Petri dishes (Falcon) in Dulbecco and Vogt's modification of Eagle's medium supplemented with 10 per cent calf serum. The medium was changed twice daily. Growth control was assayed as saturation density. (+)=Saturation density less than 8×10^4 cell/cm² with 2 changes/week of DME+10 per cent CS. (−)=Saturation density more than 5×10^5 cell/cm² with 2 changes/week of DME+10 per cent CS.

Table 2. CHROMOSOME CONSTITUTION OF MOUSE CELL CULTURES

Cell	Chromosome number: Median/cell	Chromosome number: Median range, fraction	Metacentrics (abnormal): Number of cells with metacentric chromosomes: 0	1	2	3	⩾4	Counted metacentrics/100 cells
MEF	40 (=2 n)	1·0	100	0	0	0	0	0
3T3	73	0·85	89	9	2	0	0	13
3T6	76	0·72	84	13	3	0	0	19
F13T6	195	0·58	63	29	7	1	0	46
SV3T3	74	0·97	74	21	4	1	0	32
F1SV3T3	118	0·95	9	32	28	22	9	196
ReSV3T3	72	0·90	91	6	2	0	1	14
Py3T3	70	0·74	91	8	1	0	0	10
F1Py3T3	60,120	0·50, 0·50	4	18	16	17	45	353
RePy3T3	65	0·79	69	8	4	4	15	115

For preparation of chromosome spreads, a cell line was inoculated at about one-tenth of its saturation density, to give a dividing population. Medium was changed one day after transfer. On the second day, cells were exposed to 0·1 μg/ml. Velban (Vinca Leucoblastine, Lilly) for 4 h, suspended in 0·25 per cent trypsin for 10 min, centrifuged at 900 r.p.m. for 10 min, resuspended, swelled by exposure to 20 per cent foetal calf serum in distilled H_2O for 30 min, recentrifuged at 500 r.p.m. for 10 min, fixed for 30 min at 4° C in cold Carnoy's fixative, air-dried on slides, and stained with 1 per cent acetic acid–orcein. A histogram for each cell line was obtained from exactly 100 well-spread metaphases. In addition about 1,000 cells were examined under low power (100×) and classified according to whether or not their number was in the median class for that cell line. This was done to eliminate the bias against counting cells with higher chromosome numbers, where inadequate spreading and multiple overlaps made accurate counts difficult. The metaphase preparations that were counted exactly for total chromosome number were also counted for metacentrics. Because few unstable configurations of the sort that might interfere with mitosis (dicentrics, rings) were present, it is safe to assume that most of the cells examined were capable of further division and representative of the population.

Back Revertants of T-antigen Positive Flat Lines

A few dense foci inevitably arose in cultures of F1Py3T3 and F1SV3T3 kept at saturation for more than a week. These foci were cloned to provide stable populations with saturation densities of more than 5×10^5 cells/cm². The back revertant lines derived from the flat revertants were called ReSV3T3 and RePy3T3. The back revertant of F1SV3T3, like F1SV3T3, remained positive for SV40 T-antigen. Neither back revertant line was selected by re-infection with a transforming virus; both arose spontaneously.

Chromosomes in RePy3T3 and ReSV3T3 returned in both cases to distributions close to those of the respective dense transformed parent lines SV3T3 and Py3T3 (Fig. 1*f*, *i*). The SV40 transformed series shows this most clearly (Fig. 1*d*, *e*, *f*). Only about 2 per cent of the F1SV3T3 cells are in the median range of SV3T3. Presumably a cell of this minority was the ancestor of the ReSV3T3 population.

In the polyoma transformed series, RePy3T3 lost the hyperploid peak but did not return to the distribution of Py3T3. Apparently, RePy3T3 arose from one of the low mode cells of F1Py3T3 (Fig. 1*g*, *h*, *i*).

The back revertant populations lost metacentrics also, to return to the range of their transformed parents. A

few hyperploid cells contained most of the metacentrics in RePy3T3 (Table 2).

These data show that increase in chromosome number accompanies reversion, and decrease in chromosome number accompanies back reversion. The cell lines studied here were selected as discrete variants in at most two selective steps. Yet in each case the revertant population had a hyperploid chromosome distribution that overlapped only slightly with the parental distribution. Selection in each case was exerted on growth control, yet in each case the chromosome constitution was markedly changed. We conclude, therefore, that increase in chromosome number is a necessary part of the mechanism for maintenance of the reverted state of these cell lines.

Is hyperploidy sufficient, as well as necessary, to maintain the revertant state in flat cells? Hyperploid cells are larger than their transformed parents; they therefore have a lower surface/volume ratio. This renders them less susceptible to the stressful loss of synthesized metabolites to the medium[1,28,32,33,35]. This may be the factor behind the induction of hyperploid revertants by culture at low cell density. Such revertants are very unstable, as they grow to form visible colonies[34]. Many back revertants arise after only 10 cell divisions, an instability much greater than the frequency of re-reversion of the flat revertants selected for by passage in FUdR.

An origin consistent with the transient nature of those revertants would be that the cells first became hyperploid in response to the stressful conditions of high cell dilution, and that polyploidy resulted in reversion. Once the cell number had increased enough to permit the cells to condition the medium, hyperploidy would no longer be selective, and any cell with the standard subtetraploid complement of a back revertant would be able rapidly to overgrow the culture.

If one can select for reversion by requiring polyploidy or select for polyploidy by requiring reversion, then any mechanism for maintenance of the reverted state should require hyperploidy as a necessary step. At present the mechanism of maintaining reversion is largely unknown, as is the mechanism for maintaining transformation. Recent findings seem to indicate that the maintenance of the transformed state in viral and spontaneous transformation is mediated through alterations in the glycoproteins of the cell surface. Correspondingly, flat cells complete the normal surface, and are unagglutinable[23,24]. These alterations, assayed with plant agglutinins, are detected as an increase in agglutinability of cells accompanying a loss of growth control[36–41]. Increases in agglutinability occur as well in cells lytically infected with SV40 as polyoma[19,42].

Two mutants of polyoma virus have recently been described of genes which affect the maintenance of the transformed state through interference with cell surface. One is a host range mutant that cannot grow on 3T3, but that can grow on polyoma-transformed 3T3[20]. This mutant cannot transform either rat or hamster cells, and is unable to induce an increase of agglutinability in lytic infection of mouse cells[19]. The other transformation mutant is temperature-sensitive. When BHK hamster cells are transformed with this mutant and kept at low temperature, they resemble other transformants and are agglutinable. When transformed cells are shifted from low to high temperature, they regain orientation and lose agglutinability[21].

The agglutinins apparently detect a cell surface structure responsible for maintenance of growth control, and a polyoma gene can affect this structure. The surface structure detected with agglutinins on transformed cells, however, cannot itself be a viral gene product, because (*a*) spontaneous, chemical, and X-ray induced transformants from 3T3 and BHK, as well as tumour cells of spontaneous origin, agglutinate as well as viral transformants[36,37,39]; (*b*) brief treatment with very low concentrations of proteolytic enzyme can bring about a change in the cell surface similar to that occurring in the transformation of normal to malignant cells[38]; (*c*) the SV40-induced surface antigen (S-antigen) is apparently a component of the normal cells, since cell lines transformed spontaneously or by polyoma virus and non-transformed lines can be made to display SV40 S-antigen by mild proteolytic digestion of their cell surfaces[42]; and (*d*) a transient loss of growth control resembling transformation can be induced directly in a 3T3 population by the addition of small amounts of trypsin to the confluent culture[43].

Because our flat revertants are unagglutinable, hyperploidy must in some way act to permit the revertant cell to mask agglutinin receptor sites, thereby reconstructing a normal cell surface. Increase in cell size alone thus is not the cause of reversion.

What role does the increase in metacentrics have in reversion from viral transformation that is not required for reversion from non-viral transformation? A virus-transformed cell may revert with chromosome loss, eliminating all viral genes[15,22], or with chromosome gain, despite the continued presence of the viral genome. Translocation, with chromosome gain, may be a way for virus-transformed cells to revert by inactivating[1,15,21,28,44] viral gene products responsible for maintenance of transformation, rather than by losing[15,21] the entire viral genome. Whatever the mechanism, inactivation of some but not all viral gene products must occur in revertants, since they contain T-antigen, but are growth controlled. Only the gene products necessary for the cell surface alteration and the maintenance of the transformed state would need to be inactivated, so the virus-specific T-antigen should have no part in maintenance of the transformed state (Table 1).

Does the relation of hyperploidy to reversion predict the behaviour of hybrids between malignant and non-malignant cell lines? All viable mouse × mouse hybrids are, in a chromosomal sense, revertants: both types of cell lines contain more mouse chromosomes than any typical established mouse cell line. Recent reports show that hyperploidy of mouse chromosomes accompanies growth control in hybrids. When mouse × mouse hybrid lines were prepared[47] between 3T3 ($M = 70$) and a spontaneous mouse tumour line with little growth control ($M = 53$), the hybrids had between 100 and 120 chromosomes and were as growth controlled as 3T3. With time in culture, chromosomes were lost and growth control of the hybrid population dropped, variants with higher saturation density appearing.

Two hybrid lines[47] were isolated from mixtures of SV40-transformed human cells ($M = 71$), and 3T3 mouse cells ($M = 70$) contained the SV40 T-antigen (and presumably the SV40 genome), but both hybrids were as growth controlled as the 3T3 parent. Both hybrids had 15 human and 135 mouse chromosomes. This increase in mouse chromosomes (70 to 135/cell) is very similar to the increase seen in the F1SV3T3, which are also growth controlled despite the presence of the SV40-T-antigen. If the generalization is accepted that malignancy *in vivo* and growth control *in vitro* are causally related[1,3,8–10,12], then an *in vivo* study is consistent with the hypothesis that hyperploidy is related to reversion. Malignancy was suppressed when malignant and non-malignant cells were fused[48] to give rise to viable hybrids with between 85 and 128 mouse chromosomes/cell. These hybrid lines gave rise to spontaneous malignant re-revertants with fewer chromosomes.

Is Reversion of Clinical Interest?

The most significant test of this hypothesis would be by inversion, determining whether agents causing hyperploidy (for example, low concentrations of velban or DNA base analogues) revert transformed and malignant populations. Recent observations on the suppression of

malignancy in melanotic melanoma cells with subtoxic levels of BUdR may be relevant here[49]. BUdR damages chromosomes[50] and induces hyperploidy[52]. BUdR-treated melanoma cells revert, lose pigment and become growth controlled. Revertant cell nuclei are at least twice the diameter of nuclei of the melanoma cells (ref. 49, Fig. 5). It would be interesting to see whether these "suppressed" cells are also hyperploid.

Selective killing of cancer cells by agents that interfere with cell division has been the rationale of most chemotherapy of tumours. X-irradiation, imuran, cytoxan, vincaleukoblastine, amethopterin, dactinomycin and nitrogen mustard are all effective against leukaemias and solid tumours because they are preferentially toxic to dividing cells[52]. Chemotherapy is, however, rarely a complete success. Remission is often followed by relapse, with the reappearance of tumour cells, especially in the leukaemias. Non-proliferative leukaemia cells have been implicated as the source of regrowth in relapse[53,54].

If it is true *in vivo* as well as *in vitro* that hyperploid variants can be more growth controlled than their parents then these drugs and others used for cancer chemotherapy must be assayed for the possible induction of hyperploidy in the tumour cells that they do not actually kill. Should revertant hyperploid cells be induced, they would then (as non-dividing cells) be resistant to further doses of the drug, and re-revertants would be able to grow out from them once the drug was removed.

BUdR[49], FUdR[13], dactinomycin[55], X-rays[56], and colcimide[1] have already been shown to induce polyploidy in cells surviving low doses. Therefore these agents must be suspected of inducing revertant hyperploid cells *in vivo*. Such revertants would be non-proliferative cells from which relapsing tumours might grow out, by back reversion.

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Field Control of Plant Growth and Development through the Laser Activation of Phytochrome

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Helium–neon lasers emitting at 632·8 nm can influence the phytochrome controlled growth and development of plants more than a quarter of a mile away. It may therefore be possible to control the growth of crops using lasers to activate the phytochrome system.

THE action spectrum and photoreversible nature of the phytochrome system were first described by Borthwick *et al.*[1,2]. It is now known that this photoreceptor occurs throughout the plant kingdom, and that its activation, which can be brought about by low energies and/or brief periods of illumination, affects several aspects of plant growth. Although this means that control of some phases of development is theoretically possible, little attempt has been made to explore the practical implications of this discovery in the field.

In 1966 we reported an experiment in which floodlamps were used nightly to interrupt the dark period of a field-

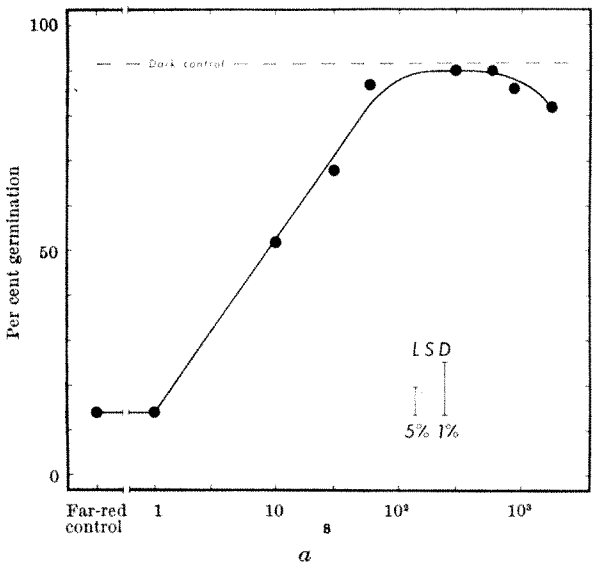
grown crop of barley[3]. The results of that work, which demonstrated that the period to field maturity could be significantly shortened (in one variety by up to three to four weeks), prompted a search for new light sources which might be capable of activating the phytochrome response system.

This communication deals with an examination of the effects of a helium–neon gas laser (emitting continuous light at a wavelength of 632·8 nm) on the induction of photomorphogenesis in several different plant response systems. The ability of the laser to activate phytochrome responses was first explored in laboratory experiments and then examined in field experiments at distances of about one-quarter mile.

Laboratory Experiments

The initial aim of this work was to demonstrate that a helium–neon laser could activate phytochrome initiated responses. The beam from a Spectra-Physics model 115 laser (rated single mode output of 3 mW), the first laser used in these experiments, was passed through a Wratten No. 22 gelatine filter to eliminate emission in the blue region of the spectrum. It was then diverged with a 6·3 cm focal length lens 25 cm from the laser port, and deflected 90° by a front surfaced mirror 40 cm from the lens. All the plant tissue was thus irradiated from above (about 18 to 26 cm below the mirror).

The intensity at the surface of the tissue was measured with a Spectra-Physics power meter and varied from 0·1–0·15 mW/cm². The diameter of the effective area was about 2·5 cm and all manipulations were carried out under green safe-light provided by an Ilford bright green filter No. 909.



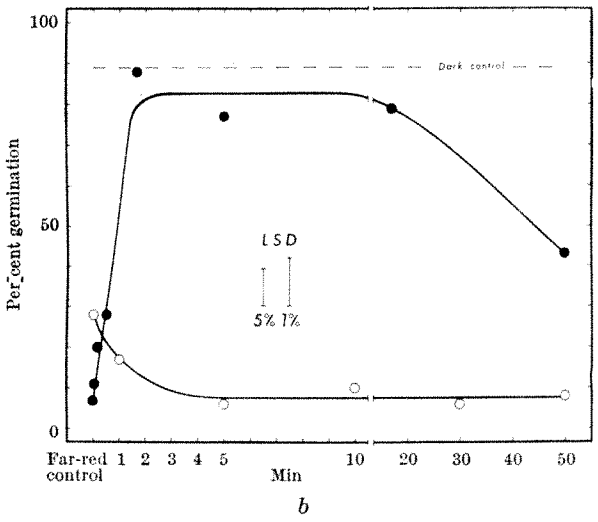


Fig. 1. Laser-induced promotion of lettuce seed germination. *a*, 1967 Great Lakes lettuce seed, fifty seeds per 5 cm Petri dish on two Whatman No. 1 filter papers and 1 ml. H_2O, exposed to far-red light (incandescent filtered through Westlake FRF-700 filter) for 36 h at 25° C. Seed then irradiated with laser for varying periods; germination measured 24 h after start of irradiation. *b*, Same seed irradiated in the same way the following year. Open circles: seed irradiated for 30 s with laser and then for indicated periods of time with far-red light to reverse effects of laser stimulation. Three replicates of fifty seed each were used for each treatment in both experiments.

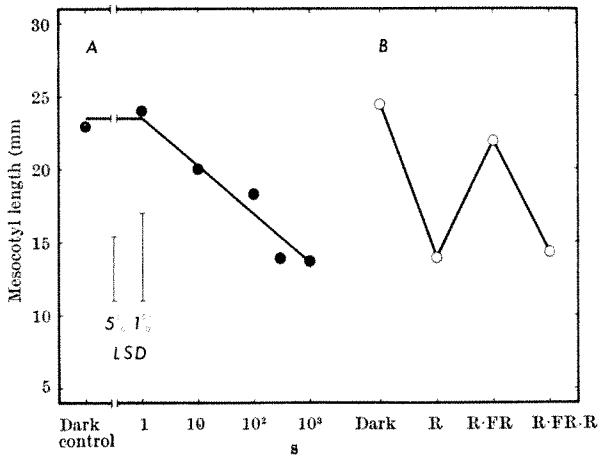


Fig. 2. Inhibition of oat mesocotyl elongation by laser irradiation. *A*, Effect of increasing exposure to laser irradiation on mesocotyl elongation; *B*, reversibility of laser-induced inhibition by successive irradiations of 5 min laser and 5 min far-red. *Avena sativa* cv. Swan dehusked seed germinated in dark at 25° C for 41 h in 9 cm Petri dishes containing two Whatman No. 1 filter papers and 4 ml. H_2O. Uniformly germinated seeds were transferred to waxed paper cups containing a central plastic 2 cm diameter, 3 cm high platform covered with 1–11 cm Whatman No. 1 filter paper moistened with 7 ml. H_2O. Ten seeds were placed on each platform, embryos facing outwards, with primary roots in contact with the filter paper. All embryos were exposed simultaneously to the laser irradiation which started at 43 h and ended at 46 h after the start of inhibition. The cups were covered with 7 cm Petri dish lids and the seedlings subsequently grew in darkness at 25° C for 4 days. Each treatment consisted of three replicates of ten seeds.

One of the most commonly examined phytochrome controlled responses is the red light activation of lettuce seed germination. In this test, the response of lettuce seed from a single harvest was examined in successive years (Fig. 1). In the year of harvest (Fig. 1*a*), 10 s irradiation with the laser was sufficient to cause a highly significant stimulation of germination, though 1 s had no effect at all. The response was maximal after 1 min of exposure and it seemed that 30 min of irradiation resulted in a decreased germination percentage.

When the experiment was repeated the following year, the responsiveness of the seeds to short irradiations had decreased although the overall nature of the response was the same (Fig. 1*b*). In this case, though the effect of 1 and 3 s of exposure was significant, it was appreciably less than in the previous year. The response reached a maximum at about 100 s and after 1,000 s there was significant inhibition (Fig. 1*b*). Moreover, the stimulation produced by 30 s irradiation with the laser was significantly reversed by exposure to far-red light for 1 min or longer.

A different kind of phytochrome controlled response is stem elongation, which involves changing the rates of tissue growth. The ability of the helium–neon laser to prevent mesocotyl elongation in rapidly growing seedlings is illustrated in Fig. 2. Irradiation for 1 s did not inhibit mesocotyl elongation, though increasing lengths of exposure produced an essentially linear response. Further, a demonstration of the reversibility of the response is included (Fig. 2*B*) which indicates that the response can

be repeatedly reversed by alternating exposures to the laser and far-red light.

The last physiological response tested in the laboratory differed from the dormancy breaking and growth altering effects examined above. This test involved the determination of the capacity of the laser to alter morphological development by inhibiting the long night induction of flowering of Japanese morning glories (*Pharbitis nil Chois.* var. Violet).

At the time of irradiation with the laser, each cotyledon was about 3 cm long from base to lobe tip. Because the beam was not large enough simultaneously to encompass both cotyledons, they were fastened together and the base of each was exposed. During the 1,000 s irradiation, however, the lobes were also occasionally exposed to the beam.

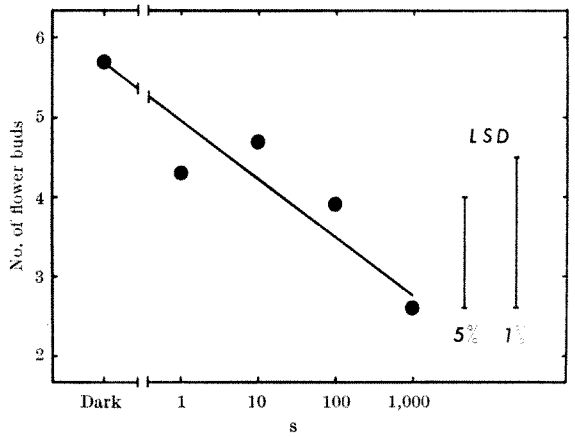


Fig. 3. Laser-induced inhibition of flowering of *Pharbitis nil. Chois.* Seed acid treated and washed[4] and planted in waxed cups with 'Perlite'. Seedlings grown at 27° C under 24 h fluorescent illumination and watered daily with half strength Hoagland's solution. After 7 days seedlings transferred to dark and irradiation started in the middle of the long (16 h) imposed night. (Treatments finished 3 h later, so were close to, but not quite in, the middle of the dark period.) At the end of the dark period the seedlings were transplanted into compost-filled clay pots, watered daily and grown at 27° C in 16 h fluorescent light (1,700 foot candles) for 20 days. Each treatment contained seven replicates and axillary and terminal buds were examined with a magnifying lens.

Fig. 3 shows the linear decrease in the number of flower buds formed as a result of increasing exposure to the laser. This response seemed to be more sensitive than the previous ones tested although the results were rather variable. But four of the seven plants which received the long night treatment formed terminal flower buds whereas none of those which were irradiated for 1 s or longer did so. Although *Pharbitis* seemed to respond to 1 s of irradiation, the maximum effect was not achieved by 1,000 s of exposure.

Field Experiments

The results of the laboratory experiments indicated clearly that a helium–neon laser could activate the phytochrome system, and encouraged us to attempt to reproduce the effects at greater distances. In these experiments a different laser was supplied by the Laser Development Group of the Weapons Research Establishment, Salisbury, South Australia. It was not a commercial unit and delivered a total multimodal output of 8 mW. The laser was placed at a window on the third floor of the Waite Institute and, because the ground slopes away from the building, the height of the laser and its angle of inclination both altered between experiments. Because the blue emission which accompanies the red is not projected in a parallel beam, we did not filter it out in the field experiments.

Pharbitis was chosen for the experiments on inhibition of flowering, because this response is more sensitive in *Pharbitis* than in the other species investigated. In the first experiment the *Pharbitis* seedlings were placed at a distance of 170 feet and the angle of laser inclination was 15°. The spot size was 15 cm and intensity (as measured with the Spectra-Physics power meter) averaged about 0·1 mW/cm². Only one treatment was imposed and the results (Table 1) indicated that exposure of the seedlings for 1,000 s was sufficient to activate the phytochrome and inhibit flowering.

Table 1. EFFECT OF NIGHT BREAK ON FLOWERING OF *Pharbitis*

Treatment	No. of flowers induced on 28 *Pharbitis* plants
Control—16 h night	38
1,000 s night interruption	2

Pharbitis seed acid treated and washed[4] and planted in moistened vermiculite for 48 h. Seedlings transplanted to 2·5×5·0 cm vials containing half strength Hoagland's solution for 2 days and grown under 24 h fluorescent irradiation (1,700 foot candles) at 27° C. Seedlings supported by 2·5 cm square of plastic foam in vial mouth. Vials placed into circular wooden racks placed, in turn, into enamelled pots which were covered at the start of the long inductive night. The pots were taken to the irradiation site and each rack was exposed as a unit to the laser beam. At the end of the dark period, the seedlings were transplanted to 13 cm compost-filled pots (two per pot) and grown for two weeks at 27° C in a 16 h, 1,700 foot candles fluorescent day.

The experiment was repeated at a distance of about a quarter mile (1,230 feet), and with a laser angle of inclination of 2·5°. To achieve this distance a collimating lens was attached to the laser, producing a spot size of about 15 cm; maximum intensity was 0·15 mW/cm². With a more extensive range of treatments (each of two replicates of ten plants), results (Fig. 4) very similar to those obtained in the laboratory experiment with *Pharbitis* (Fig. 3) were obtained. A linear relationship between length of exposure and inhibition of flower bud formation was found, so distance is not a factor in the effectiveness of laser illumination provided that the beam does not diverge too much.

The last experiment, with barley, was not really a field experiment but is included in this section for convenience. We used the same laser as was used in the field experiments, although it was placed only 60 feet from the plants. The laser beam was focused through a 10 cm focal length lens at 40 cm from the plants, and spot size at the plant position was 12 cm. Because the laser and the plants were at the same level, there was zero angle of inclination of the incident irradiation, and intensity was 0·20 mW/cm².

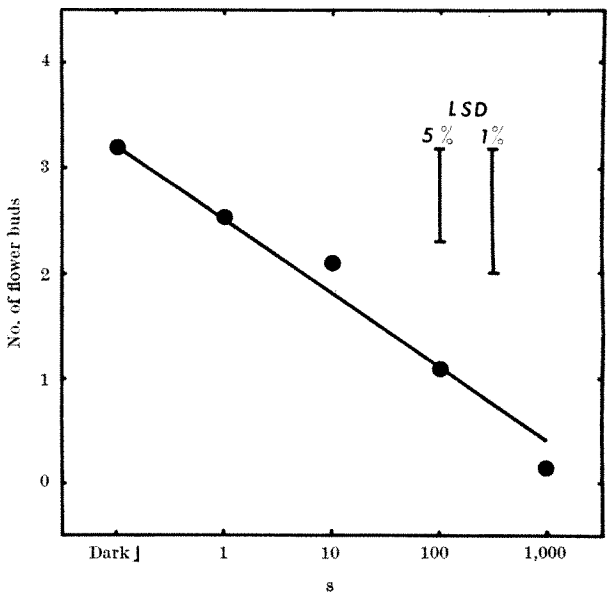


Fig. 4. Inhibition of flowering of *Pharbitis nil. Chois.* induced by laser illumination at a distance of 1,230 feet. Seed acid treated and washed[4] and planted in vermiculite for 48 h. Seedlings transplanted and grown in 2·5×5 cm vials containing small plastic foam supports and half strength Hoagland's solution for 2 days under continuous fluorescent light at 27° C. Ten vials, in circular wooden racks inside covered enamelled pots, taken to irradiation site and exposed as a replicate to the laser beam. At the end of the dark period the seedlings were transplanted into compost-containing pots and grown for 2 weeks at 27° C in a 16 h fluorescent day (1,700 foot candles).

Table 2. EFFECT OF NIGHT BREAK ON BARLEY DEVELOPMENT

	Mean stage of development		
	One exposure	Two exposures	Three exposures
Control	2·2	2·6	2·6
10 s	3·3	2·6	3·1
100 s	2·7	2·9	3·0
1,000 s	2·8	3·1	4·0

Least significant difference ($P = 0.05$) = 0·7.

Barley seed was germinated in 9 cm Petri dishes with two Whatman No. 1 filter papers and 4 ml. H_2O at 20° for 24 h. Uniform seedlings were transplanted (five per pot) to Perlite-filled pots and watered daily with half strength Hoagland's solution. Plants were grown at 23° C in a 12 h fluorescent-incandescent daylength (1,700 foot candles) for 21 days. At the time of irradiation the pots were removed from the constant environment cabinet, irradiated, and then replaced in the same environment. Identical procedures were followed on successive nights for treatments requiring two and three exposures. At harvest, 8 days after the initial irradiation, the plants were dissected and the stage of development[5] of the apex was recorded. Each value is the mean of ten replicates.

Barley is not notably responsive to a few cycles of night interruption. But because floodlights had affected the growth and development of several varieties[3], it was of interest to compare the effects of the laser with the earlier results. *Hordeum distichum* cv. C15611 was chosen because it was more sensitive than the varieties tested before. The results of this experiment (Table 2) demonstrate that, in spite of the fact that the laser output energy level was just over the threshold for Wintex barley[6], significant stimulation of apical development was produced in just eight days. The fact that significant effects were produced by three night interruptions illustrates that in some cases successive nightly irradiations at low intensity levels have significantly greater effect than a single exposure of greater length or higher intensity.

Energy Relationships in the Laser Activation of Phytochrome

The effective use of laser energy in the control of crop plant growth and development will depend on several aspects of both the response and the laser. Energy requirements for phytochrome activation of different crops differ markedly. The results of the present work substantiate previous results which indicate not only that there is a large energy difference between the lower threshold response level and the response saturation level, but that a particular process, as in barley, can be influenced both by the intensity and by the number of exposures. The absolute energy required to produce a response with the laser is very close to that required with other light sources. In 1952, Borthwick *et al.*[1] determined the incident energy requirement for the promotion of lettuce seed germination (Grand Rapids). A two-prism spectrograph with 35 Å/cm dispersion at 600·0 nm and a slit width of about 9·0 nm, $2{\cdot}11 \times 10^{-3}$ J/cm² of incident energy between 653·0 and 663·5 nm produced 50 per cent germination. An estimate of the energy required to reach this level of germination at a wavelength of about 632·8 nm was made from their graph and is $4{\cdot}5 \times 10^{-3}$ J/cm². This agrees well with our results. Assuming an incident energy from the laser at the seed surface of 0·25 mW/cm², Fig. 1*a* indicates that the incident energy requirement for 50 per cent germination (achieved with a 10 s irradiation) was $2{\cdot}5 \times 10^{-3}$ J/cm². Because of the ageing of the seed and their lowered sensitivity, the energy required for 50 per cent germination in the experiment illustrated by Fig. 1*b* was greater by a factor of five, that is, $12{\cdot}5 \times 10^{-3}$ J/cm². Similar results were obtained from a comparison of energy requirements reported for barley[6] and those found in this work.

Specific Advantages of Lasers

There are several possible reasons for using lasers in agricultural or plant physiological investigations: (1) the spectral purity of the emitted light may be important in studies dealing with specific photoreceptors[7]; (2) the intensities possible at specific wavelengths are frequently greater than can be obtained with other sources of monochromatic light; (3) the small cross-section of the beam may be valuable for producing highly localized intracellular injuries, or (4) for intracellular localization of specific photoreceptors.

In this work, the underlying aim was to determine whether a laser emission of 632·8 nm could activate the phytochrome system, and, if so, whether it was possible to produce phytochrome responses at large distances. So here, the important characteristic of laser light is that it is not subject to the inverse square law (that is, intensity does not decrease inversely as the square of the distance). (Laser beam intensity diminishes because of beam divergence, or absorption by or reflexion from intervening matter.) Also, though other sources of light may be more efficient than at least some lasers in the conversion of electrical into light energy, the proportion of energy produced at phytochrome-activating wavelengths is extremely small. With helium–neon lasers, all the emitted light is of the desired wavelength.

Laser Control of Crop Growth and Development

The experiments reported here demonstrate that helium–neon laser light can activate the red photoreceptor of the phytochrome system in plants, and that effects can be seen at distances of one-quarter mile and perhaps more. In practical terms, this means that the photoperiodic responses of crops are potentially controllable with lasers.

The clearest and perhaps only present example of photoperiodic control of field crop growth is with the chrysanthemum[8]. In the western United States chrysanthemums are grown outdoors under incandescent lights to interrupt the night period and control the reproductive behaviour of the plants. Here, as with our Japanese morning glories, flowering is inhibited by the activation of the phytochrome system during the night. The results obtained with barley in this work and earlier[3], and with other crops such as pineapple, however, illustrate that a phytochrome-activating night break can also be effective in stimulating the initiation and development of reproductive growth. Photoperiodic experiments with other types of plant have indicated that vegetative growth, as well as reproductive growth and development, can be influenced[9].

The lasers used in this work were not particularly powerful in comparison with those already available commercially. Reciprocity experiments[10] have indicated that time of exposure can be compensated for by intensity so that, for example, the minimum effective exposure for Japanese morning glories will be reduced to 0·1 s with a laser that has a power output ten times greater than the one used. Unfortunately, however, little information is yet available on the energy requirements of the phytochrome system of most crop plants, or of the sorts of useful responses that can be induced. As these kinds of data accumulate and as laser technology develops, the potential for laser-mediated control of the growth and development of crops may be exploited.

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Magnetism and Opaque Mineralogy of Basalts from the Mid-Atlantic Ridge at 45° N

by

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The volcanic layer may be only 200 m thick at 45° N and oxidation of titanomagnetite may be responsible for the decrease in amplitude of the magnetic anomalies away from the ridge axis.

THE parameters measured in palaeomagnetic work are total intensity, declination and inclination. These define the natural remanent magnetization (NRM) of rock samples. In this article we summarize our observations of intensity and inclination (but not declination) of basalts collected from the Mid-Atlantic Ridge at 45° N and describe their opaque minerals. Detailed accounts will be given elsewhere and some have already appeared[1-3].

At 45° N the Mid-Atlantic Ridge[4] has a median valley about 9 km in width whose floor is at an average depth of 2,900 m. Between 45° N and 46° N the valley lies in a direction 19° E of N. The valley has deep depressions and ridges elongated in the direction of the valley. It is possible to draw a line defining the topographic axial depression (called the axis) of the ridge to an accuracy of 1 or 2 km. The crest mountains on either side of the median valley have an average width of 50 km, and the peaks are commonly 1,800 m above the median valley. The high fractured plateaux form the flanks of the ridge. Photographs show the exposed hard rocks to be mostly pillow basalts; samples from the median valley are very fresh, both to the eye and geochemically. Many samples from the crest mountains and high fractured plateaux are entirely weathered and have not been studied. In other samples, visible weathering is confined to the surface, and fresh rock can be cut from their centres. In this way, material, which to the naked eye appears fresh, has been obtained at distances up to 150 km from the axis.

Remanent Intensity

The mean intensity of NRM based on thirty-eight samples is 0·0092 egs units cm^{-3}. The mean susceptibility is $8{\cdot}8 \times 10^{-5}$ so that the magnetization induced by the present field is negligible compared with the NRM. Twelve samples from the high fractured plateau have a mean NRM intensity of 0·0041, but within the median valley the intensities are much higher (mean of nine samples is 0·0574). In samples from the axis itself, the intensity reaches 0·1 (Fig. 1).

The magnetic properties of samples show several striking features. Blocking temperatures (the temperature at which remanence components are destroyed by heating in the laboratory) of less than 300° C are characteristic of most samples from the median valley[1]. The mean blocking temperature of samples from the crest mountains and high fractured plateau is 450° C (see ref. 1). Curie temperatures also increase with distance from the axis[2,3]. Various experiments show that the iron minerals in these basalts are very readily oxidized by heating, resulting in an increase in Curie and blocking temperatures and in saturation magnetization, but a decrease in thermoremanent magnetization.

Under alternating field (AF) demagnetization the directions of magnetization are exceedingly stable, commonly being little changed in 1,000 oersted peak field. The directions in several samples are changed by less than 5° in fields as high as 2,000 oersted. This directional stability is very much higher than that normally encountered in subaerial basalts in which fluctuations in direction commonly set in during AF demagnetization at about 500 oersted[5]. The stability of intensity is also high; the average field required to reduce the intensity by 50 per cent is 320 oersted, whereas values for subaerial basalts are typically about 200 oersted[6].

The saturation isothermal remanent magnetization (IRM_{sat}) ranges from 0·17 to 0·85 cgs. units cm^{-3}. For the high intensity axial basalt the IRM_{sat} is 0·57, so that the ratio NRM/IRM_{sat} is 0·18. This is exceedingly high,

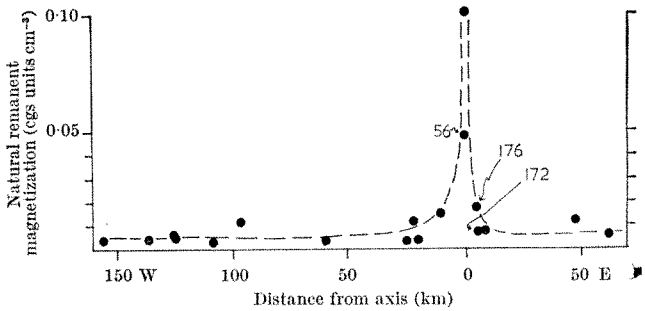


Fig. 1. Graph of natural remanent magnetization against distance from the axis (zero point) of the Mid-Atlantic Ridge at 45° N. The points are averages for dredge and core stations and are each based on results from one to five separate samples. The stations mentioned in the text are indicated.

and shows the natural magnetization process to be a very efficient one indeed. The coercivity of IRM_{sat} averages 430 oersted, which is about 100 oersted higher than that commonly found in subaerial basalts[5].

Inclination Estimates

The original attitude of dredged pillow basalts can sometimes be determined from their form and internal structure so that a rough estimate of inclination is possible[7,8]. Estimates from six pillows dredged at station 56 (45° 13′ N, 28° 0′ W) have a spread of 20° and a mean of +44° (Table 1). The error in determining attitude is probably not more than 20°, and replicate values from the same pillows obtained independently agree to 10°. The polarities are normal.

Table 1. MAGNETIC INCLINATION IN PILLOW BASALTS AT STATION 56

Sample No.	Inclination (°) F.A.	E.I.
1	+38	
2	+39	+32
3	+34	+44
4	+44	
5	+46	
6	+53	

The horizontal is assumed parallel to the equatorial plane of the pillow. These observations were made by us independently.

Table 2. RESULTS FROM BORE CORES 172 AND 176*

Piece	Natural remanent magnetization (cgs units cm^{-3})	Initial inclination I_0 (°)	Inclination after demagnetization in 100 oersted I_{100} (°)
172 (5)	0·0051	−25	−32
176C (1)	0·0081	−24	−27
176B (3)	0·0163	−39	−41
176A (2)	0·0199	+26	+24

* Each core piece has been cut into standard cylinders the number of which is entered in parentheses. The data are averages for cylinders.

Inclination information from the first basalt drill core using the new 'Brooke' drill[9] during the cruise of CSS Hudson (May to July 1969[10]) is summarized in Table 2. The vertical axis of the core may not be defined to better than 20° although it is believed to be much better than this. Core 172 (45° 5·3′ N, 27° 58·4′ W) is a single piece 15 cm long. Core 176 (45° 5·2′ N, 27° 59·3′ W) is in three pieces (A, 8 cm; B, 10 cm; C 4 cm) which do not articulate. After magnetic cleaning in 100 oersted AF the directions within each piece agree to better than 2°. This is the first cored basalt from the crest of an ocean ridge and there are many uncertainties in interpreting the data. It is entirely possible, for instance, that the drill sampled boulders embedded in sediment so that the inclination and polarity results are meaningless. If this is so, then the inclinations at each station would differ widely. There is, however, approximate agreement between cores (mean of 176 is 31°, 172 is 34°). Moreover, positive inclinations decrease and negative inclinations increase after magnetic cleaning as is expected of rock *in situ* (Table 2). Piece 176A has positive inclinations (normal or negative polarity). The rest of core 176 is reversed.

The Earth's field during the past few million years has been accurately that of an axial geocentric dipole when averaged over periods of tens of thousands of years[11], and the expected inclination at 45° N is 63°. The estimates from pillows and core are 20° to 30° lower. The effect is interesting, but the evidence is not unequivocal, and it is too early to speculate as to its cause. Some evidence of low inclinations has also been reported from the Reykjanes ridge[8].

Opaque Minerals

The predominant opaque minerals in these submarine basalts are skeletal titanomagnetite and ilmenite. Sulphide minerals (pyrite, pyrrhotite, marcasite and chalcopyrite), so rare in subaerial basalts, are generally present. Titanomagnetite is responsible for the magnetization, but the ilmenite and sulphides are interesting because the ilmenite is always in the lowest state of oxidation (R1 on an ilmenite oxidation scale R1–R7) and the sulphides indicate a reducing environment during cooling. The size of the titanomagnetite is generally below 1–5 μm (there is sometimes a coarser fraction ranging up to 100 μm) and less than that typical of subaerial flows. Twenty-five of the thirty-four samples studied are in the lowest oxidation category C1 (titanomagnetite oxidation scale C1–C6) and none is in the high grade pseudomorphic oxidation categories (C4–C6). The high temperature deuteric oxidation, so common in subaerial basalts, is subdued or absent. A further remarkable feature of the titanomagnetites is that they have often been partially altered to titanomaghemite. This is a low temperature oxidation effect. It is absent in axial samples.

Axial Intensity

From the general appearance of the basalt pillows and the observations just described it is certain that they cooled very quickly as they were extruded onto the ocean floor. Contact with very cold[12] bottom water (3° C) would cause the almost instantaneous formation of an impervious crust, which, together with the confining pressures of overhead water, would cause the retention of volatiles and sulphides, and the rapid crystallization of skeletal titanomagnetites. The blocking temperatures of the axial rocks are low (less than 300° C) so that it is likely that the titanomagnetites were in their present form when the remanent magnetization was acquired. This situation is one of the most favourable in nature to the formation of thermoremanent magnetization (TRM), that is, the acquisition of remanence by cooling of already formed crystals through their critical blocking temperature[13]. This would explain the high ratio NRM/IRM_{sat}. In contrast subaerial lavas cool over periods of years, lose their volatiles, and may undergo, to varying degrees, subsolidus oxidation at temperatures below the characteristic blocking temperatures[14,15]. Hence their magnetization is, to some extent at least, a chemical remanent magnetization (CRM) formed by grain growth through the critical blocking diameter at temperatures below the Curie temperature[15–18]. Experiments[17] show that TRM is some ten times more intense than CRM for the same material in the same ambient field. We therefore suggest that to a first approximation the very high intensities in these axial submarine basalts is a consequence of their TRM, in contrast to subaerial basalts whose remanence may be partly a CRM and of lower intensity.

Intensity Away from Axis

The next item to consider is the decrease of intensity with distance from the axis, most of which occurs within about 5 km (Fig. 1). In terms of time this means a period of order 10^5 years[19]. There appears to be some physical or chemical process which demagnetizes a rock as it moves sideways from the axial volcanic source. Viscous decay due to thermal agitations is one possibility and occurs as the logarithm of time, but the observed decay is more rapid (Fig. 1). The chemical demagnetization processes which seem the most likely are the oxidation of titanomagnetite by its partial replacement with titanomaghemite, or the incipient submicroscopic unmixing of magnetite and ilmenite rich phases; both processes may occur. By the first process (which may take place at the surface through the absorption of seawater) parts of strongly magnetized grains may have been replaced by titanomaghemite with a comparatively weak CRM. By the second process (which might occur during even very mild hydrothermal alteration of buried flows in the volcanic zone and which is perhaps simulated in the experiments mentioned above) the original TRM is replaced by a CRM in the unmixed magnetite rich phase. This causes a decrease in the intensity and an increase of Curie and blocking temperatures with distance from the axis. Schaeffer and Schwarz[5] have also independently invoked a similar process.

Ridge Model

These results relate to magnetic models of the ocean ridges. The thickness of the magnetized layer required to generate the magnetic pattern may be calculated using the mean intensities for each physiographic province. The thickness obtained is 200 m for the median valley and 1 km for the crest mountains and high fractured plateaux. This suggests that igneous activity outside the median valley has thickened the magnetized layer by a factor of five. The remarkable preservation of the reversal sequence in the geomagnetic anomaly profiles indicates that the magnetic layer is formed within a few km of the axis[20,21], and that lateral thickening of the magnetic layer has been negligible. If, moreover, the decay of intensity in surface samples is due to chemical demagnetization through extended contact with sea water, then only a proportion of flows will be affected. Most will soon be covered and subject only to mild hydrothermal effects for the short time they remain in the warm volcanic zone. The mean intensity of magnetization of the extrusive layer may then fall between the observed surface curve and the decay curve for bottom flows. The proposed model is set out in Fig. 2. The thickness of the magnetized layer is about 200 m and approximately constant across the entire ridge. The growth lines define successive age slices, and in their upper parts these are composed of comparatively weakly magnetized[1] intrusive rocks (gabbro and diabase) and metamorphic rocks (greenstones, serpentinized peridotite) rocks capped by a thin volcanic layer which is more magnetic by a factor of ten.

The model is based on magnetic evidence, but is consistent with the dredge haul stratigraphy and seismic velocities (to be published by D. Barrett and F. Aumento). In this model it is the oxidation of the titanomagnetics (as recorded by the increase in Curie and blocking temperatures) that is mainly responsible for the axial anomaly being about twice as large as those on either side, so that over the more rapidly spreading ridges in which material moves rapidly sideways out of the warm volcanic zone this anomaly contrast should be less. It is perhaps noteworthy that over the fast spreading East Pacific Rise the axial anomaly has a magnitude very similar to those on either side, and the intensities of magnetization calculated from the anomalies themselves (by the inverse method) show no systematic variation with distance from the axis[22].

The opaque mineralogy and magnetic properties of submarine and subaerial basalts are so different that they should (when taken together with many other geological factors) prove to be a useful tool for the identification of old oceanic crust, which is a problem of great general interest. The hypotheses of continental drift and seafloor spreading predict that if two plates of lithosphere containing oceanic crust are driven together, one over the other in a subduction zone, then their present boundary should be marked by remnants of the ocean floor. These remnants may then be used to map the boundaries of old plates. In this way large quantities of primitive oceanic basalt could have been incorporated into the continental crust. These basalts we observe to contain abundant disseminated sulphides which later may be concentrated into useful deposits by hydrothermal processes. Many greenstones were originally submarine lavas so that the ultimate source of the metalliferous deposits, which are commonly associated with them, is to be sought in an ancient spreading rift system.

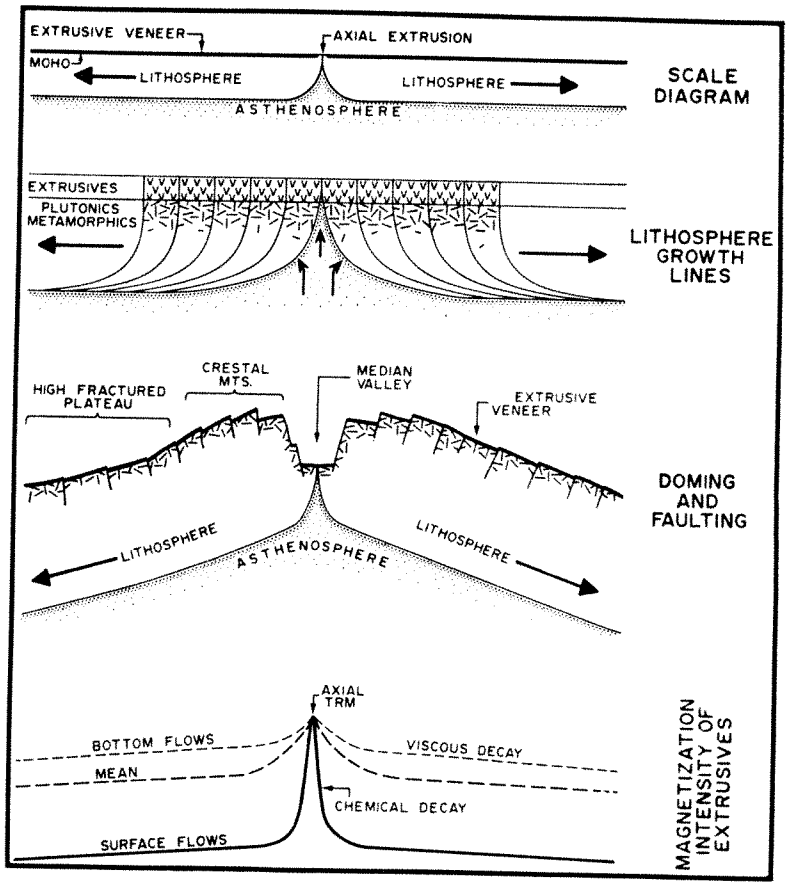


Fig. 2. Proposed model for the Mid-Atlantic Ridge at 45° N. The top two diagrams illustrate the growth of the lithosphere, and the third diagram the effect of doming and faulting. The thin volcanic veneer responsible for the marine magnetic anomalies is about 200 m thick and relatively non-magnetic plutonic and metamorphic rocks are exposed along the infacing escarpments. At the bottom the observed (thick line) and assumed intensity distribution within the volcanic layer is given. The mean curve is constructed assuming that the upper quarter of the flows have been affected by maghemitization due to sea water contamination, and three-quarters of the flows have been affected by very mild hydrothermal alteration (unmixing of titanomagnetite) due to burial in the warm volcanic zone. Beyond the median valley only very slow viscous decay occurs. The intensity values can be scaled from Fig. 1.

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Formation of the Indian Ocean

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Palaeomagnetic results suggest a possible sequence for the fragmentation of the land masses around the Indian Ocean.

PALAEOMAGNETIC data from the southern continents[1] are now sufficiently detailed to confirm that du Toit's reconstruction of Gondwanaland[2] is essentially correct. The data are compatible with Gondwanaland remaining as a unit for the whole of the Palaeozoic, for the palaeomagnetic poles all lie on a common polar-wander path with the continents reconstructed[1]. Whether or not Madagascar should be placed to the west or east of India was indeterminate and controlled entirely by the precision with which the position of India could be located, because there are no Palaeozoic results for Madagascar. For Africa and South America, it has been shown that the agreement of the pole paths continues up to the end of the Triassic, with the divergence occurring between the Late Triassic and Early Cretaceous[3]. The first opening in the South Atlantic thus took place within these limits.

Recently, some K–Ar ages have been determined for the Rajmahal Traps of India[4], and, surprisingly, these show that they are only 100 to 105 m.y. old (Albian). These volcanics had previously been regarded as Jurassic in age, a factor that led most analysts of Mesozoic palaeomagnetic results[5–8] to suggest that India was adjacent to Western Australia rather than East Africa in the original configuration of Gondwanaland. For each of the four principal circum-Indian Ocean fragments of Gondwanaland there are now palaeomagnetic results that fall in the time range 95 to 110 m.y. (see Table 2), and for Madagascar new results for the Late Cretaceous have been reported[9]. These measurements relate to the time either just preceding the break-up of the super-continent or else during which the dispersal of the fragments was taking place, and they enable some suggestions to be made regarding the history of the formation of the Indian Ocean and the future interpretation of sea-floor spreading data.

Gondwanaland during the Palaeozoic and Early Mesozoic

The essential features of the Palaeozoic and Early Mesozoic (Triassic and Jurassic) palaeomagnetic results from Gondwanaland are summarized in Table 1, and I have used them to derive the Gondwanic configuration of Fig. 1. The palaeomagnetic poles tend to group about four positions during the Palaeozoic, rather than to lie on a continuous curve. These "quasi-static" intervals[6] (designated as groups *A*, *B*, *C* and *D*) are separated by short intervals of time during which the pole moved from one stable position to the next. From a recent analysis of the Phanerozoic polar-wander path for India[4], it is now possible to place a further constraint on this continent's position in Gondwanaland. The reconstruction of Fig. 1 enables the Indian Triassic poles to fall in the same region as Triassic poles for South America, Mesozoic poles for Africa and Jurassic poles for Antarctica, without seriously upsetting the agreement of the Indian Palaeozoic poles with the common polar-wander curve. This restricts the position of Madagascar to the region between the west coast of India and the east coast of Africa. The reassembly so obtained is then the same as that confirmed by a computer fit of the continental shelves[10].

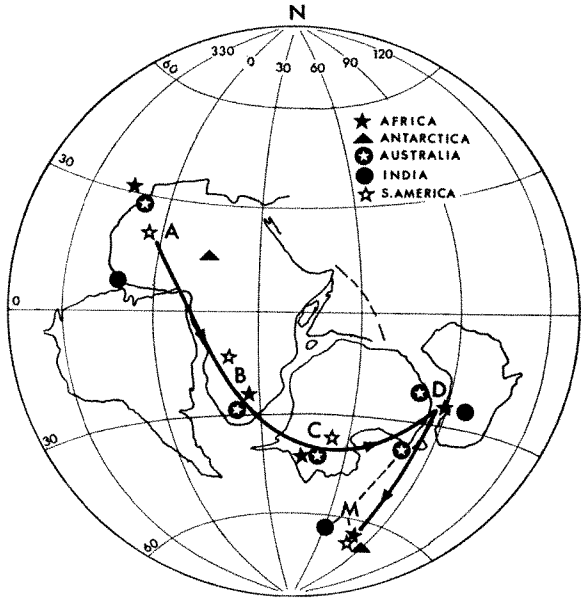


Fig. 1. Reconstruction of Gondwanaland based on the mean palaeomagnetic poles listed in Table 1. Africa is placed in its present coordinates on an equatorial equal area projection. The common polar-wander curve is shown, commencing at the group *A* poles in Cambrian times through to group *M* in the Triassic–Jurassic. Note that the Australian group *M* pole diverges from the rest.

Although the Mesozoic poles for Australia form a close group, their mean does not overlap with those from the other continents as illustrated in Fig. 1. One is forced to conclude that Australia broke away from the rest of Gondwanaland during the Early Triassic. This is puzzling because sea-floor spreading data from the south-east Indian Ocean between Australia and Antarctica[11] suggest that the break between them took place only 43 m.y. ago. Cretaceous palaeomagnetic results from Australia

Table 1. GROUP MEAN PALAEOMAGNETIC POLES FOR THE GONDWANIC CONTINENTS FOR THE PALAEOZOIC AND MESOZOIC

Continent	Group *A*	Group *B*	Group *C*	Group *D*	Group *M*
Africa[7]	31 N, 18 W (3)	26 S, 26 E (1)	46 S, 40 E (1)	27 S, 89 E (1)	64 S, 81 E (9, Tr-K)
Antarctica[8,24]	28 S, 10 E (1)	—	—	—	54 S, 140 W (4, Jur)
Australia[23]	12 S, 4 E (2)	77 S, 12 W (3)	73 S, 147 E (1)	44 S, 135 E (6)	48 S, 151 E (10, Tr-K)
India[4]	28 S, 32 E (1)	—	—	26 N, 132 E (2)	20 S, 128 E (3, Tr)
S. America[22,25,26]	15 N, 38 W (5)	28 S, 34 W (1)	62 S, 14 W (4)	—	82 S, 154 W (7, Tr)

Number of poles making up each mean is given in parentheses. Approximate ages are: Group *A*, Cambrian to Silurian; Group *B*, Devonian to Lower Carboniferous; Group *C*, Upper Carboniferous; Group *D*, Permo-Carboniferous; Group *M*, Mesozoic (as indicated).

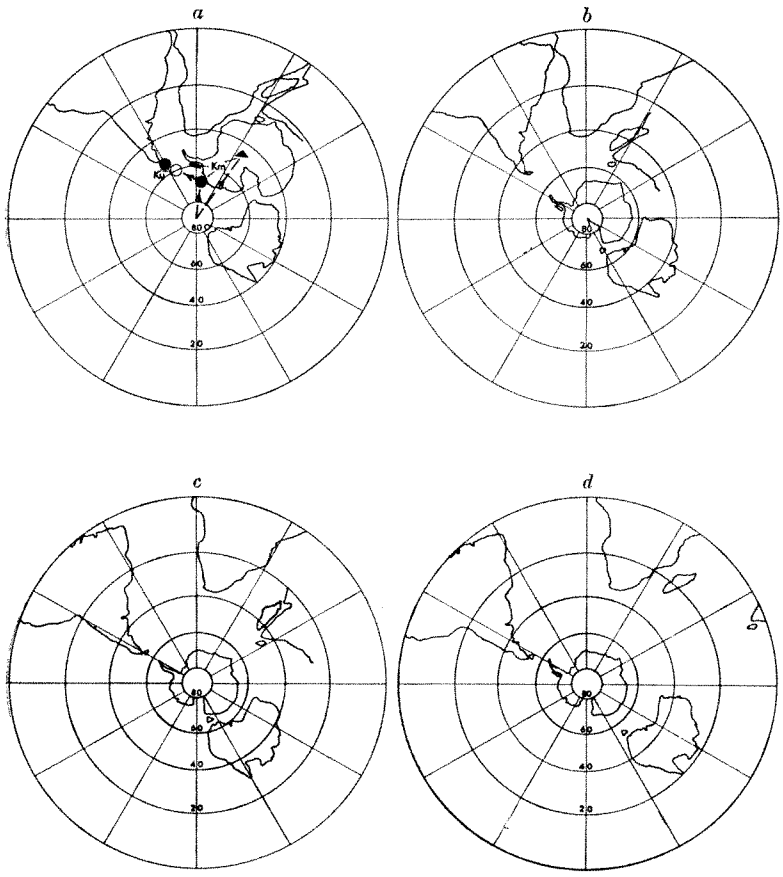


Fig. 2. The break-up of Gondwanaland showing approximate positions of the fragments at four stages. Polar stereographic projections. *a*, Triassic to mid-Jurassic, based on the group *M* poles of Table 1. The subsequent mid-Cretaceous (Km) poles for India and Madagascar (open circle) are indicated using the symbols of Fig. 1 and data of Table 2. *b*, Mid-Cretaceous, using poles in the range 95–110 m.y. from Table 2. *c*, Upper Cretaceous to Palaeocene, where India and Madagascar are located using the poles of Table 2, and the other continents are located from sea-floor spreading data[11,16] as also in (*d*) Miocene.

and Antarctica (discussed later) are again compatible with the Australia–Antarctica link as in Fig. 1, but the Jurassic data are not. Because all the Mesozoic palaeomagnetic measurements in Australia are from rocks in eastern Australia, Irving and Robertson have suggested[12] that eastern Australia detached itself from the rest of Australia and Antarctica during the Early Triassic and then rejoined it again later. Although this might seem fortuitous, Rod has suggested[13] possible geosutures in eastern Australia that could be compatible with this suggestion, but, in making a possible reassembly for the Triassic to mid-Jurassic, the whole of Australia has been moved to conform with the palaeomagnetic results as shown in Fig. 2*a*.

Cretaceous

Results from the four circum-Indian Ocean continents in the time range 95 to 110 m.y. are summarized in Table 2. The Andean Intrusive Suite, Antarctica, previously cited as Upper Cretaceous to Eocene, is now associated with isotopic dates around 100 m.y.[14]. For South America, there are only results from the Lower Cretaceous Serra Geral formation[15], but this continent will not be considered further in relation to the formation of the Indian Ocean. The successive positions of South America shown in Figs. 2*b*, *c*, *d* are those inferred from sea-floor spreading in the South Atlantic[16]. The positions of the other continents can only be related to a single (or mean) palaeomagnetic pole for each period. The continents are placed in latitude and azimuthal orientation with respect to the pole at the centre, but the longitude remains indeterminate. Thus the successive positions of the circum-Indian Ocean continents shown in Fig. 2 during the break-up of Gondwanaland have arbitrary longitude.

Table 2. CRETACEOUS PALAEOMAGNETIC POLES FROM GONDWANALAND

Continent	Mean pole position	Age
Africa[7]	62 S, 80 E (1)	106–111 m.y.
	60 S, 82 E (1)	116–128 m.y.
Antarctica[8]	86 S, 2 W (1)	96 m.y.[14]
Australia[8]	54 S, 147 E (2)	93–104 m.y.
India[4]	10 S, 117 E (3)	100–105 m.y.
	30 S, 107 E (1)	Late Cretaceous
	33 S, 101 E (2)	65 m.y.[19]
Madagascar[9,27]	76 S, 44 E (3)	Late Cretaceous
S. America[15]	78 S, 54 E (1)	120–135 m.y.

Number of poles making up each mean is given in parentheses.

The pole positions for both Africa and Australia remain unchanged throughout the Mesozoic, whereas the mid-Cretaceous poles for India and Antarctica differ from their Triassic and Jurassic poles respectively as illustrated in Fig. 2*a*. Included on the same diagram are the Late Cretaceous poles for India and Madagascar (Table 2). The important point to note is that the Late Cretaceous Madagascar pole not only plots close to the Late Cretaceous Indian pole, but also lies on the path joining the Indian mid and Late Cretaceous poles. This suggests that the proximity of India and Madagascar was maintained at least up to the latter part of the Late Cretaceous (say 70 to 80 m.y. ago).

The diverging pole positions for India and Antarctica and Africa for the mid-Cretaceous show two things. First, the India–Madagascar–Antarctica block must have broken away from Africa between the mid-Jurassic and mid-Cretaceous, opening up the Indian Ocean for the first time. This estimate is compatible with those made by Le Pichon and Heirtzler[11] and Smith and Hallam[10]. Second, the India–Madagascar block must have subsequently separated from Antarctica. It is possible that this last break may be related to the extrusion of the Rajmahal Traps around 100 m.y. ago. By the mid-Cretaceous, the palaeomagnetic poles indicate a situation as in Fig. 2*b*. The important point that arises is that both Antarctica and the India–Madagascar block are situated farther south of Africa, so that although Antarctica and India are separating, this fragment of Gondwanaland has moved southwards. Most discussions of the limited sea-floor spreading data for the Indian Ocean[10,11,17] tend to assume that India moved always in a northerly direction to its present position from one adjacent to East Africa.

Late Cretaceous to Palaeocene

By the close of the Mesozoic era, the opening between Antarctica and Africa is almost complete and the south-west part of the Indian Ocean has opened (Fig. 2*c*). This is, of course, consistent with Vine's suggestion[18] that the south-west part of the Indian Ocean ridge is a dead ridge and has not been the site of measurable spreading since the Mesozoic. The subsequent motion of India and Madagascar is northwards relative to Antarctica during the Late Cretaceous, and the break between them must occur near the Mesozoic–Cainozoic boundary. It is tempting to relate this to the extrusion of the Deccan Traps, which K–Ar dates now place firmly at around 65 m.y.[19]. Davies sup-

poses[20] that India and the Seychelles parted company some time in the Palaeocene, relating this to the Deccan Trap vulcanism. One might surmise that the Seychelles formed part of an India–Madagascar block and that rupture of the Seychelles and India was also accompanied by the rupture of India and Madagascar. This occurred at a latitude of 30° S as shown in Fig. 2*c*, at which time the Carlsberg ridge was establishing itself.

Cainozoic

On the scheme just outlined, spreading from the Carlsberg ridge during the Cainozoic involves the separation of India on one side and Madagascar and the Seychelles on the other. Note that Africa is not involved. The rapid northward movement of India occurs during the Cainozoic[21], so that by the Miocene (Fig. 2*d*) it had crossed the equator. The final phase is the separation of Australia and Antarctica, which sea-floor spreading data suggest began 43 m.y. ago[11].

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Flysch-type Sedimentation in the Alboran Sea, Western Mediterranean

by

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The Quaternary deposits of the Alboran Sea and associated sediment dispersal patterns, and geographic and tectonic setting of the region are closely similar to those of some ancient flysch basins preserved in the geological record.

Flysch is a popular geological term applied to distinct sedimentary deposits exposed in many of the world's tectonic belts. Most geologists in the Alps, where the term was coined[1], would designate as flysch the type of sequences described by Tercier[2] and other workers[3,4]: (*a*) thick accumulations of detrital or terrigenous deposits; (*b*) variable lithologic complexes usually consisting of regular alternations of sandstones and micaceous shales with interbedded conglomerates or limestones; and (*c*) formations of marine origin. Furthermore, the palaeogeographic setting as inferred from diverse stratigraphic and tectonic investigations suggests that flysch units in the Alps and other regions such as the Apennines and Carpathians would be characterized by deep, partially isolated marine basins bordered by narrow shelves and relatively steep dissected slopes and by discontinuous and tectonically active cordilleras. These marine basins generally received large volumes of terrigenous deposits during stages of advanced orogenic development[5]. Agreement, however, as to an exact definition of the term has yet to be reached[6]. Most flysch units described to date are consolidated formations of pre-Pleistocene age.

Because of the widespread and volumetric importance of flysch in the rock record and because the depositional history of most flysch units is still poorly understood it is essential to seek potential analogues of such deposits that may now be accumulating in present-day oceans or that may have been deposited in marine basins in the recent geological past. In this respect, preliminary results of a study of the Quaternary sedimentation patterns in the Alboran Sea in the western Mediterranean are most useful.

Geographical and Geological Setting

The partially land-locked Alboran Sea, approximately 160 km wide and 320 km in length, lies between the Betic Cordillera on the Iberian peninsula and the Moroccan Rif on the North African margin. It connects with the Atlantic at the Strait of Gibraltar to the west and with the Balearic Basin to the east. Sea floor topography of this region is particularly complex. There is almost no shelf platform, and submerged ridges, seamounts, basins and submarine valleys characterize the region (charts in ref. 7, and C. E. G., C. B., D. J. S., Blavier, P.,

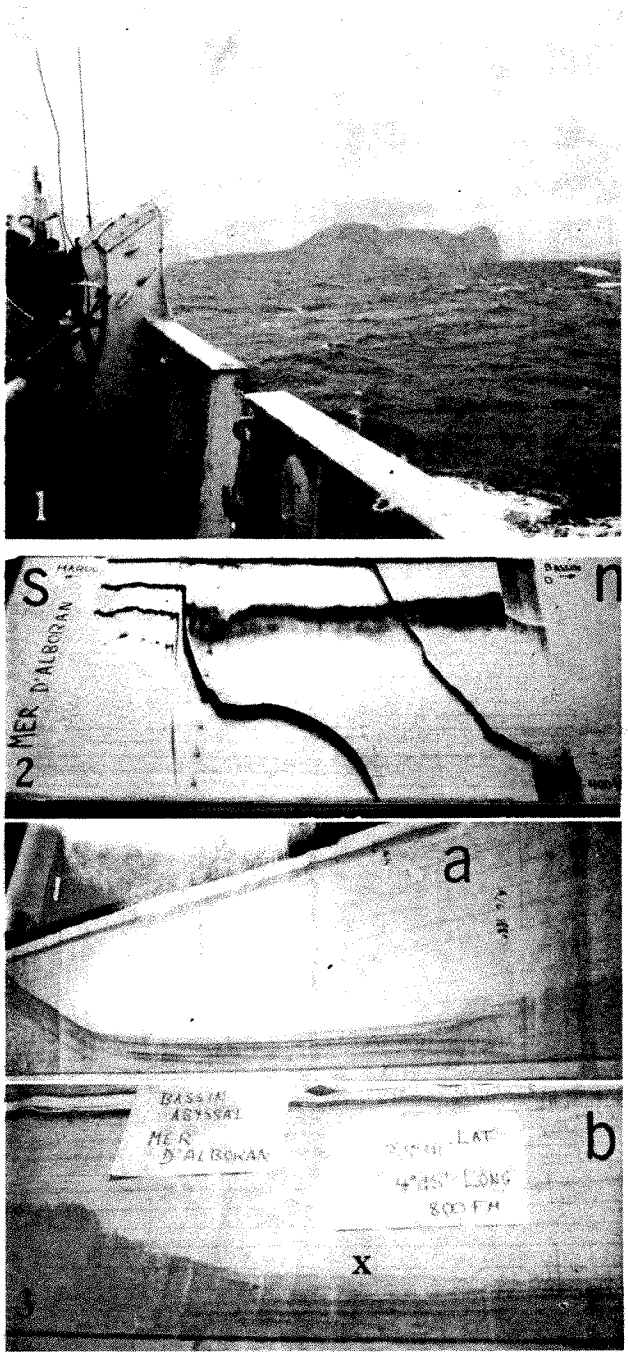


Fig. 1. (1) Alboran Sea east of the Strait of Gibraltar. Ship is positioned above head of Gibraltar Submarine Valley in which much sediment is funnelled south-eastward to the Western Alboran Basin. Gibraltar and Great Europa Point are in the distance. (2) Steep slopes (> 10°) as recorded on PDR record obtained north of Xauen Bank; note dense deep-scattering layer. (3) Two N–S oriented PGR profiles across the Western Alboran Basin plain and basin aprons. Note flysch-like alternating sequence of laterally continuous reflectors (thin sand turbidites) and acoustically transparent mud section. Wedge of sediment at base of slope (×) is a large allochthonous slump mass that has been emplaced on the southern margin of the basin by gravity sliding.

and Tonarelli, B., in preparation). Slopes to 11° are encountered (Fig. 1). Attention has previously been paid to the predominant NE–SW trend of large morphological features including the volcanic Xauen Bank–Tofiño Bank–Alboran Ridge extension. This lineation undoubtedly reflects NE–SW structural trends including major faults[8] and volcanic extrusives[9]. The region has been subjected to almost continuous tectonic activity since the late Mesozoic[10]. Compressional development affected the region during the Cretaceous and Tertiary as a result of movement of the African against the European blocks, and possibly the counter-clockwise rotation of the Iberian peninsula. Opening of the Alboran Sea in Ponto-Pliocene time[11,12] reflects distension accompanying lateral displacement of the Afro-European blocks. Volcanic activity[9] became important during this down-drop and separation, or *effondrement*, and seismic surveys show that more than 1 km of terrigenous sediment fill has accumulated in this region since the beginning of the Pliocene[13,14]. Local folding and faulting of the Plio-Pleistocene fill indicates that the region has once again been subjected to orogenic compression. Submergence of 2,000 year old archaeological sites was caused by recent downward displacement resulting from tectonic movements[15]. The presently active seismic belt extending eastward from the Azores to North Africa[16] continues to affect this region. Concurrent tectonic mobility and rapid sedimentation, essential prerequisites for flysch deposition, have thus been predominant characteristics of the Alboran Sea since the Pliocene.

Nature of the Depositional Fill

Of particular interest is the sediment fill in the Western Alboran Basin, the largest (24 by 37 km) and deepest (just over 1,500 m) basin plain (Fig. 2) in this sector of the Mediterranean. This oval basin lies approximately 110 km east-south-east of the Strait of Gibraltar and 100 km south of Malaga, Spain. The NW–SE trending Gibraltar Submarine Valley (80 km long and 5 to 10 km wide) links this basin plain with the Ceuta and Gibraltar Canyons near the Strait. A large seamount chain lies north-east of the basin; isolated mounts (probably volcanic necks) are found north of the basin. Extensive high resolution Precision Graphic Recorder (PGR) coverage (2,220 km) and dense coring (49 large diameter, 120 mm) sphincter cores[17] collected during several Nato-SACLANT cruises serve to define the unconsolidated sediment pond (Fig. 2A). The dense PGR net consists of twelve N–S profiles extending from the African to Spanish shelves and spaced 8 or less km apart, six E–W profiles, an NW–SE profile close to the Gibraltar Submarine Valley and an NE–SW profile along the Spanish margin (profiles detailed in ref. 18). These profiles highlight the geometry and attitude of subbottom strata. The PGR used with an EDO echo sounder transmitting pulses at 12 kHz provides extremely high resolution to a depth of about 30 m below the surface of the sediments.

The survey defines an assemblage of terrigenous, gravitational and hemipelagic deposits that recall those of typical ancient flysch. Thin sand layers that extend across the basin plain and basin aprons alternate with fine-grained acoustically transparent sediments (Fig. 1). Also noted on PGR profiles are allochthonous 10 to 15 m thick slump masses, covering at least 36 km², concentrated on the edge of the basin plain and in the basin apron at the base of 1° to 6° slopes. Where cored, these large-scale gravity slide deposits reveal anomalously steeply dipping (to 20°) sand layers and some contorted strata. Distinct reflectors concentrated in the basin plain are recognized in cores as thin graded and laminated sand units of relatively uniform thickness (1 to 15 cm) and subbottom depth. These units are believed to represent short-term gravitative (turbidity current, bottom flow and traction) events. Most sand layers, laterally continuous across most of the basin, serve as precise stratigraphic horizons. The nature of these layers is described in detail elsewhere (to be published by C. E. G., C. B., D. J. S., P. Blavier and B. Tonarelli, and in ref. 18).

Mud, predominantly silty clay, accounts for at least 95 per cent of the Holocene fill of the Western Alboran Basin. This mud, not deposited from a simple rain of pelagic material onto the basin floor, has a multiple origin. Mud distribution, determined from cores and high-resolution seismic records, is closely related to topography. Total thickness of the Holocene section is

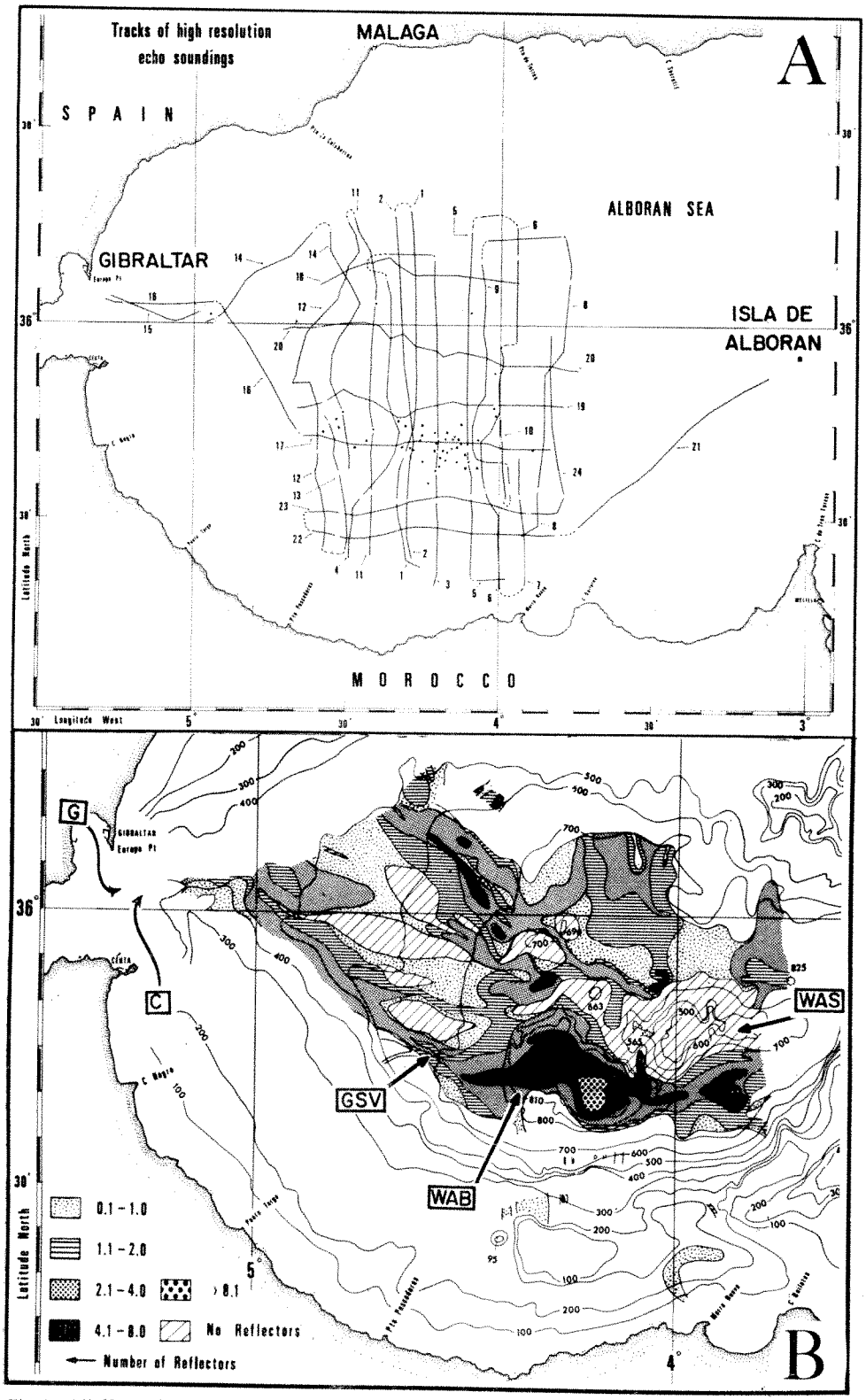


Fig. 2. (*A*) Chart of study area showing the net of available PGR profiles and cores (shown as dots); (*B*) chart showing distribution and total number of sand layers per 20 m section as obtained from the PGR records. Note the close relation of number of beds with topography. *G*, Gibraltar Canyon; *C*, Ceuta Canyon; GSV, Gibraltar Submarine Valley; WAB, Western Alboran Basin; WAS, Western Alboran Seamount chain. Base chart by courtesy of SACLANTCEN.

approximately 2 m on basin slopes and increases to more than 4 m (sedimentation rates to about 35 cm/1,000 years) in the near-horizontal basin plain. Thickest mud deposits are therefore localized in lows where thin sand layers most abound. These deposits are hemipelagic in composition: lutite with planktonic and benthonic foraminifera, deep and shallow water ostracods, and plant fibres. Components are, at least in part, near-shore in origin.

At least 10 to 20 per cent of mud core sections are parallel and cross-laminated as shown in X-radiographs[18], suggesting that bottom traction transport may be important in the deposition of mud in the basin. Bottom currents have also truncated the top of sand layers and concentrated microfossils in thin laminae. A few subtly graded mud units are probably mud-turbidites. Another facies type consisting of homogeneous mud layers with scattered microfossils (comprising more than half of the Holocene fill) reflects more regular deposition from less dense suspension layers[18].

Dispersal Patterns

Coarse-fraction provenance. Sandy layers represent only about 5 per cent of the total late Pleistocene and post-glacial sequence but are most valuable in interpreting the paths of sediment transport into the Alboran Sea. The regional distribution and number of sand reflectors per upper 20 m of section as noted on PGR records has been plotted (Fig. 2*B*). The resulting distribution pattern indicates concentrations of finger and tongue-shaped sand units oriented normal to the slope. The concentration of sand layers is highest in the Western Alboran Basin plain and other topographic lows including submarine valleys and small depressions between seamounts. From these data it is possible to infer the predominant dispersal path of silt and sand (Fig. 3). Arrows showing transport directions of coarser material have been assigned on the basis of (*a*) attitude of subbottom reflectors, including imbrication of layers, and (*b*) termination of reflectors, usually near the base of slopes. A multiple source origin

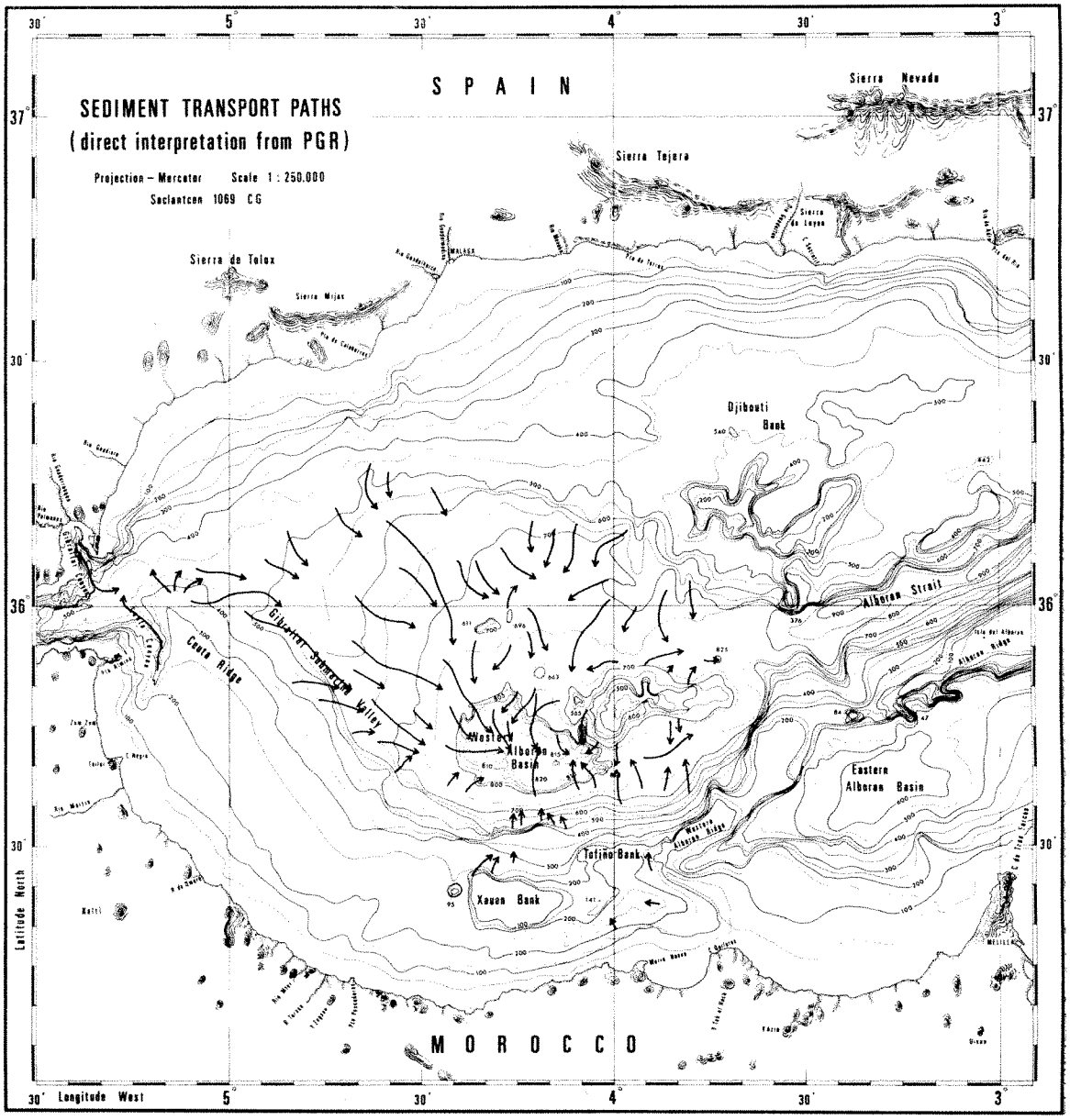


Fig. 3. Sediment dispersal (arrows determined from analysis of PGR records in Fig. 2*B*), showing inferred transport paths of silt and sand into the western part of the Alboran Sea. This style of ponding, including the multi-source origin of sediments, calls to mind terrigenous depositional patterns of certain ancient flysch basins. Base chart by courtesy of SACLANTCEN.

must be invoked to explain the diversity of sediment transport directions: material is transported from (*a*) extrabasinal source areas (sediments transported to the Spanish and Moroccan coasts by rivers and torrents draining the Betic and Rif (Cordilleras)) and (*b*) intrabasinal sources within the Alboran Sea (steeper upper slopes, ridges, banks and seamounts that have shed material downslope). Similar multiple-source ponding has been postulated for some ancient flysch basins[19–22].

A significantly large volume of sediment has been derived from the north-west quadrant, presumably the Spanish margin. Canyons and other linear depressions have channelized flow basinward. One turbidite layer, early Holocene in age, can be followed laterally for more than 65 km and seems to have originated in the Gibraltar region. There is good evidence that the Gibraltar and Ceuta Canyons and Gibraltar Submarine Valley funnel near-shore sediments to the Western Alboran Basin. The Moroccan margin and shallow banks such as Xauen and Tofiño Banks also provide sediment to the Alboran Sea[18].

Fine-fraction provenance: higher sedimentation rates of hemipelagic muds in the deeper parts of basin plains than on adjacent slopes must be accounted for. It is probable that deep water mass movement (dense Mediterranean undercurrent outflow) is deflected by topographic lows thus concentrating fines in such areas as the Western Alboran Basin. Currents near the bottom would account for the truncation of the upper parts of sand turbidites and for the concentration of microfossils as distinct layers. The intensity, direction and constancy of deep seawater movements in this area, still poorly known, are presently under investigation.

The predominant source of supensate in fine-grained turbid flows are floods that seasonally inject material at fluvial point sources along the mountainous Moroccan and Spanish margins. Winter storm and wave activity eroding the adjacent coast line also bring fine sediments into suspension. A probable third source of fines is extra-basinal: (*a*) fines carried by the low density surface Atlantic water entering through the Strait of Gibraltar and by deeper dense Mediterranean water entering the Alboran Sea from the east. It seems that most of the mud in the basin has been deposited from slowly moving suspension-rich layers.

Another mechanism may also account for some of the lutite-rich section: deposition from gravity controlled turbid flows. Mud transport paths may, in such cases, be similar to sand dispersal patterns. It is noted, for instance, that the thickness of mud in the lowest parts of the Gibraltar Submarine Valley is 25 per cent to 30 per cent lower than in the Western Alboran Basin as shown on PGR profiles. A possible hypothesis is that mud and sand-charged bottom flow occasionally moves through the Gibraltar Submarine Valley. Velocity of such flows may be too high for deposition of fines in the canyon; settling of lutite and associated benthonic and planktonic microfossils occurs toward the centre of the basin plain as current velocities decrease[18]. It would be difficult to distinguish between deposits from low density turbidity currents and those from low density suspension layers transported by slow moving water masses[23].

Modern Flysch Basins in the Mediterranean?

If one adheres to the cited criteria of flysch outlined in the introduction of this article it becomes apparent that most Quaternary deep-sea sediments covering the world's deep ocean floors have been deposited in geographic sites and under conditions that differ significantly from those of basin of Alpine orogenes. The large open ocean basins such as the present North Atlantic are generally bordered by more gentle slopes, larger abyssal plains and wider shelves than those of former flysch basins of the Alpine type. Such basins tend to receive smaller amounts of sediment per unit area than smaller somewhat shallower partially land-locked flysch basins of the Alpine type. The margins of ocean basins such as those off north-east North America have been tectonically quite stable. Furthermore, it is difficult to predict the future tectonical development of a present-day ocean basin, more less recognize its present orogenic phase. It is reasonable therefore to question, as some workers have[5], whether any modern oceanic deposits can be accurately designated as flysch for that term is usually applied by land geologists working in the Alps, Apennines, Carpathians and other mountain belts.

We propose that the Alboran Sea has been receiving a Quaternary flysch-type sediment fill during Quaternary time: (*a*) the terrigenous fill consists of gravitative and hemipelagic sequences which, if lithified and exposed, would be recognized as a shaly flysch of which there are many examples in the Alps and elsewhere; (*b*) sediment ponding[24,25] and associated sediment transport processes appear to be similar to those inferred for some ancient flysch basins; (*c*) the geographic setting and crustal mobility of the region directly affect depositional style and rates in a manner that also calls to mind those postulated for ancient flysch deposits.

Marine surveys have now perhaps reached a level of sophistication such that it should be possible to evaluate the modern deep-sea record so as better to understand the rock record. We believe that the Alboran Sea and other selected basins of the Mediterranean should prove to be valuable natural laboratories to test the hypothesis that there are modern flysch basin counterparts.

We thank SACLANTCEN (La Spezia, Italy) for support of this particular study, an outgrowth of a larger investigation on the Alboran Sea. We also thank the captain, officers and men of the Maria Paolina G. and our colleagues, Messrs P. Blavier and M. Catello, for their help in obtaining the data at sea, and B. Tonarelli for preparing the detailed chart used as a base for Figs. 2 and 3. Support in the form of a travel grant and funding for research in La Spezia and Washington was provided to D. J. S. by the Smithsonian Institution Research Foundation and the Office of Naval Research, Washington.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Sunspots and Planetary Orbits

THE possibility of a relationship between the periods of the sunspot cycle and the periods of the rotation of the planets has been investigated by several authors[1–5]. Such an interaction could be mediated by non-linear processes of gravitational wave conversion of the type which may, for example, be responsible for the acceleration of the equator of the Sun[6,7]. On the face of things, the most likely relationship should be that between the sunspot cycle and the period of rotation of Jupiter, gravitationally the dominant planet, but non-linear may imply that cross modulation products representing the combined effects of two or more planets may be the most easily recognizable.

What follows is a successful correlation between the harmonic periods of the sunspot cycle as revealed by Anderson's single and double period analysis of the sunspot cycle and the cross modulation products of the sidereal periods of the four largest planets, Jupiter, Saturn, Uranus and Neptune. The reciprocals of the cross modulation frequencies (in years) are shown in Table 1.

Table 1. COMPARISON OF SUNSPOT CYCLE AND PLANETARY ORBIT ANALYSIS

Planets	Frequency (days^{-1})	Periods (yr)		338 Year fundamental	Anderson's sunspot cycle analysis (yr)		
		Upper sidebands	Lower sidebands	Harmonic No.	Single period	Double period	Relative amplitude
J + S	0·000323753	8·46		40	None		
J − S	0·000137865		19·86	17	9·95	19·9	13
J + S + U	0·000356342	7·68		44	None		
J + S − U	0·000291164	9·40		36	None		
J − S + U	0·000170454		16·06	21	8·05	16·1	13
J − S − U	0·000105276		26·01	13	13·00	26·0	11
J + S + N	0·000340367	8·04		42	8·05	16·1	13
J + S − N	0·000307139	8·91		38	8·90	17·75	26
J − S + N	0·000154479		17·72	19	8·90	17·75	26
J − S − N	0·000121251		22·58	15	11·26	22·5	70
J + S + U + N	0·000372956	7·34		46	7·35	14·7	3
J + S + U − N	0·000339728	8·06		42	8·05	16·1	13
J + S − U + N	0·000307778	8·90		38	8·90	17·75	26
J + S − U − N	0·000274549	9·97		34	9·95	19·9	13
J − S + U + N	0·000187068		14·64	23	7·35	14·7	3
J − S + U − N	0·000153840		17·80	19	8·90	17·75	26
J − S − U + N	0·000121890		22·46	15	11·26	22·5	70
J − S − U − N	0·000088662		30·88	11	15·36	30·75	10

The upper sideband values match components of Anderson's single period sunspot analysis, and the lower sidebands match double period components. These modulation products were calculated and grouped together as upper and lower sidebands of the orbital period of Jupiter:

Planet		(Sidereal period)$^{-1}$
Jupiter	(J)	0·00023080895 days^{-1}
Saturn	(S)	0·00009294371 days^{-1}
Uranus	(U)	0·00003258899 days^{-1}
Neptune	(N)	0·00001661419 days^{-1}
J − S − U + N	=	0·00012189044 days^{-1}
	=	22·46 yr

Periods from the Explanatory Supplement, 1961 Epheremis (Great Britain).

Six of seven of the highest amplitude sunspot harmonic components match these sideband periods. There seems to be a definite relationship between the orbital periods of the planets and the periodic nature of the sunspot cycle, and the relationship depends on a non-linearity in the solar energy conversion system.

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Mid-latitude Atmospheric Emission of N_2^+ First Negative Bands

THE first negative bands of N_2^+ are excited in the atmosphere by energetic particle precipitation. The cross section data of Srivastava and Mirza[1] imply an associated N_2^+ ion production rate of 14·1 ion pairs per photon in the 0–0 band or 41·5 ion pairs per photon in the 0–1 band, The total ion production rate for all species may be a few times greater and depends on the relative concentration of N_2, a function of the height at which the ionizing radiation is stopped.

Intensities of several Rayleighs for the 0–0 band at 3914 Å have been reported in middle latitudes by Yano[2] and others. The associated total ionization rates of order 10^8 (cm^2 column)$^{-1}$ s^{-1} could be important in the maintenance of the night time ionosphere. Broadfoot and Hunten[3], on the other hand, placed an upper limit of 1 R on the 0–0 band.

Photometric measurements on the 0–1 band at 4278 Å have been made at Adelaide since August 1969. The photometer, with a field of view of 2·5° semi-angle, uses a 3 inch diameter, 8 Å bandwidth interference filter which is periodically tilted to scan through the 4278 Å band head. The output from the photomultiplier is fed to a signal averager. Using total scanning times of 30 min to 60 min (giving detection sensitivities of 0·05 to 0·1 R) observations through more than 10 moonless nights of low geomagnetic activity ($Kp \leq 3$) have revealed that intensities of up to 0·1 may be present during some periods.

We conclude that the night time intensity of the 0–1 band does not usually exceed 0·1 R, implying a maximum total ionization rate of order 10^7 (cm^2 column)$^{-1}$ s^{-1}. Compared with the loss rate of ions through dissociative recombination in the F region this rate is small and cannot

be regarded as a major factor in the maintenance of the ionization through the night. It is also small compared with the ion production rate due to cosmic radiation. Using data of Millikan *et al.*[4], interpolated for the geomagnetic latitude of Adelaide, the total ion production rate due to cosmic radiation is $6{\cdot}5 \times 10^7$ (cm^2 column)$^{-1}$ s^{-1}. As the ionization peaks below 30 km we assume that about 75 per cent of the ions are N_2^+ and, using the cross-section data of Srivastava and Mirza[1], we infer an intensity of approximately 1 R for the 0–1 band. But this estimate makes no allowance for quenching of the $B^2\Sigma_u^+,v'=0$ level of N_2^+ from which the emission arises. This effect reduces the intensity by a factor $(1+s[Q]/A)^{-1}$ where s is the specific rate coefficient for quenching by molecules of number density $[Q]$ and A is the total transition probability for the first negative system. Using the data of Jeunehomme[5] on quenching by N_2 we evalute $s=2{\cdot}1\times10^{-9}$ cm^3 s^{-1} and $A=1{\cdot}4\times10^7$ s^{-1}.

In the 15 to 30 km region where ion production by cosmic radiation is greatest, the number density of N_2 is greater than 3×10^{17} cm^{-3}. The quenching term $s[Q]$ is thus large compared with the emission term A and the intensity of the 0–1 band excited by cosmic radiation will be a very small fraction of 1 R. Even at heights as great as 80 km, the quenching term may be significant in estimating ion production rate or particle energy flux from intensity of the N_2^+ first negative bands. This is particularly relevant to the study of polar cap events associated with proton precipitation.

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Search of Electrodynamic Radiative Corrections for Time Variations

A SIMPLE model of the electrodynamic radiative corrections was formed recently by combining different authors' hypotheses of neutrinos of non-zero masses and non-zero electromagnetic properties with the hypothesis of a neutrino sea[1–3]. The central notion of the model is that the magnitudes of these corrections would depend on the temperature and density of the neutrino sea at the point in question and these properties of the neutrino sea would vary in space and time.

The model predicts, among other things, that the different radiative corrections R, in particular the Lamb shifts $S(n,Z)$ (in Robiscoe's notation[1]) and the anomalous magnetic moments of the electron and muon, a_e and a_μ, should show simultaneous, correlated time variations. The model can be developed further by assuming that $S(n,Z)$, a_e, and a_μ are roughly proportional to each other and, originally, to the self-energy of the particle disturbed by the neutrino sea. The smaller ingredients of R, the vacuum polarization and so on, should not perturb this proportionality markedly. The theoretical values of R, calculated from the quantum field theory, represent the secular averages of the corrections R in question. The experimental and theoretical values of R are denoted by $R_{\rm exp}$ and $R_{\rm th}$, respectively. Then the values of the "relative discrepancy"

$$d = (R_{\rm exp} - R_{\rm th})/R_{\rm th}$$

for the different radiative corrections should be roughly comparable with each other. In terms of d, the scarce experimental data can be combined to form better statistics for the search for time variations.

There are, of course, many ways of forming working hypotheses about the origin of the proposed time variation of the neutrino sea. For instance, there may exist in space regions with elevated neutrino density due to gravitational focusing[5]. If a radiative correction is measured while the Earth is passing through such a region, an abnormally large value might be obtained. On the empirical side, there is some slight experimental evidence for the local enhancement of Lamb shifts in solar active regions[1,2]. The working hypothesis that solar activity would be associated with activity in the neutrino sea was therefore accepted. Thus the transient large-scale activity of the Sun might conceivably correlate with changes in R on the Earth. The dates of the measurements reported in the literature, however, are not usually given accurately enough to warrant a correlation with any transient events. Perhaps this possibility could be checked by the authors from their daily results. Meanwhile a correlation with the 11 year cycle could be sought from the available data.

In this context a new model of the solar energy production, due to Sheldon[6], should be considered. Starting from the null result of the solar neutrino experiment of Davis *et al.*[7], Sheldon proposes that the solar nuclear reactions (and the neutrino production) are "switched on" only for a comparatively short period during each 11 year cycle. This model could be taken as the working hypothesis for the present study. The explanation of the null result need not necessarily be accepted simultaneously in the form presented by Sheldon, however, because another explanation is also possible in terms of the present model of neutrino interactions: before reaching the detector, the high energy solar neutrinos might be decelerated to energies under the detection threshold by their interactions with atoms and with the neutrino sea.

As usual I shall use the number of sunspots as the measure of solar activity, and minimum and maximum activity are denoted by $t_{\rm min}$ and $t_{\rm max}$ respectively. For the previous three cycles (numbers 18, 19, and 20 by Bonov[8]) we have approximately $t_{\rm min}=1954{\cdot}2$, $1964{\cdot}7$, and $1976{\cdot}5$, and $t_{\rm max}=1947{\cdot}3$, $1957{\cdot}7$, and $1968{\cdot}4$. The rise of sunspot number is more abrupt than the descent, so the active period may be considered to extend from $t_{\rm max}-1$ yr to $t_{\rm max}+1{\cdot}5$ yr and the quiet period from $t_{\rm min}-3$ yr to $t_{\rm min}+1$ yr. Other periods are termed intermediate.

Except for the work of Triebwasser *et al.*[9], the dates of the manuscripts are used as "first approximations" to the dates of measurements. The delay time may even be physically justifiable to some extent; Sheldon's model suggests that the appearance of sunspots would be delayed considerably with respect to the production of neutrinos.

Table 1 presents the values of d for which the total standard deviation $\sigma=(\sigma_{\rm exp}^2+\sigma_{\rm th}^2)^{\frac{1}{2}}$ is less than 900 p.p.m. In the weighted means $\bar{d}$, in which the weights are σ^{-2}, a measurement d with σ larger than 900 p.p.m. would be neglected in any case. The result of Robiscoe and Cosens[12], which is in an intermediate period, is based on two measurements of which the former was carried out during a quiet period. This complication is not important because the former measurement would not change the value of $\bar{d}$ significantly in either period.

The number of useful observations is very small, and there is only a single measurement to determine the value of $\bar{d}$ for the intermediate periods. The situation is not much better in the active and quiet periods. Only one active

Table 1. COMPILATION OF DATA

Solar period	Quantity	Measurement Authors	Year*	Ref.	$d \pm \sigma$ (p.p.m.)	Ref.	$\bar{d} \pm \sigma_{\bar{d}}$ (p.p.m.)
Quiet	$S(2,H)$	Triebwasser *et al.*	1952	9	-132 ± 76	22	-93 ± 24
	$S(2,D)$	Triebwasser *et al.*	1952	9	-161 ± 85	22	
	a_e	Wilkinson and Crane	1962	10	-82 ± 26	13	
Intermediate	$S(2,2)$	Lipworth and Novick	1956	11	-306 ± 178	22	0 ± 7
	$S(2,H)$	Robiscoe and Cosens	1966	12	-9 ± 76	22	
	a_e	Wesley and Rich	1970	13	0 ± 7	13	
Active	$S(2,D)$	Cosens	1968	14	$+104 \pm 85$	22	$+61 \pm 66$
	$S(2,2)$	Narasimham	1968	15	$+71 \pm 145$	22	
	$S(3,2)$	Mader and Leventhal	1968	†	-477 ± 262	22	
	a_μ	Bailey *et al.*	1968	17	$+249 \pm 292$	23	
	a_e	Gräff *et al.*	1969	18	$+14 \pm 259$	13	
Active: indirect measurements	$S(2,H)$	Kaufman *et al.*	1968	19	-246 ± 66	22	-148 ± 42
	$S(2,H)$	Shyn *et al.*	1969	20	-123 ± 85	22	
	$S(2,H)$	Cosens and Vorburger	1969	21	-47 ± 76	22	
	$S(3,2)$	Mader and Leventhal	1968	†	-96 ± 191	22	

* Date of the manuscript. † Private communication reported in ref. 16.

period is covered by the measurements. Some preliminary conclusions can be drawn nevertheless. First, the values of d obtained from a_e (and a_μ) agree with the values of d obtained from $S(2,1)$ for similar periods. This supports the hypothesis of proportionality already presented. Second, the results comply with the 11 year variation, irrespective of whether $S(2,1)$, a_e, and a_μ are considered separately or together. Thus, if the best results on $S(2,1)$ (refs. 9, 12 and 14) are compared with each other, $S(2,1)$ is found to increase with increasing solar activity. The two good results on a_e (refs. 10 and 13) also comply with the prediction. Because of the large weights of these good measurements, the values of $\bar{d}$ also show the 11 year variation.

There is serious disagreement with the predictions in the "indirect" determinations of $S(2,Z)$ from the measurements of $\Delta E - S(2,Z)$ (spacing $2P_{3/2}$–$2S_{1/2}$). The corresponding values of $S(2,Z)$ disagree with the theoretical values and with the two recent good experimental values[12,14], whether the hypothesis of the time variation is true or not. This suggests that the indirect measurements contain an unknown error.

In spite of the suggestive agreement between the predictions and the observations, only new experimental data can settle the problem. If the values of d, especially for a_e, become negative during 1974–77, the 11 year variation of the radiative corrections would be proved. If the opposite occurs, this particular variation would be ruled out, but other kinds of time variation could still exist.

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The Free Dynamics of Pulsars

THE rotational dynamics of freely spinning pulsars are discussed below; the effect of pulsar nonrigidity and some possible consequences of viscosity are included. Expressions given for the period of the Eulerian free oscillation (or free precession) of pulsars are of particular interest. This oscillation is a permitted solution of the free rotational dynamics because, as a result of centrifugal effects, pulsars are somewhat oblate, and any nonspherically symmetric body, which is not a perfect fluid and is spinning freely in space, will wobble about the constant direction of its angular momentum vector. It will be mentioned that this phenomenon may account for some of the amplitude and time variations seen in pulsar signals and, if observed, could give the first fairly accurate measurement of a pulsar's size.

Other articles[1–4] have already discussed the rotational dynamics of pulsars under the action of torques caused by the electromagnetic forces present; they have looked into the alignment of the magnetic dipole in the oblique rotator model and the decay of the spin itself which indicates the pulsar age. The work presented here will supplement these calculations by presenting the solutions to the homogeneous equations of motion and, because the actual torques are so weak, will more clearly represent a pulsar's rotational motion over a short time. The free motion of pulsars has previously been discussed[5,6] but only in quite different contexts. Several researchers[7–9] have recently discussed similar problems.

The equilibrium shape of a homogeneous, freely rotating fluid body is, in general, that of a MacLaurin spheroid for slow rotations[10]. The spheroid's flattening $f \equiv (c-a)/a$, where c and a are, respectively, the polar radius and equatorial radius of the body) is a unique function of the parameter $\omega^2/2\pi\rho$, in which ω is the body's spin frequency and ρ its density. In the case of nonhomogeneous bodies with a given initial density distribution, a series solution can be found[11] giving f as a function of a nondimensional surface acceleration

$m = \omega^2 a/g_e$ where g_e is the gravitational acceleration at the equator $r = a$. This solution shows that to $0(f^2)$, f lies between $m/2$ and $5m/4$ where the lower bound corresponds to a hypothetical case in which all the mass is concentrated at $r = 0$ and the upper bound represents the exact solution for a homogeneous body.

Thus, assuming that over long periods of time pulsars react like fluids and ignoring any deformation caused by magnetic effects (because it will be relatively small[7-9] for expected values of the magnetic field),

$$f \sim m = \omega^2 a/g_e = 4\pi^2 a^3/(GMP^2) \quad (1)$$

where P is the pulsar's spin period, assumed to be the period of its signal, and M is its mass; G is the universal gravitational constant. For typical models of the Crab pulsar NP 0532, equation (1) gives $m \sim 10^{-3}$.

Before considering the effects of fluidity and viscosity, we discuss the motion of a rotationally symmetric rigid body which is spinning freely. The solution[12,13] of the Euler equations shows that such a body (and its attached coordinate system) has a constant angular velocity about its symmetry axis z while the vector $\omega_x \mathbf{i} + \omega_y \mathbf{j}$ is of constant magnitude and precesses uniformly about z with the Eulerian free oscillation period

$$P_e = 2\pi I/(\omega_z \Delta I) = (I/\Delta I)P \quad (2)$$

where I is the moment of inertia about any axis lying in the equatorial plane and $(I + \Delta I)$ is the moment of inertia about the z axis. Thus a free body rotates or wobbles about the direction of its constant angular momentum vector with a constant amplitude if there is no energy dissipation.

For the slightly distorted shape of an ellipsoid[10]

$$\Delta I/I = 1/2[1 - (c/a)^2] \sim f \quad (3)$$

The effect of nonrigidity is to lengthen the Eulerian free precession period[11,14,15] while leaving unchanged the qualitative picture of the dynamics. This occurs because any portion of the rotational bulge which is able to follow the instantaneous rotation axis will not affect the free precession; for instance, in the limiting case of a rotating perfect fluid, no precession will occur for ω will always be aligned along the principal axis. Quantitatively, the Eulerian free oscillation period, given by equation (2), is replaced for a nonrigid body by[11,15]

$$P'_e = (2\pi/\omega_z)\left(\frac{A + k\omega_z^2 a^5/3G}{C - A - k\omega_z^2 a^5/3G}\right) \quad (4)$$

where k is the Love number which specifies the fractional disturbance of the surface potential due to a second degree tidal potential. We may calculate k for a particular body, and for an incompressible homogeneous sphere it is $3/2(1 + \mu)$ in terms of the nondimensional rigidity $\mu = 19\bar{\mu}/(2\rho g_e a)$ with $\bar{\mu}$ the rigidity and ρ the density[15].

Ruderman[16] has shown that the outer layers of neutron stars will cool very rapidly by neutrino emission processes to form an outer crust which will be relatively thick for all but the most massive neutron stars. The crust is comprised of nuclei arranged into a body centred cubic lattice. This knowledge permits an estimate[17] of the crustal rigidity: $\bar{\mu} \sim 10^{30}$ dynes cm^{-2}. Thus for NP 0532, $\mu \sim 10^{-3}$, which indicates that the pulsar surface, although extraordinarily strong by terrestrial standards, behaves much like a fluid in the presence of the pulsar's enormous gravitational field. Considering $\mu \ll 1$ and assuming the pulsar superfluid core to have a negligible effect, we find $P'_e = r^{-1} P_e$ and thus the free precession period would be increased substantially by the fluidlike character of the crust.

Equation (4) assumes an inviscid body, but for completeness we must ask whether viscous effects can prevent the body from acting like a fluid and cause it to maintain its rigid configuration. This can happen only if frictional forces dominate the inertial forces, that is, if the Reynolds number $2\pi a^2/\mu\omega_z \nu \ll 1$ with ν the kinematic viscosity. For a typical pulsar this requires $\nu \gg 10^{14}$ cm^2 s^{-1}. Unfortunately, little information is available on neutron star viscosities beyond an estimate[16] of the viscosity of the inner core. More is known about the terrestrial solids; for instance, $\nu \sim 10^{13}$ cm^2 s^{-1} for glacial ice and the value for the Earth's mantle is $\sim 10^{22}$ cm^2 s^{-1} (see ref. 11). If the crust's viscosity is large (or if there is a very strong magnetic field[18]), the pulsar will respond as though it is a rigid body for the high frequency spin and precession motions. In this case the solution (2) is once again valid; in analogy with the simple mass spring dashpot system, there is little time delay in the response of the body and little energy dissipation. The intermediate case of $\nu \sim 10^{14}$ cm^2 s^{-1} will have large energy dissipation but the dynamics are too difficult for discussion here.

From equations (2) and (3), we see that the body axes system for a slightly distorted viscous spheroid precesses with a period

$$P_e = f^{-1} P \sim m^{-1} P$$

or about 10 s for the Crab pulsar. In the inviscid case ($\nu \ll 10^{14}$ cm^2 s^{-1}), equation (4) gives $P'_e \sim (m\mu)^{-1} P$ or several hours for the Crab pulsar if the asphericity is determined solely by rotation.

It is not possible to specify the amplitude of the Eulerian oscillation without knowing initial conditions from which to derive the constants of energy and angular momentum; however, the amplitude is known to be small in the case of the Chandler wobble of the Earth. Whatever the initial amplitude, it will decay in time because of viscous dissipation and electromagnetic torques and, if this oscillation is to be significant, there must be an impulsive energy input from time to time. This same argument has been used in the case of the Earth; it is difficult to understand why the Earth's axis should wobble at all[15], but recent evidence[19] suggests that earthquakes may be the energy source for the Earth's wobble. It is therefore of some interest that the abrupt jumps $\Delta\omega$ in frequency observed in several pulsars (for example, NP 0532 and PSR 0833) have been explained in terms of starquakes which decrease the pulsar's moment of inertia and thereby cause ω to increase so that angular momentum is conserved. In fact, starquakes can be thought of as being generated as the pulsar attempts to go from an oblate shape to a more spherical one because of the slowing spin velocity[17,20]. Recently Ruderman[6] published an extension of his idea on starquakes in which he discusses the very low frequency wobble of the Crab pulsar as being a result of precessional motion and tries to relate changes in the wobble period after the starquake with changes in the moment of inertia caused by the starquake. During the discussion, he assumes that the pulsar's shape responds immediately to any change in the rotation axis.

Whatever the mechanism causing the frequency jumps, the spin rotational kinetic energy E is increased by an amount $\Delta E \sim (\Delta\omega/\omega)E$. If energy of the same order of magnitude is put into the precessional mode, large amplitudes are possible for the precessional motion. The amplitudes seen, however, may be just a remnant of the initial condition; the precessional decay rate should not be too dissimilar from the spin decay rate.

Our picture of the rotating neutron star, as seen over the short period when the weak torques can be neglected, is a body whose angular momentum vector is constant from an external point of view. It is, however, a picture in which the magnetic moment axis (which may be considered as piercing the surface at a fixed point) appears to wobble in space because of the combined motions of spin and precession. This wobble can produce time variations in the signal period, as has already been described by Ruderman[6].

The wobbling will also permit the emissions from a narrow angular source on the pulsar to sweep out a larger angular area of the sky during one precession period. This will increase the probability of seeing any pulsar which has a narrow emission cone, and the pulsar number density may be less than is currently thought by those who believe the emission is beamed. Correspondingly, because the amplitude of the received signals from such a pulsar will depend on the portion of the emitting region seen, the signals will be modulated according to the orientation of the magnetic axis relative to the spin axis and the magnitude of the precession angle. This modulation may account for part of the amplitude variations[21] seen in pulsar signals. The period of this modulation will be P_e (or P'_e); modulations of pulsar signals have been sought[22,23] but only at much shorter periods.

Even if the amplitude of this precession is small, it may be of considerable interest if its period is measurable. If a period of modulation can be obtained, we can reverse the procedure described above for f is expected to be very small. Thus, because $m \sim f$, we have a measure of the nondimensional surface acceleration as given in equation (1). Using this, we can write the pulsar radius as

$$a = [GM/4\pi^2 P_e]^{1/3} P$$

for the viscous case where everything is known except M, which, according to current models, must be near one solar mass. In this way, a pulsar radius could be measured for the first time. This result should be useful as a constraint on future neutron star models.

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Electron Intensity Variations in the Inner Belt following a Geomagnetic Storm

Loss characteristics of inner belt electrons have been studied since the Starfish and USSR nuclear explosions in the upper atmosphere in 1962[1-3]. The solar flares of September 2, 1966, and May 23, 1967, were the first natural events to increase the electron population above the artificial level[4,5]. We have studied the electron flux levels following the May 1967 event for a three month period starting in August 1967. Electron flux levels above 120 keV were monitored by ionization chambers also used as solar X-ray monitors on OGO IV in polar orbit. Supplementary electron data above 280 keV were obtained from the C1 detector on the 1963 38C satellite also in polar orbit. Details of the 1963 38C detectors have been described previously[1].

Table 1. OGO IV IONIZATION CHAMBER CHARACTERISTICS

Detector	D1	D2
Window thickness (mg cm^{-2} of beryllium)	243	23·5
Chamber gas effective thickness (mg cm^{-2})	18·9	4·52
Geometric factor (cm^2 sterad)	0·42	0·35
Field of view	±8·8°	±8·8°
Electron cutoff energy for chamber window (keV)*	550	120
Proton cutoff energy for chamber window (MeV)†	12·5	3·5

* 10 per cent of maximum response.
† Theoretically determined range (experimentally verified to within 1 MeV).

Two OGO IV ionization chambers provided night-time particle data in the region of the South Atlantic Anomaly. The ionization chambers are identical except for window thickness[6,7] and have been calibrated for proton and electron response[8] (Table 1). In addition to the ionization current induced by electrons and protons penetrating the chamber windows, some ionization current is produced by high energy protons (> 40 MeV) which penetrate the 0·25 cm stainless steel chamber walls. Proton flux data[9] which were time invariant over the period of interest[10] indicate that more than 80 per cent of the D1 ion current may be attributed to penetrating protons and can be used as a proton background monitor for the D2 chamber. The D2 ionization current is dominated by low energy electrons (> 120 keV) passing through the chamber window. Low energy protons passing through the windows

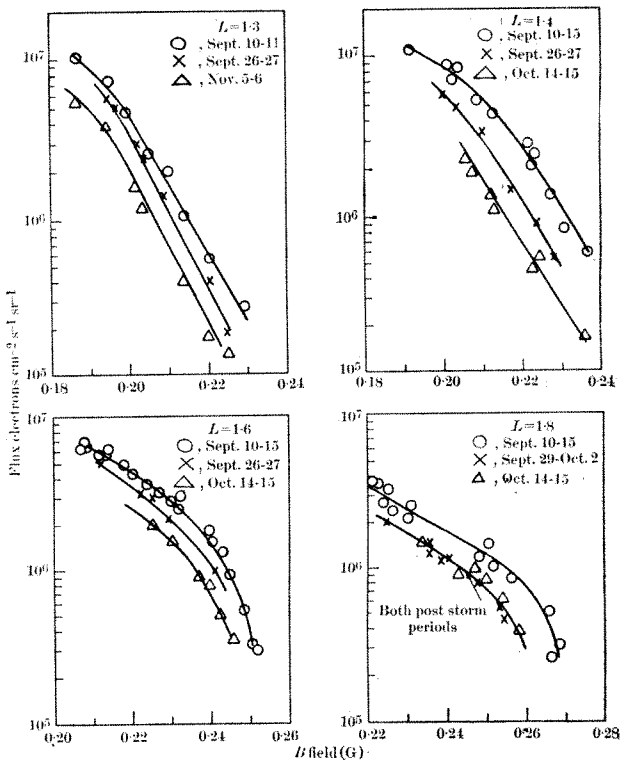


Fig. 1. D2 electron flux against mirroring field at selected L shells. Each curve is fitted to data of the three selected time intervals except at $L = 1{\cdot}8$ where no significant difference appeared between the flux at the two selected post storm periods.

of either chamber do not make a significant contribution to the ionization currents observed[11].

OGO IV data cover L shells from 1·3 to 1·8 and B fields from 0·18 to 0·25 G. Somewhat smaller fields were covered by 1963 38C due to its higher orbit. The OGO IV ionization chamber look direction was established by reference to the orbital plane experiment platform. Data were taken only when the detectors were oriented within $\pm 10°$ to the normal plane of the local field, as determined by the forty-eight coefficient field model of Jensen and Cain. The field value at which the particles would mirror was obtained by using the adiabatic invariance of the electron magnetic moment.

A check on the field value computed by the Jensen and Cain model was provided by the D1 chamber sensitive to omnidirectional protons. Plots of D1 current against field were made using data over the 3 month observation period and a least squares fit established a nominal relationship between the field and the D1 current for each L shell. At $L = 1·3$ the standard deviation was $\pm 0·002$ G over the field range considered here. At $L = 1·8$ the deviation was $\pm 0·006$ G with somewhat smaller errors at intermediate L shells. Since the D1 and D2 currents were observed simultaneously, the Jensen and Cain field was corrected by using the nominal field value corresponding to the D1 current on that L shell. This method prevents errors in the field values which may be a function of altitude and hence a function of time.

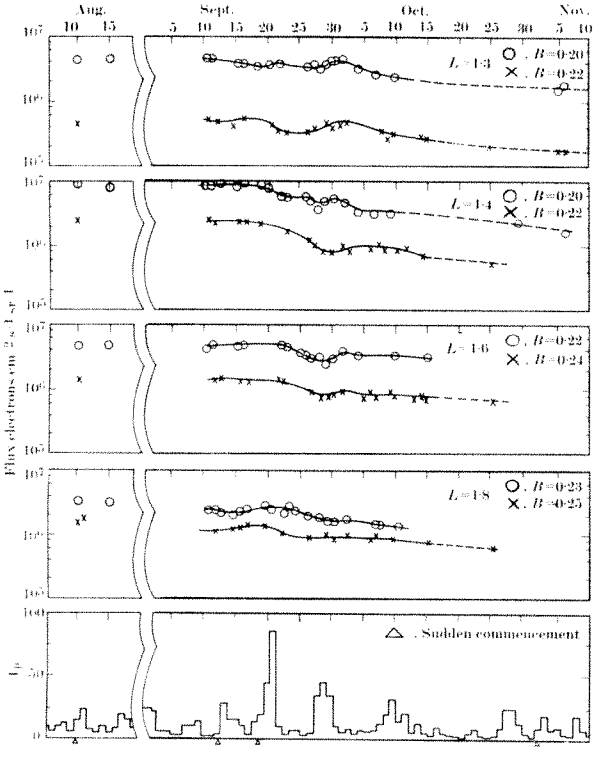


Fig. 2. Daily averages of D2 flux over the two month period during which significant variations occurred. The data shown are selected from mirror fields $\pm 0·005$ G given from the field values to which the data were normalized. The index A_p is also shown.

The electrons mirroring at field values reported here remain above 350 km as they drift eastward through the Anomaly. Previous work[12,13] indicates that no significant losses should occur due to atmospheric scattering. OGO IV electron data (> 120 keV) obtained on both sides of the Anomaly did not indicate any electron loss above 350 km.

Solar activity was considerable during late May 1967. The 3B flare of May 23 seems to be associated with the geomagnetic storm of May 26 ($A_p = 146$). Between June 6 and September 19, 1967, the A_p daily index remained below 29. The September 21 storm ($A_p = 85$) seems to be associated with two 2B flares occurring 3 days before. A smaller disturbance occurred on September 29 with $A_p = 45$.

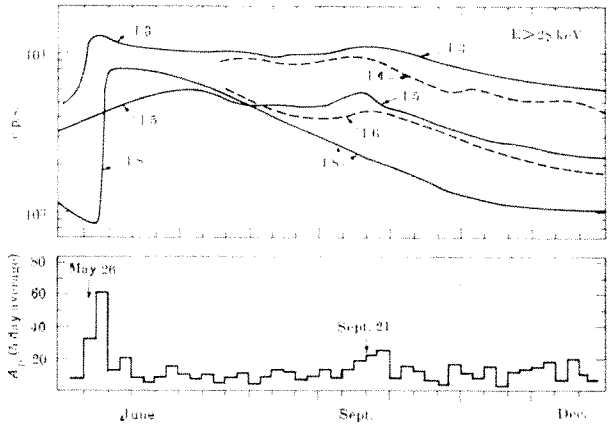


Fig. 3. C1 data from 1963 38C taken from refs. 5 and 10. The curves shown represent a best fit to the ten day average flux values. The mirror field ranges from which the data have been selected are also shown along with the five day average values of A_p.

L shell	B range
1·3	0·165–0·175
1·4	0·180–0·190
1·5	0·190–0·200
1·6	0·195–0·205
1·8	0·200–0·210

Each plot in Fig. 1 indicates the flux variation with the mirroring field for electrons on a given L shell at the specified time observed with the D2 chamber on OGO IV. Most striking is the rapid change in the electron flux following the September 21 storm. Largest decreases occur at the largest fields for which data are available, particularly at $L = 1·3$ and 1·4. Decreases are generally smaller at larger L shells but not as confined to the region near the loss cone. Twenty-five days following the storm the flux had decreased on all L shells and fields covered by OGO IV.

The field dependence of the flux is removed in Fig. 2 by making plots similar to those shown in Fig. 1 for short time periods over which the flux variation is not significant. Using the derived flux field relationship appropriate to the time period, the flux is normalized to one of the two field values indicated for each L shell. Only data requiring a correction of less than 0·005 G are used in these plots. The largest response to the September 21 event is at $L = 1·4$ and $B = 0·22$ G where the flux is reduced to one-third of its initial value in 8 days. At $L = 1·8$ a small fluctuation coincident with the September 21 events occurs; it does not, however, cause a significant change in the general decay rate observed for this L shell before the storm. In addition to the general flux decrease following the September 21 event, the effects of a smaller storm on September 28 are also observable. The electron population seems to respond more rapidly to this second storm, particularly at weaker fields.

Fig. 3 indicates the C1 detector data on 1963 38C for the time period of interest[10]. The data for each L shell are the 10 day average for electrons mirroring within the range of field values specified. For $L < 1·6$ the decay in the electron flux (> 280 keV), initiated after the large increase in May, had ceased before September 1967. A modest increase, particularly at $L = 1·5$, is observed. The uniform decay, observed in the OGO IV data at lower energies, seems to initiate in late September and continue for at least 3 months. The flux at $L > 1·7$ seems to be unaffected by the September event at the weaker fields and

higher energies covered by 1963 38C. This marked difference in response to the September event is also observable in the OGO IV data. The 1963 38C data do not have adequate time resolution to associate the decay observed by the C1 detector with the September 21 event except at $L = 1{\cdot}5$ where the flux was reduced by more than a factor of two in the ten day period following September 21.

Until recently, short term electron population decay characteristics had only been studied in the inner belt following the high altitude nuclear bursts in 1962. Van Allen's[3] measurements at $L < 1{\cdot}5$ indicate that 0·5 MeV electrons mirroring at large B fields decayed more rapidly than equatorially mirroring electrons. This is consistent with the trend reported here.

The May 23, 1967, flare and subsequent geomagnetic storm are relevant to the observations reported here. This solar event caused an observable increase in the trapped electron flux for $E > 280$ keV over the entire inner belt[9]. The May 1967 increase was large and well defined for $L > 2{\cdot}0$ and $L < 1{\cdot}4$. At $L = 1{\cdot}5$ the data of Bostrom *et al.*[5] indicate the $E > 280$ keV flux required more than 30 days to reach maximum value. It would seem that those L shells which responded slowly to the initial particle population increase were most strongly affected by the September event.

For electrons mirroring at latitudes appreciably off the geomagnetic equator, the flux levels in the inner belt seem not to be characterized by simple decays from the one or two large events which cause major increases in the inner belt each year. Detailed consideration must be given to the smaller geomagnetic events subsequent to the major storms for a proper description of the temporal variations of electrons mirroring at intermediate latitudes in the inner belt.

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Extrapolation of the Shear Elastic Moduli of Face Centred Cubic Solids to the Molten State

The principal characteristic of liquids is their zero elastic resistance to shear. The change in the shear elastic moduli of solids near their melting point is therefore of some interest in connexion with the process of melting. The data available[1] show that the elastic stiffness in general decreases with temperature, but does not reach zero at the melting point. It seemed that a more informative way of treating the data than plotting the moduli against temperature might be to plot them as functions of the volume (at constant pressure). When this was done it was found that the points fell very nearly on a straight line, and it became clear that in the case of close packed solids where the structure of the melt is known to be similar to that of the solid it would be possible to obtain an estimate of the shear modulus of the melt by extrapolating this line to the volume of the melt.

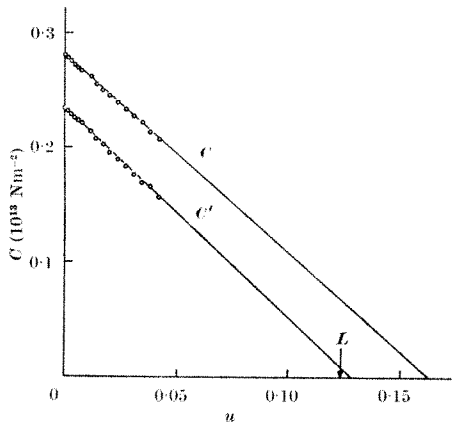


Fig. 1. The shear elastic moduli of aluminium plotted against u, the fractional increase in volume (relative to 0° C). The arrow at L indicates the value of u for the melt.

This procedure was first tried for aluminium for which values of the shear moduli C ($= c44$) and C' ($= (c_{11} - c_{12})/2$) are available nearly to the melting point[2]. In Fig. 1 these are plotted against values of $u (= (v - v_0)/v_0)$, where v is the specific volume at a given temperature, and v_0 is the specific volume at a convenient reference temperature, usually 0° C. It will be seen that the experimental values lie on straight lines and that when extrapolated the line for C' falls to zero very near the value of u corresponding to the melt. The line for C reaches zero at a higher value of u.

For most other f.c.c. melts elastic data were only available over the range 0–300 K so that the data had to be extrapolated over a much wider gap. For lead some data have recently been obtained between 0° and 120° C (ref. 3) and these have been used together with the earlier values[4] to give similar results to those obtained for aluminium.

Table 1

Element	Value of u at which the shear modulus becomes zero on extrapolation	Value of u for the melt
Al	0·125	0·123
Cu	0·113	0·127
Ag	0·110 ($C' = 0$)	0·127 (ref. 7)
Au	0·114	0·117
Pb	0·059	0·064
A	0·215 ($C = 0$) 0·245 ($C' = 0$)	0·243 (ref. 9)

The values for copper[5], silver and gold[6] have been treated in the same way, but owing to the wide extrapolation involved must be treated with some reserve. In Table 1, the value of u for which the linear extrapolation of the shear modulus reaches zero is compared with the value for the melt[7].

The recent measurements of the elastic moduli of solid argon[8] enable us to apply the same method of plotting the shear moduli to this case. We now take the reference temperature as 0 K and using the volume data of Dobbs and Jones[9] we obtain the plots shown in Fig. 2. These show a pronounced curvature especially at the low temperature end of the curve. Linear extrapolation of the high temperature parts of the curves for both C and C', however, leads to zero values in the region of the value

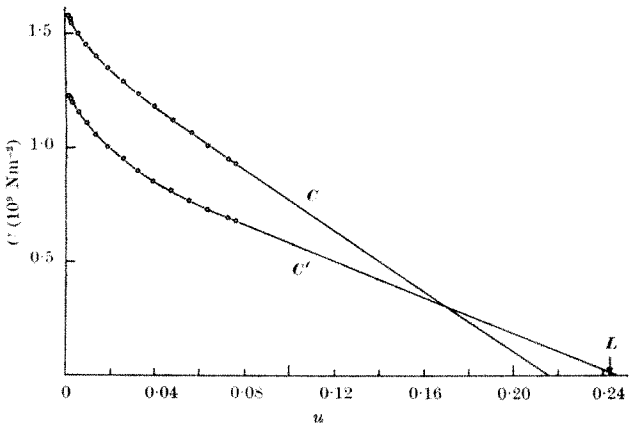


Fig. 2. The shear elastic moduli of argon plotted against u, the fractional increase in volume (relative to 0 K). The arrow at L indicates the value of u for liquid argon.

of u for the liquid, though in this case C reaches zero at a slightly smaller value of u.

The errors in the values of u were estimated by comparing independent estimates. The figures in the third column were taken from the review article by Borelius[7]. The main uncertainty comes from the expansion on melting. Comparison of the figures in the literature suggests that an error of about 0·01 in the values in column 3 would be reasonable. The figures in column 2 were obtained from several estimates using different treatments of the expansion data. These varied by about 0·005. It seems therefore that differences between column 2 and column 3 are not significant, but the correlation between the two sets of values near 0·06 (Pb), 0·12 (Al, Cu, Ag, Au) and 0·24 (A) is significant.

These results suggest that the zero value of the shear modulus of liquids is a consequence of their low density. In other words, if the atoms of the liquid were to be placed on an f.c.c. lattice at the same density they would form a "solid" of zero shear modulus.

It would be of interest to test this suggestion by further studies of the high temperature variation of the elastic contents, and it would also be important to extend these measurements to high pressures.

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BIOLOGICAL SCIENCES

N-Terminal Sequences of Immunoglobulin Heavy and Light Chains from Three Species of Lower Vertebrates

It has been established that immunoglobulin heavy (H) and light (L) chains have a variable amino terminal region of about 110–120 residues[1–9]. This region presumably makes up the antibody binding site and is responsible for the high degree of antigenic specificity that is a characteristic feature of these molecules. In spite of the growing body of amino-acid sequence data on immunoglobulin H and L chains, the genetic mechanisms by which an almost infinite array of specific antibodies can be expressed in response to antigenic stimulation remain to be established. It has been proposed, as one possible explanation, that the genes coding for the immunoglobulin polypeptide chains, are present in the germ line[10–11]. This study was prompted by this concept, as well as the structural similarities that have been demonstrated among various H and L chains, suggesting that genes coding for these chains evolved from a common ancestral gene[12,13]. We have investigated immunoglobulins from three lower vertebrates in the hope that sequence data of the variable region of these chains will provide an insight into the genetic mechanisms responsible for antibody variability.

For these studies antibodies were isolated against *Salmonella typhosa* "O" antigen from three species of animals; an amphibian, the marine toad (*Bufo marinus*), two types of bony fish, the paddlefish (*Polyodon spathula*) —a chondrostean representative—and the gar (*Lepisosteus osseus*)—representing the holosteans. Purification of the immunoglobulins was achieved by a combination of chloroform : methanol extraction, ion exchange chromatography and gel filtration. Antibody activity was followed throughout the purification process. The homogeneity of the purified material was established in each preparation by immunoelectrophoresis and ultracentrifugation.

For further studies the isolated immunoglobulins from each species were totally reduced and alkylated in 7 M guanidine–HCl and the H and L polypeptide chains separated by gel filtration on 'Sephadex G-200' columns in the presence of 5 M guanidine–HCl. The isolated H and L chains were homogeneous on electrophoresis in acrylamide gel containing 5 M urea.

Table 1. COMPARISON OF PHYSICAL AND CHEMICAL PROPERTIES

Species	$s^{\circ}_{20,w} \times 10^{-13}$	Mol. wt.	Percentage carbohydrate	Chain mol. wt. H	Chain mol. wt. L
Marine toad	17·9	850,000	9	72,000	23,000
Marine toad*	7·3	173,000	n.d.	63,000	23,000
Paddlefish	14·2	630,000	7	72,000	23,000
Gar	13·6	600,000	9	72,000	23,000

* Low molecular weight antibody.

In Table 1 some of the physical and chemical studies of these molecules are compared. From the marine toad, two types of immunoglobulins were isolated: a macroglobulin resembling mammalian IgM in molecular weight, carbohydrate content and chain molecular weight, and a low molecular weight antibody which cannot be equated with a mammalian class of immunoglobulin[14]. The amino-acid composition, peptide maps and antigenic analysis revealed that the two types of antibodies in the marine toad represented distinct classes.

The paddlefish and gar were found to contain macroglobulins that were significantly lower in molecular weight than that found in the marine toad. These molecules did, however, have H and L chains of the same size as in the macroglobulin of the marine toad. Preliminary evidence indicates that these molecules have a tetrameric molecular form similar to the macroglobulins recently isolated from another bony fish, the carp (*Cyprinus carpino*), by Shelton and Smith[15]. This is in contrast to the pentameric configuration common to man, rabbit, mouse, sheep, chicken and dogfish shark[16–18].

The amino-acid compositions of the macroglobulin H and L chains from all three species closely resembled each other and are very similar to human IgM[19]. This is especially evident in the number of methionine and cystine residues and a high content of serine, threonine and acidic amino-acid residues which seems to be a characteristic feature of all these macroglobulins.

N-terminal sequence analyses of the isolated H and L chains were carried out by the Edman degradation method as described by Waterfield and Haber[20]. These analyses are shown in Table 2. The L chains from all the species examined as well as the H chain from the marine toad low molecular weight antibody were found to be non-reactive in the Edman procedure. We have assumed that the amino terminal residue is pyrrolidonecarboxylic acid (PCA), which has been found at the N-terminus of most H chains and also the lambda type L chain from mammalian immunoglobulins[1-9,21,22]. The blocked H and L chains of the marine toad low molecular weight antibody and the paddlefish macroglobulin L chain were digested with pronase and the blocked N-terminal pronase peptides from each of the chains were isolated from a 'Dowex 50–X2' column as described by Bennett[22]. The results in Table 2 are suggested sequences for these blocked N-terminal peptides based on amino-acid compositions of the principal peptides deduced from molar yields. It is possible in this case that other blocked N-terminal peptides are present in much lower yields. The H chains from all three species of macroglobulins had a free N-terminal residue. The yield of the residues on a relative molar basis indicates that the sequence shown for each of these chains represents its principal N-terminal sequence.

Table 2. COMPARISON OF AMINO TERMINAL SEQUENCES

Source	Chain type	Residue position 1	2	3	4	5	6	7
Marine toad macroglobulin	Heavy	—	Ser-	Ile				
Marine toad macroglobulin	Light	PCA						
Marine toad LMWA	Heavy	(PCA,Ser,	Asx,Glx)					
Marine toad macroglobulin	Light	(PCA,Ser,	Asx,Glx,Thr)					
Paddlefish macroglobulin	Heavy	Asp-	Val-	Val-	Val-	Thr	Leu	
Paddlefish macroglobulin	Light	(PCA,Ser,	Glx,Glx,Thr,Thr,Tyr)					
Gar macroglobulin	Heavy	Asp-	Val- Ile Ala- Leu-	Val- Leu	Val			
Gar macroglobulin	Light	PCA						

The amino terminal residue was nonreactive in the Edman procedure and we have therefore assumed the presence of pyrrolidonecarboxylic acid.

Even the limited sequence data reported here suggest a degree of homology among these chains, as well as homology to immunoglobulin chains from mammals. These data are similar to those reported for human mu and lambda chains and for rabbit gamma chains[21-23]. The sequences of the paddlefish and gar H chain seem to be very similar to those of the leopard shark and rabbit L chain sequences[24,25]. It is also of interest relative to our H chain data that a free N-terminal residue of glutamic acid has been reported from a few mu, gamma and alpha heavy chains of man[8,9]. Such similarities provide additional evidence that immunoglobulin H and L chains had a common evolutionary origin.

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Very Lysine Rich Histone of Echinoderms and Molluscs

THE similarity of the histone component F2a1 from calf and pea[1] casts doubt on the hypothesis that it acts as a selective repressor of gene activity. Such a function may, however, be fulfilled by the very lysine-rich fractions F1 because they are significantly heterogeneous in vertebrate species[2,3]. Alternatively, the heterogeneity might be explained in terms of gene multiplicity and/or a modification of some amino-acid residues[4] and may have no implication in gene regulation. It would then have an evolutionary significance, similar to that of the heterogeneity in proteins such as haemoglobin and cytochrome. We thought that it would be of interest, therefore, to study the very lysine-rich component in different species, particularly those far apart on the evolutionary scale. We chose a mollusc and an echinoderm and found that their F1 histones differed from each other in a limited but significant way and that they also differed from the equivalent F1 fraction from calf thymus.

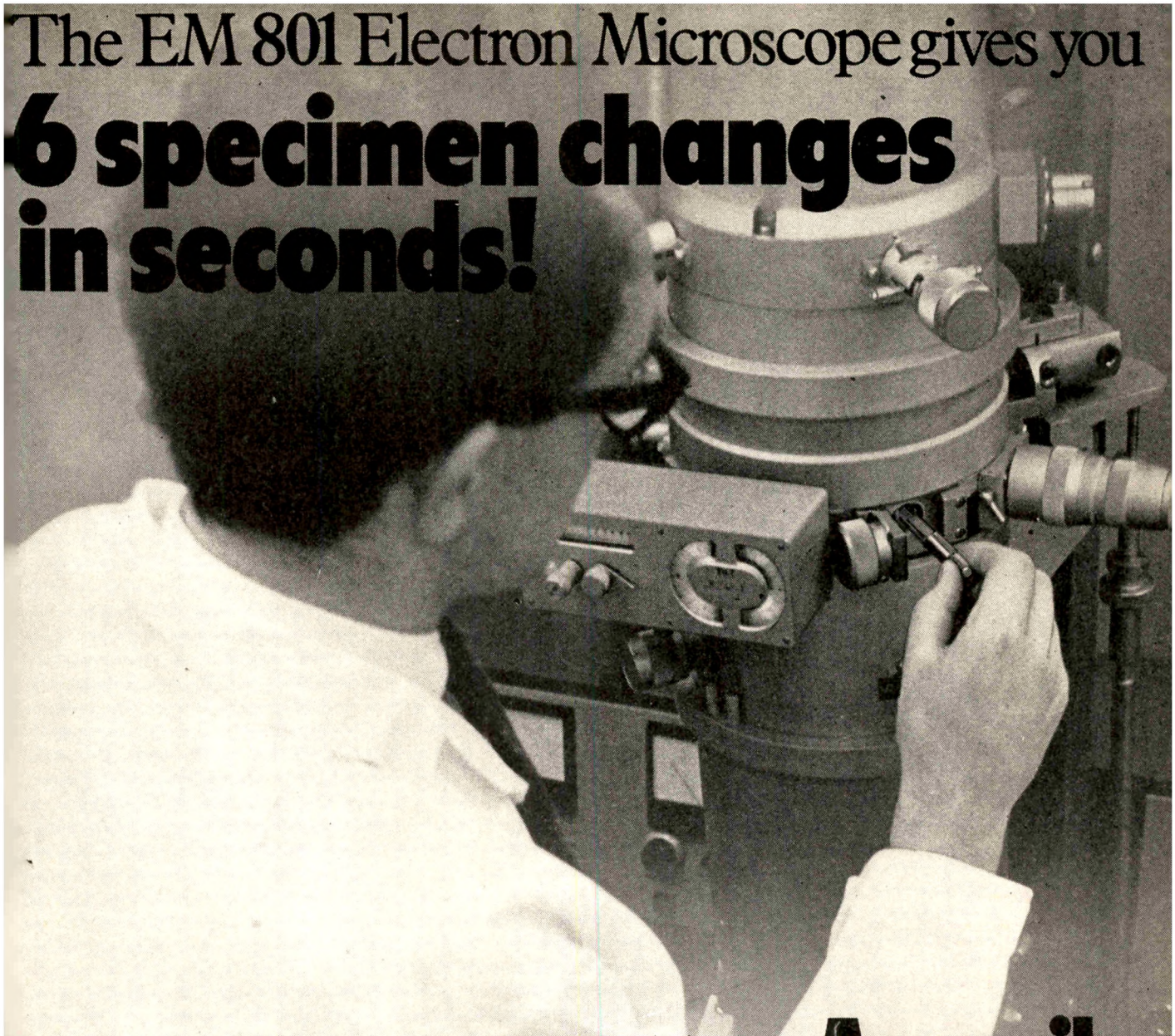
We obtained the nuclei by homogenizing and centrifuging the tissue in 0·25 M sucrose–3 mM $CaCl_2$. In some cases purer nuclei were obtained using 2 M sucrose–3 mM $CaCl_2$–0·5 per cent 'Triton X 100'. They were washed with 0·1 M Tris buffer (*p*H 7·6–8·0) and with 0·15 M NaCl. Sodium bisulphite was also used to minimize proteolytic degradation[5].

We prepared histone fractions by the methods described by Johns[6]. We encountered difficulties when applying these methods to invertebrate tissues; in particular it was difficult to prepare fraction F2b from echinoderms. Fractions F2a and F3 were very similar to the analogous fractions from calf thymus. The fraction F1 used in these studies was isolated by method 1 in the case of the sea urchin embryos and the mollusc hepatopancreas. Method 2 was found more suitable in the experiments with the sea cucumber.

The very lysine-rich fraction was prepared from the haemal system of the sea cucumber *Holothuria tubulosa* (mainly a myoepithelial tissue), *Arbacia lixula* embryos at the late blastula stage and hepatopancreas from the

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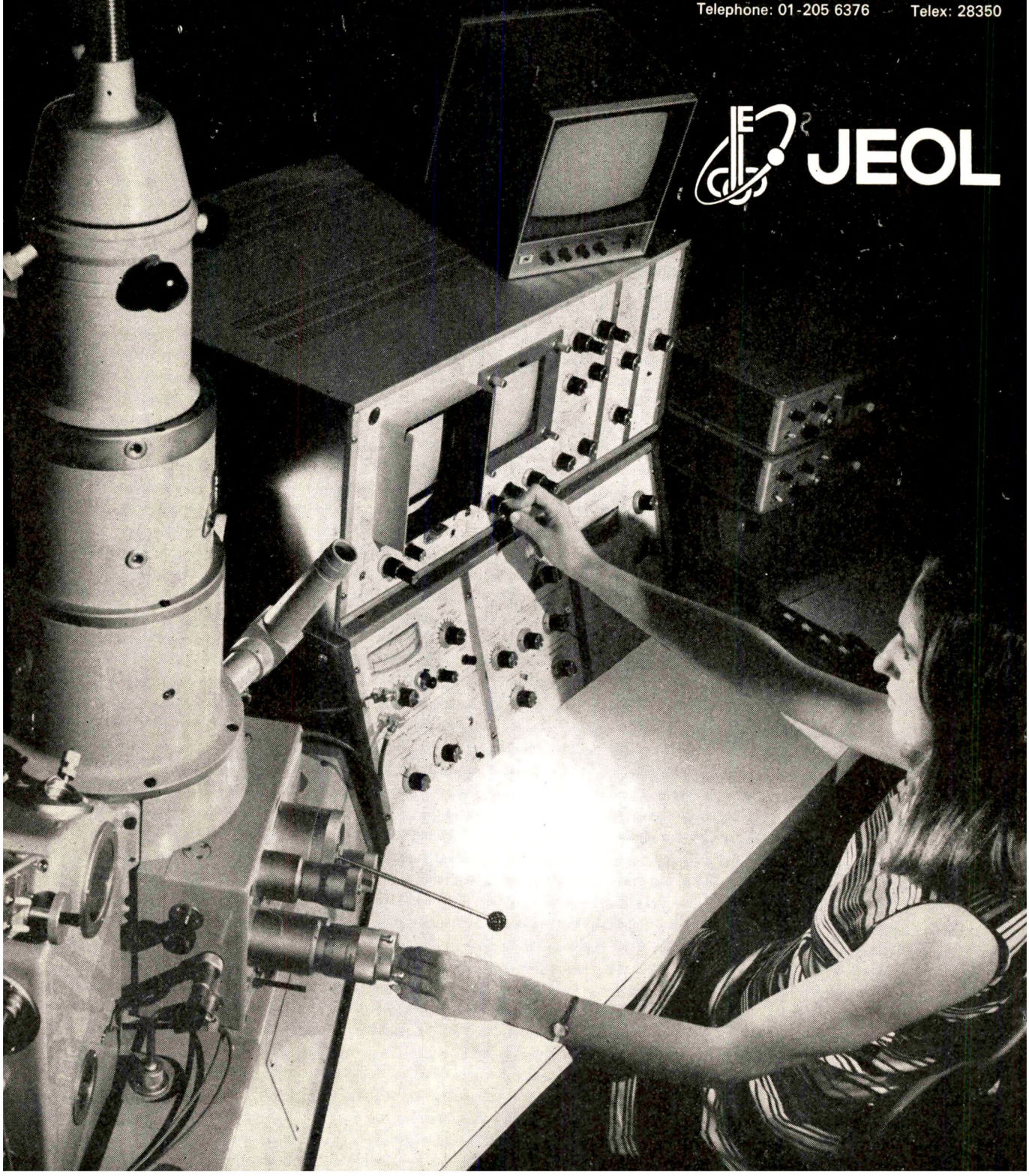
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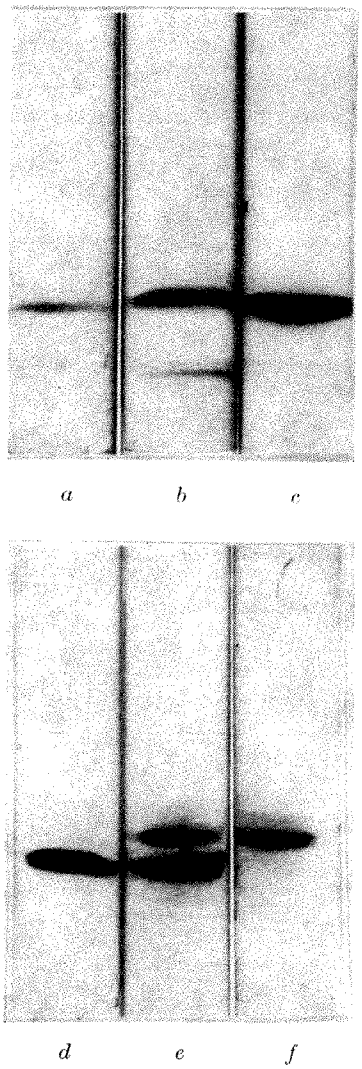
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mollusc *Mytilus edulis*. The amino-acid analyses of the fractions are given in Table 1. Comparison with the data for calf thymus histones reveals an analogy in the relatively high amounts of lysine and alanine and the low content of arginine of this protein. A significant difference is found, however, in the amount of proline, an amino-acid of great structural importance. This difference is most prominent in the echinoderm histones; the mollusc histone has an intermediate proline content. From the values shown in Table 1 it can be concluded that at least 10 per cent of the amino-acids of this protein are different in calf and the echinoderms studied. Furthermore, Fig. 1 shows that the electrophoretic mobility of this fraction is similar in the three cases studied and higher than in calf thymus. These differences seem to be more marked than those found in this component in calf and pea[7]. The results show that the very lysine-rich histones vary significantly in different invertebrates and that the variation is much greater than that reported for other histones. Evidently the structural requirements for the biological activity of this fraction are not so stringent as those for the histone fraction F2a1.



a *b* *c*

d *e* *f*

Fig. 1. Polyacrylamide gel electrophoretic pattern of very lysine-rich histones: (*a*) from *M. edulis* hepatopancreas; (*b*) from the haemal system of *H. tubulosa*. Slow moving minor components are present in this fraction; (*c*) from *A. lixula* embryos; (*d*) from calf thymus; (*e*) a mixture of the components from *M. edulis* and calf thymus; and (*f*) from *M. edulis* hepatopancreas. The gels *a*, *b* and *c* were run simultaneously, whereas the gels *d*, *e* and *f* belong to another electrophoretic run. A result analogous to that shown in *e* was found with mixtures of the components from calf thymus and from the echinoderm tissues studied. The gels were in 8 M urea and contained 12 per cent polyacrylamide. The electrophoretic buffer was 0·025 N sodium acetate, adjusted to *p*H 4·2 with acetic acid.

Table 1. AMINO-ACID COMPOSITION OF THE VERY LYSINE-RICH HISTONE F1

	Calf thymus	*H. tubulosa* haemal system	*A. lixula* embryos	*M. edulis* hepato-pancreas
Lys	25·7	30·1	29·1	25·9
His	0·0	0·7	1·5	1·1
Arg	2·5	2·4	2·4	2·9
Asp	3·1	4·4	4·8	5·4
Glu	5·3	6·5	6·4	4·3
Thr	5·6	4·4	6·4	4·1
Ser	6·4	4·4	4·7	8·0
Pro	9·7	5·9	5·7	7·5
Gly	7·4	6·5	4·7	7·0
Ala	23·3	22·1	21·9	20·0
Met	0·0	0·1	0·4	0·5
Val	4·5	4·1	2·8	3·8
Ile	0·9	3·1	2·9	2·8
Leu	4·0	3·6	4·2	3·6
Tyr	0·6	0·7	1·0	1·2
Phe	0·7	1·2	0·8	1·6

An amino-acid analyser (Unichrom, Beckman) was used in the analyses. No corrections have been made for hydrolytic losses. The amino-acid compositions are expressed as moles/100 moles of all amino-acids found. The analysis of calf thymus F1 is reproduced from Kinkade and Cole[8]. All the analyses are average values obtained from two or more fractions prepared in different operations. Independent analyses coincide within experimental error. The fraction obtained from *H. tubulosa* was purified by reprecipitation with three volumes of acetone.

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Ribosomal RNA Synthesis and RNA Polymerase

MULTIPLE forms of RNA polymerase activity have been isolated from developing sea-urchin embryos, rat liver nuclei and calf thymus[1–3]. The molecular basis of this heterogeneity is at present unknown but, whatever its basis, the crucial question seems to be whether different enzymes copy different genes. We report here the isolation and purification of two different RNA polymerases from *Xenopus* oocytes; only one of them is localized in the nucleolus. On the basis of localization, binding to ribosomal DNA, and response to inhibitors, we conclude that the nucleolar RNA polymerase is responsible for ribosomal RNA synthesis *in vivo*.

We report also the effect of α-amanitin on the RNA synthesis stimulated by microinjecting *Escherichia coli* σ factor in stage 4 oocytes and in early cleavage embryos. In normal conditions, the first class of cells produces almost exclusively ribosomal RNA; but the second does not produce ribosomal RNA at all. Our results suggest that in stage 4 oocytes as well as in early cleavage embryos both polymerases are present, even if only one of them is producing measurable amounts of RNA.

Xenopus laevis offers a great advantage for studying RNA polymerases from higher organisms because pure ribosomal DNA can be isolated in large quantities[4]. This DNA[5,17] comprises repetitive units, each consisting

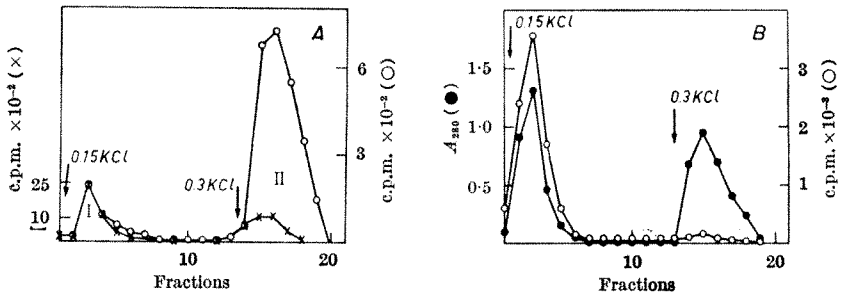


Fig. 1. DEAE-cellulose chromatography of *Xenopus laevis* RNA polymerase Tissues were homogenized at 4° C in 4 volumes of buffer containing 10^{-3} M Tris (*p*H 7·9) and $1·4 \times 10^{-3}$ M β-mercaptoethanol and the homogenate was centrifuged at low speed after filtration through cheese-cloth. Chromatin was precipitated by increasing the ionic strength to 0·1 M KCl and the precipitate was collected by centrifugation at high speed. RNA polymerase activity was solubilized by sonication in 0·75 M KCl and the subsequent steps involved reduction of the ionic strength to 0·125 M and chromatin removed by centrifugation for 1 h at 15,000 r.p.m.. DEAE-cellulose was equilibrated with 10^{-3} M Tris (*p*H 7·9) and $1·4 \times 10^{-3}$ M β-MSH. The chromatin eluate was applied to the column and after washing with standard Tris-MSH buffer the ionic strength was raised to 0·15 M KCl. The subsequent step elution was at 0·3 M KCl. The fractions were assayed for incorporation activity (○—○) and the assay mixture (0·3 ml.) contained, per ml., 100 μmoles Tris-HCl (*p*H 7·9), 3 μmoles $MnCl_2$, 1·8 μmoles ATP, GTP and CTP, 2·5 μCi ^{14}C-UTP (395 mCi/mmole), 0·526 mg phosphoenolpyruvate, 8·8 μg pyruvate kinase, 30 μmoles $(NH_4)_2SO_4$. The mixture was incubated for 30 min at 30° C. Binding capacity (×—×) was determined by measuring the retention on the filter of ^{3}H labelled ribosomal DNA[4]. The binding assay incubation mixture (0·3 ml.) contained 0·5 μg of rDNA, 0·1 ml. of the fraction to be assayed, 10^{-2} M Tris, 10^{-2} M $MgCl_2$, 10^{-2} KCl. The mixture was incubated for 10 min at 30° C and the reaction was stopped by dilution with 10^{-2} M Tris–10^{-2} M $MgCl_2$–10^{-2} M KCl (binding buffer). The content of the tube was filtered through 'Millipore' filters and the filters subsequently washed with binding buffer. *A*, DEAE-cellulose profile of enzymes extracted from ovaries; *B*, DEAE-cellulose profile of enzymes extracted from purified nucleoli.

of cistrons coding for the 18S and 28S ribosomal RNA and a spacer region of high G+C content.

Ribosomal DNA is a very efficient template when challenged with core RNA polymerase or core plus sigma from *E. coli*. Moreover, the bacterial core polymerase can copy this template asymmetrically[5]. The availability of rDNA prompted us to attempt the isolation of RNA polymerase from this organism.

RNA polymerase was isolated from ovaries as described in Fig. 2. Fig. 1*A* shows that two peaks of RNA polymerase activity can be eluted respectively with 0·15 M (peak I) and with 0·3 M KCl (peak II). The two peaks were also assayed for their capacity for retaining ^{3}H-labelled rDNA on 'Millipore' filters[6]; the distribution of rDNA capacity coincides with the polymerizing activity.

It has recently been reported that α-amanitin[7–10], a toxin from *Amanita phalloides*, is a potent inhibitor of the calf thymus RNA polymerase activity eluting at high ionic strength. Table 1 shows that α-amanitin completely inhibits the oocytes RNA polymerase peak II and is completely inactive against the activity eluting at 0·15 M KCl.

Table 1. EFFECT OF α-AMANITIN ON *Xenopus laevis* RNA POLYMERASES

Enzyme source	Peak	α-Amanitin	c.p.m.
Total ovary	I	—	285
		+	290
	II	—	766
		+	10
Purified nucleoli	I	—	344
		+	350

The incubation mixture and the incubation conditions were as described under Fig. 2. When indicated, the samples contained 10 μg/ml. of α-amanitin.

Roeder and Rutter[11] and Jacob *et al.*[10] reported that one of the activities common to all animal systems so far analysed is preferentially localized in the nucleolus. We now show that for *Xenopus*, the activity eluting with peak I is localized in the nucleolus and only peak I is capable of preferentially binding rDNA.

Young *Xenopus* oocytes are a good source of clean nucleoli because each cell contains about 800–1,000 extrachromosomal copies of this organelle which can therefore be isolated with little nucleoplasm contamination. Nucleoli were isolated from ovaries manually dissected from young tadpoles[12]; RNA polymerase was extracted as described above. Fig. 1*B* shows that the only RNA polymerase activity which can be extracted from nucleoli is the one eluting from DEAE-cellulose at low ionic strength (peak I). This activity was completely α-amanitin-insensitive (Table 1).

The nucleolar localization of activity I suggests[10,11] that this polymerase is involved in ribosomal RNA synthesis; because of the availability of pure rDNA we were able to confirm this hypothesis.

We found that *Xenopus* polymerases, like bacterial polymerases, retain DNA on 'Millipore' filters. Activity I and activity II were challenged with different kinds of DNA: ribosomal DNA (68 per cent G+C)[5]; *Xenopus* bulk DNA (42 per cent G+C)[13] free of ribosomal cistrons. The molecular weight of these DNAs was about 6×10^6.

Fig. 2 shows that the first peak eluted retains 90 per cent of rDNA but only 20 per cent of *Xenopus* bulk DNA free of ribosomal cistrons. The second peak can retain only 20 per cent of the DNAs tested. When the fraction of ribosomal DNA not retained by peak II was challenged with peak I, 90 per cent of the input was again retained. By contrast, no further retention was obtained when the same experiment was performed using peak II in the second assay. These results demonstrate that in purified ribosomal DNA there are sites that are recognized only by peak I and suggest that the nucleolar RNA polymerase is involved in rRNA synthesis *in vivo*.

Table 2. EFFECT OF α-AMANITIN ON STAGE 4 ENDOGENOUS AND σ STIMULATED RNA SYNTHESIS

σ Factor	α-Amanitin (μg/cell)	c.p.m. × 50 oocytes
	—	7,980
—	5	8,123
	+10	8,000
	20	8,029
	—	27,600
	5	15,427
+	+10	16,061
	20	15,743

Stage 4 oocytes were injected as previously described[10] and incubated for 8 h after injection. The oocytes were then collected and homogenized in 0·1 M acetate (*p*H 5·2). The homogenate was TCA precipitated, filtered on 'Millipore' and counted.

If the nucleolar RNA polymerase is responsible only for ribosomal RNA synthesis, the latter should be insensitive to α-amanitin *in vivo*. We tested this prediction by

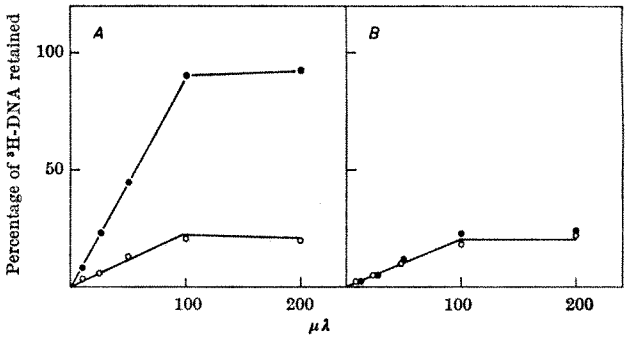


Fig. 2. Retention on 'Millipore' filters of *Xenopus* ribosomal DNA and *Xenopus* bulk DNA free of ribosomal cistrons by peak I and peak II. The incubation mixture (0·4 ml.) contained 0·5 μg of ^{3}H-DNA, increasing amounts of enzyme, 10^{-2} M Tris, 10^{-2} M KCl and 10^{-3} $MgCl_2$. The ribosomal cistrons were purified from *Xenopus* erythrocytes DNA. The purified cistrons banded as a homogeneous peak at a density of 1·724 g cm^{-3} and when hybridized with purified *Xenopus* ribosomal RNA showed a saturation plateau of 23 per cent[17]. The *Xenopus* bulk DNA was free of ribosomal cistrons as detected by hybridization with high specific activity *Xenopus* ribosomal RNA. ●, Retention of rDNA; ○, retention of *Xenopus* bulk DNA. *A*, Retention of ^{3}H-DNA by peak I (tube 31); *B*, retention of ^{3}H-DNA by peak II (tube 42).

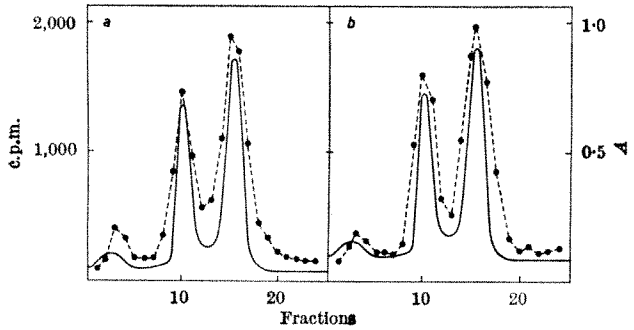


Fig. 3. Sucrose density analysis of RNA synthesized by stage 4 oocytes in the absence and presence of α-amanitin. Stage 4 oocytes were injected as described previously[19]; after injection the oocytes were incubated for 8 h and the RNA was extracted according to the procedure of Brown and Littna[18]. The RNA was precipitated with alcohol, dialysed against 0·1 M acetate (*p*H 5·0) and displayed on 15–30 per cent sucrose gradient. The gradient was centrifuged for 18 h at 22·5 r.p.m. in an 'SW 25·1' Spinco rotor. The gradients were collected with a continuous recording apparatus; the RNA was precipitated with TCA and counted. *a*, Control; *b*, α-amanitin was injected to reach a concentration of 10 μg/ml. in the oocyte.

micro-injecting α-amanitin into *Xenopus* stage 4 oocytes and early cleavage embryos. Stage 4 oocytes synthesize predominantly (more than 97 per cent) ribosomal RNA[14]. On the other hand, cells from early cleavage embryos do not synthesize any ribosomal RNA[15]. Table 2 shows that α-amanitin does not inhibit incorporation of ^{3}H-uridine into TCA precipitable material when injected into stage 4 oocytes. When the RNA synthesized in the presence of α-amanitin is extracted and analysed on a sucrose density gradient, the classical profile of ribosomal RNA is observed (Fig. 3). The possibility that α-amanitin is inactivated when injected into stage 4 oocytes is ruled out by the fact that the σ stimulated RNA synthesis in this cell is partially sensitive to α-amanitin, as previously reported[19]. Table 3 shows that α-amanitin completely inhibits RNA synthesis in early cleavage embryos, suggesting that peak II polymerase operates in those cells.

Table 3. EFFECT OF α-AMANITIN ON EMBRYONIC ENDOGENOUS AND σ STIMULATED RNA SYNTHESIS

Factor	α-Amanitin	c.p.m. ×50 eggs
−	−	6,725
	+	44
+	−	13,941
	+	7,844

Early cleavage embryos were injected as described[19] and then allowed to develop until early blastula. The cells were then collected and homogenized in 0·1 M acetate (*p*H 5·2). The homogenate was precipitated with TCA, filtered on 'Millipore' and counted. Where indicated α-amanitin was injected to reach a concentration of 10 μg/ml. in the egg.

We have tested two other inhibitors of RNA synthesis: 4-benzylidensiazo-4-deoxyrifamycin S (BDS) and methylbenzilidenaminopiperazine[16]. BDS is active on *E. coli* RNA polymerase and an enzyme extracted from a rifampicin-resistant mutant is relatively resistant. Methylbenzilidenaminopiperazine, on the contrary, is completely inactive against the bacterial enzyme. Both compounds inhibit RNA synthesis in stage 4 oocytes as well as in early cleavage embryos.

Because in stage 4 oocytes RNA synthesis is completely insensitive to α-amanitin, only the α-amanitin insensitive activity, that is, activity I, might be present in these cells. Our RNA polymerase is always prepared from oocytes of different stages and therefore the fact that we found two activities does not prove that this situation also refers to stage 4 oocytes. But by microinjecting *E. coli* σ factor into stage 4 oocytes, we found that 50 per cent of the RNA synthesis occurring under σ direction is α-amanitin-sensitive (Table 2). Because the bacterial σ probably functions by interacting with a cellular core enzyme like component, it seems likely that in stage 4 oocytes, besides the nucleolar polymerase (activity I), activity II is also present. Presumably the RNA synthesized by polymerase II under bacterial σ stimulation in stage 4 oocytes is non-ribosomal; it does indeed seem polydispersed when analysed by sucrose density gradient.

An opposite situation occurs in early cleavage embryos where all the RNA synthesis is α-amanitin-sensitive. When σ factor is injected into fertilized eggs, which are then allowed to develop until early blastula, a stimulation of 1·5–2-fold of incorporation of ^{3}H-uridine into TCA precipitable material is observed (Table 3). In this situation α-amanitin can no longer inhibit RNA synthesis completely; we therefore conclude that nucleolar RNA polymerase is present in these cells.

In stage 4 oocytes, as well as in early cleavage embryos, both polymerases I and II are present even if only one of them is producing measurable amounts of RNA. That the injection of *E. coli* σ factor stimulates both polymerases suggests that the endogenous initiation factor(s) could be limiting.

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Synthesis of Serum Albumin, Prealbumin, α-Foetoprotein, α_1-Antitrypsin and Transferrin by the Human Yolk Sac

BOTH in the chick embryo and in the rat foetus, the yolk sac is an important, biologically active membrane throughout almost all of antenatal development. In the chick, the yolk sac envelops the great mass of yolk present in the egg and absorbs yolk material for embryo nutrition. The yolk sac in the rat forms one of the foetal membranes that encompass and protect the foetus, and during the last quarter of gestation it lies in direct apposition to the maternal endometrium. In both these species, the yolk sac synthesizes serum proteins[1,2]: in both, a serum iron-binding globulin and a serum α-globulin specific for the embryo or foetus are produced. The yolk sac of the chick also synthesizes serum albumin[2], a feat apparently not matched by the rat yolk sac[1]. The yolk sac of the human embryo, on the other hand, is a relatively small vesicle at the height of its development, and it soon degenerates to become atretic by the end of the

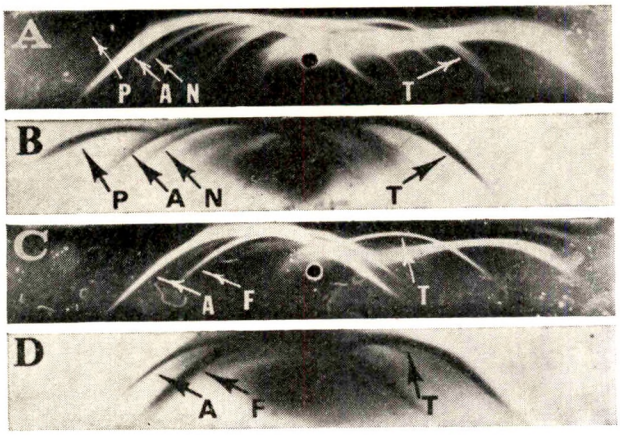


Fig. 1. Immunoelectrophoresis (*A*) and autoradiograph (*B*) of yolk sac culture fluid, embryo C9–68 of 5·5 weeks' gestation, with added adult serum and developed with rabbit antiserum against adult serum proteins. Immunoelectrophoresis (*C*) and autoradiograph (*D*) of yolk sac culture fluid of embryo C9–68 with added foetal serum and developed with rabbit antiserum against foetal serum proteins. Arrows P, A, N, F and T indicate prealbumin, albumin, α_1-antitrypsin, α-foetoprotein and transferrin, respectively.

first trimester of gestation[3]. The observation that the chick and rat yolk sacs can synthesize serum proteins prompted the present investigation into the possibility that the human yolk sac, too, might do so during its relatively brief existence as a well developed structure.

Table 1. PRESENCE OF RADIOACTIVE SERUM PROTEINS IN YOLK SAC CULTURES AS DETERMINED BY RADIO-IMMUNOELECTROPHORESIS

Conceptus	Gestational age (weeks)	Prealbumin	Albumin	α-Foetoprotein	α_1-Antitrypsin	Transferrin
C5–68	5·5	++++*	++	++++	++	++++
C9–68	5·5	++++	++	++++	++	++++
C11–68	8·5	+	++	+++	+	++
C7–68	11·5	0	+	+	0	+

* 0 = not detected; + to ++++ = increasing degrees of intensity of precipitation line on autoradiograph.

Through the cooperation of Dr Valdemar Madsen of the Amts Sygehuset in Gentofte, Denmark, yolk sacs were obtained from four human embryos which ranged from 5·5 to 11·5 weeks of gestation (Table 1). In the three younger embryos, each yolk sac was vesicular and had an excellent blood supply; the yolk sac of the 11·5 week embryo was solid and atretic. The yolk sacs were minced with scissors and cultured in roller tubes for 2–4 days at 37° C with 2 ml. of Eagle's basal medium containing Hanks's solution, 5 per cent horse serum and 2–4 μCi of L-^{14}C-leucine (specific activity 210 μCi/mmole). The cultures were dialysed against water for 3 days, lyophilized and then reconstituted to approximately 0·1 ml. with 0·1 M borate buffer of *p*H 8·6. Aliquots of human adult and foetal serum were added to aliquots of the concentrated culture fluids, and immunoelectrophoresis of the mixtures was performed in agar at *p*H 8·6 in 0·1 M borate buffer. The slides were developed for 3–7 days with rabbit antisera prepared against human adult or foetal serum proteins and rabbit antisera specific for either human serum albumin, α-foetoprotein, α_1-antitrypsin or transferrin[4]. The slides were then washed in 0·1 M NaCl for 2–3 days, dried, and inverted on 'RS Pan' film (Eastman Kodak, Rochester, New York) for 4–12 weeks for autoradiography. As controls, normal foetal and adult human sera were incubated with sterile culture medium containing radioactive leucine, and the mixtures were then studied in the same manner as the yolk sac cultures.

Relatively large amounts of radioactive prealbumin, albumin, α-foetoprotein, α_1-antitrypsin and transferrin were found in the yolk sac cultures of the embryos of 5·5 weeks' gestation (Fig. 1), and somewhat smaller amounts were present in the yolk sac culture of the 8·5 weeks' embryo (Table 1). No radioactive prealbumin or α_1-antitrypsin and only small amounts of radioactive albumin, α-foetoprotein and transferrin were found in the yolk sac culture of the 11·5 week embryo (Table 1). The radioactivity associated with the serum proteins in these cultures was not attributable to simple binding of radioactive leucine, for unlabelled foetal and adult serum proteins incubated with labelled leucine and culture medium in the absence of yolk sac did not become radioactive.

The nature of the radioactivity associated with serum albumin in the yolk sac culture was further investigated. Aliquots of concentrated yolk sac culture fluid of embryo C5–68 were mixed with 50 μg of purified human albumin in 1 ml. of 0·1 M NaCl, and the albumin was then precipitated with specific rabbit antiserum. The specific precipitates were washed three times with 0·1 M NaCl, resuspended in distilled water, adjusted to *p*H 8·0 with 0·1 M NaOH, heated to 100° C for 1 h and hydrolysed in the *p*H-stat[5] at *p*H 8·0 with α-chymotrypsin. The hydrolysates were concentrated by lyophilization, and subjected to high voltage electrophoresis on Whatman No. 1 filter paper. The papers were then dried and placed on 'RS Pan' film for 8 weeks: the autoradiographs revealed five radioactive bands (Fig. 2). Purified human albumin was also heat-denatured and hydrolysed with α-chymotrypsin; when the resulting peptides were separated on filter paper by high voltage electrophoresis and the peptide bands were developed with ninhydrin, five of the bands corresponded in relative position to the five radio-labelled bands (Fig. 2). As can be seen, the purified albumin hydrolysate contained peptides which did not have radioactive counterparts, though this was to be expected because leucine was the only labelled amino-acid in the yolk sac culture medium. The data indicate that labelled amino-acid was incorporated into serum albumin, and therefore that serum albumin was synthesized by the yolk sac.

At approximately 3 weeks of gestation, the human yolk sac is a pouch still broadly attached to the midgut; the liver at this stage is a thickened diverticulum of the gut just rostral to the yolk sac attachment[3]. By 4 weeks of gestation, the yolk sac is a well defined vesicle; and the liver is represented by hepatic buds and ducts[3] synthesizing prealbumin, albumin, α-foetoprotein, α_1-antitrypsin and transferrin in addition to many other serum proteins[4]. The youngest embryo in which the yolk sac was studied in the present report was 5·5 weeks in gestation: the amount of radioactivity incorporated into prealbumin, α-foetoprotein, α_1-antitrypsin and transferrin by the yolk sac at this stage as judged from the intensity of film blackening on autoradiography seemed to be equal to or greater than that incorporated *in vitro* by equivalent quantities of liver from the same embryo[4], although later in gestation the amounts were much less

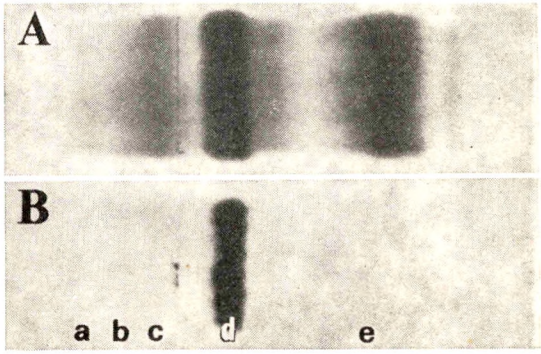


Fig. 2. High voltage electrophoresis (*A*) of peptides obtained from purified albumin by α-chymotryptic hydrolysis; pattern developed with ninhydrin. Autoradiograph (*B*) of high voltage electrophoresis of peptides obtained from the radioactive albumin specific precipitate from yolk sac culture fluid, embryo C5–68. Radioactive bands are marked *a* to *e*; bands *a*, *b* and *c* were very faint and difficult to reproduce photographically.

than in the liver cultures[4]. In none of the yolk sac cultures was as much radioactivity incorporated into serum albumin as in equivalent liver cultures.

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Transmission of Mouse Neuroblastoma by a Cell-free Extract

A MOUSE tumour line, C1300, has been in serial transplantation since 1940 at the Jackson Laboratory, Bar Harbor, Maine. This tumour arose spontaneously from the region of the spinal cord in the body cavity of an albino mouse and was diagnosed tentatively as neuroblastoma; but until recently this could not be confirmed. Klebe *et al.* showed that C1300 tumour cells *in vitro* differentiate into neurone-like structures[1]. Similar results have been reported by others[2,3]. One of the identifying characteristics of cells derived from the sympathetic nervous system is the presence of the enzyme tyrosine hydroxylase. This communication reports the presence of tyrosine hydroxylase in the C1300 tumour, confirming the diagnosis of neuroblastoma. We also report that cell-free extract (CFE) of neuroblastoma tumour induces a similar neoplasm in the dermis, mesentery, spinal cord and sympathetic chain of mice after subcutaneous injections.

Male A/J mice inoculated with C1300 tumour tissue were obtained from the Jackson Laboratory. We are maintaining this neural tumour by serial transplantation in our laboratory. CFE was prepared as follows. Two weeks after transplantation the tissue-induced tumours were removed, minced or passed through a tissue press, and thoroughly homogenized in 0·25 M sucrose–isotonic–saline solution using a Potter–Elvehjem homogenizer with a 'Teflon' pestle. A 10 per cent homogenate was prepared and centrifuged at 3,000*g* for 10 min. The supernatant was examined under an inverted light microscope, found to be completely free of whole cells, and used as CFE. Unlike the light microscope, the inverted microscope allows one to scan the entire contents of CFE for the presence of whole cells. It is therefore unlikely that the presence of whole cells escaped our observation and, even if a few did, they would not have induced palpable tumours within the period of our observation (20 to 40 days). In our experience, subcutaneous injections of 5×10^5 cells induce palpable tumours between 15 and 20 days. In addition, the homogenate was centrifuged at 10,000*g* for 10 min and filtered through a 'Millipore' filter paper (pore size 0·45 μm). This filtrate (0·8 ml.) was used as CFE in nine series of experiments involving ten to thirteen animals per series. All procedures for the preparation of CFE were carried out at 4° C. CFE (0·4 ml.) was injected subcutaneously into the right axillary region of each mouse (4 to 8 weeks of age). Control mice received an equivalent volume of sucrose–saline solution. Tyrosine hydroxylase activity was assayed in 0·25 M sucrose homogenates of CFE and tissue-transplanted tumours using L-^{14}C-tyrosine according to the method of Nagatsu[4]. Tyrosinase activity was measured using the method of Pomerantz[5]. Protein content of the homogenate was determined by the method of Lowry *et al.*[6]. Both CFE and tissue-induced tumours were then examined for viruses by electron microscopy.

CFE-induced tumours were compared with those of tissue-transplanted neoplasms on the following criteria: (*a*) tumour induction time after transplantation, (*b*) histology, (*c*) serial transplantability of CFE, (*d*) cell differentiation *in vitro*, and (*e*) tyrosine hydroxylase and tyrosinase activities.

CFE-injected mice showed palpable tumours in the dermis in 18 to 40 days after injection; but the mice which received tissue transplants showed tumours of similar size between 8 and 10 days after transplantation. Table 1 shows the incidence of tumours in tissue and CFE-injected mice. Most CFE-injected mice developed tumours under the skin and on the same side as the injection; but four tumours appeared on the opposite side from the initial injection. In addition, three tumours developed in the superior region of the neck and three within the intraperitoneal cavity. After 30 days of subcutaneous injection, animals with intraperitoneal

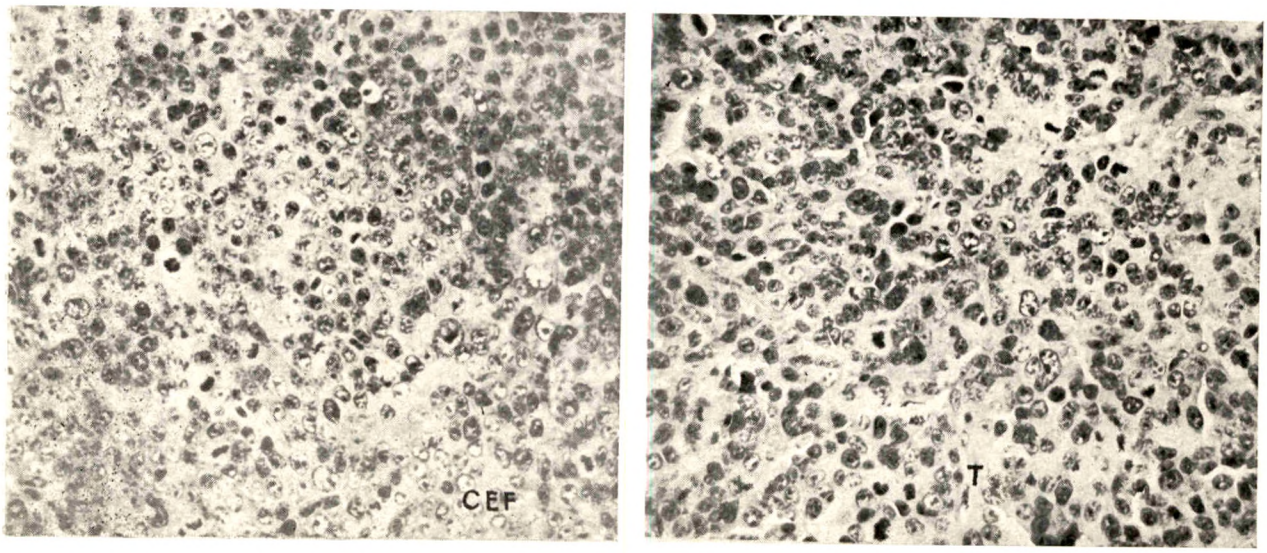


Fig. 1. Sections of tissue (T) and cell-free extract (CFE) induced tumours stained with haematoxylin–eosin. Note round cells, frequent mitoses and occasional giant cells in both tumours (×132).

tumours had distended abdomens filled with reddish fluid. The entire mesentery beginning from stomach down to the large bowel had a sheet of nodular tumour mass. The mesentery covering the ventral surface of the spleen also had a tumour mass extending the entire length of the spleen. Other areas involved included the spinal cord and sympathetic chain. To establish whether or not mice with tissue-induced tumours also had lesions within the peritoneal cavity, five mice were killed and examined 30 days after transplantation. The mesentery, spinal cord and sympathetic chains were not involved. One mouse showed a tumour in the liver and right kidney, whereas another showed a tumour in the left adrenal. This may be due to metastasis of the original tumour or induction of a new tumour by virus.

Table 1. INDUCTION OF NEUROBLASTOMA TUMOURS BY CELL-FREE EXTRACT (CFE)

No. of animals	Treatment	Incidence of tumour (per cent)
35	Piece of tissue-induced tumour	100
59	CFE of tissue-induced tumour	75
15	Piece of CFE-induced tumour	100
20	Sucrose–saline	0

A 10 per cent homogenate of neuroblastoma tumour was centrifuged at 3,000*g* for 10 min. The supernatant was used as CFE. Each mouse received 0·4 ml. of CFE subcutaneously.

CFE obtained after filtration through a 'Millipore' filter produced neuroblastoma tumour (40 to 70 per cent) in five series, whereas in the other four series no tumour was detected. This kind of variable result was expected, because most of the viruses are enclosed within the endoplasmic membranes (Fig. 3); centrifugation at high speed and filtration through a 'Millipore' filter might therefore reduce the viral contents of the filtrate. The amount of virus in the CFE would thus vary markedly from one preparation to another.

Grossly, both CFE and tissue-induced tumours appeared as soft pink masses with nodular growths. A histological study of tumour tissues (three animals from each group) was carried out after fixation in 10 per cent neutral buffered formalin. Paraffin sections of these tumours stained with haematoxylin–eosin showed that both tissue and CFE-induced tumours had similar cellular features (Fig. 1). The cells were round or polyhedral and not present in any recognizable pattern; giant cells with oval nuclei were occasionally seen. Mitosis appeared more frequently in CFE-induced than in tissue-transplanted tumours.

The CFE-induced tumours were transplantable with the same efficiency as the tissue-transplanted tumours; and CFE of CFE-induced tumours also produced similar neoplasms, suggesting that causative agents are not lost during the experimental procedure.

To compare their cellular behaviour *in vitro*, cells from CFE-induced tumours and tissue-transplanted tumours were cultured according to the method described by Klebe *et al.*[1]. Briefly, cells were grown in F12 medium containing 10 per cent agammaglobulin newborn calf serum and maintained at 36° C in a humidified atmosphere of 5 per cent CO_2 and air. After 12 days in culture, cells in the falcon plastic flask were fixed with 3 per cent glutaraldehyde for 10 min and stained with 0·1 per cent cresyl violet for 10 min. Fig. 2 shows the cells of CFE and tissue-induced tumours *in vitro*. The cellular features were identical in both tumours. In a given population undifferentiated round cells, unipolar and bipolar neuroblasts, and neurones with long dendritic processes are seen.

Tyrosine hydroxylase and tyrosinase activities were measured in 14 day old tissue-transplanted tumours and 35 day old CFE-induced tumours. Both groups of tumours were of similar size at this time. As seen in Table 2, the C1300 tumour had a high tyrosine hydroxylase activity indicating its neural origin. The presence of similar amounts of tyrosine hydroxylase in both the tissue-transplanted and CFE-induced tumours shows that

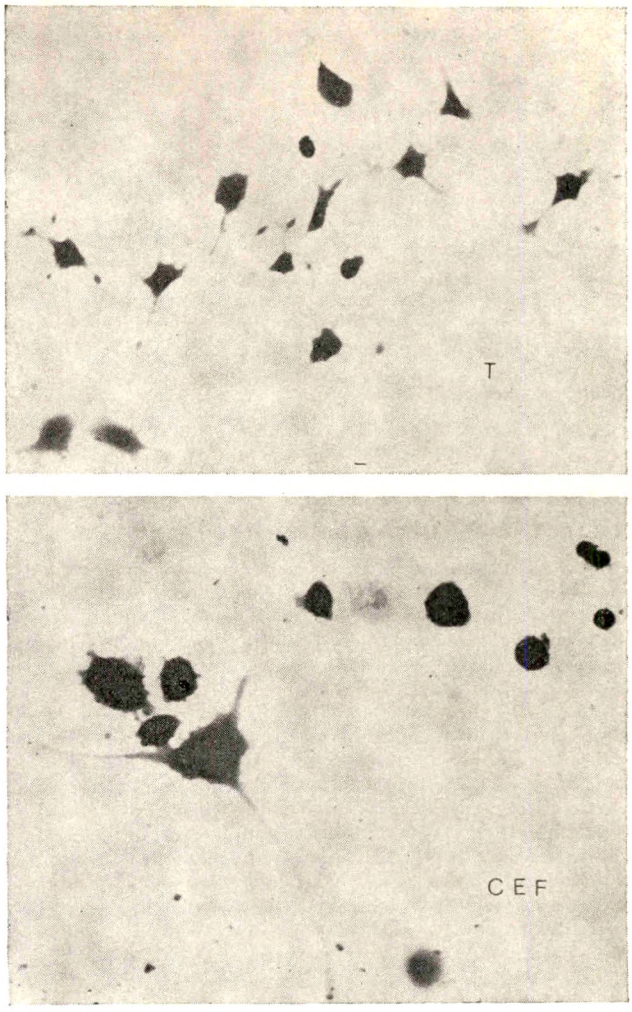


Fig. 2. Cellular morphology *in vitro*. Cells from tissue (T) and CFE-induced tumours stained with cresyl violet. Note undifferentiated round cells, unipolar and bipolar neuroblasts and neurones with dendritic processes in both tumours (×40).

the same tumour is transmitted by the CFE. Because the CFE could be converting dermal melanoblasts (derived from neural crest) into neuroblastoma cells, tyrosinase was also measured but was not detectable in any of these tumours (Table 2).

Table 2. ANALYSIS OF TYROSINE HYDROXYLASE AND TYROSINASE IN C1300 TUMOURS

Tumours	No. of animals	Tyrosine hydroxylase (nmoles of tyrosine/h/g protein)	Tyrosinase
Tissue transplanted tumour	4	30·5±0·9*	0
CFE-induced tumour	4	35·6±2·6*	0

* Standard error of the mean.

Fragments of transplanted and CFE-induced tumour tissues were fixed in 4 per cent glutaraldehyde in S-collidine buffer (*p*H 7·45), post-fixed in 1 per cent OSO_4 plus S-collidine buffer, embedded in 'Epon 812', and stained with lead citrate for an electron microscopic observation. The virus-like particles were present in both CFE and tissue-induced tumours. Fig. 3 shows the presence of several virus-like particles in the cytoplasm. Some of the viruses seem to bud off from endoplasmic reticuli, and several mature viruses are enclosed within endoplasmic reticuli. Neuroblastoma tumours also contained a few electron-dense vesicles which may represent storage sites of catecholamines (not seen in picture).

Transmission of tumours by CFE has been reported for other neoplasms[7–10]; but this is a first demonstration of the induction of a neuroblastoma tumour by CFE.

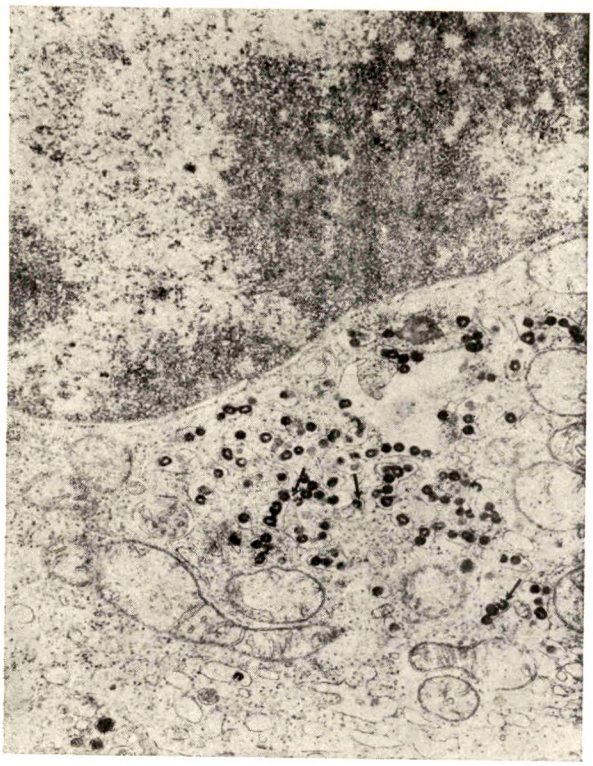


Fig. 3. Electron micrograph of tissue-induced tumour. Note the abundance of virus-like particles in the cytoplasm. Viruses appear to bud off from endoplasmic reticuli (indicated by arrows). Mature viruses are enclosed within the endoplasmic membranes (×17,364).

The presence of virus-like particles in the neuroblastoma tumour suggests a viral origin for this tumour. The most sensitive target areas, which are transformed to neuroblastomas, are the dermis, mesentery, spinal cord region and sympathetic nerve cells. It is apparent that certain nerve cells are being transformed; but whether other cell types can also be transformed remains to be ascertained. Studies are now in progress to elucidate the mechanism of induction of mouse neuroblastoma by CFE.

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Effect of L-Dopa on the Sleep of Man

NORMAL sleep in man consists of two alternating phases which may be distinguished by electroencephalographic (EEG), electromyographic, electro-occulographic, autonomic and psychologic criteria[1-3]. The initial period of nocturnal sleep is characterized by EEG spindles and slow waves, partial preservation of muscle tone, and regularity of pulse and respiratory rates. After about 90 min, this non-rapid eye movement (NREM) phase of sleep gives way to a period of irregular, low voltage electrocortical activity, bursts of rapid eye movements, profound hypotonia of certain muscles, rapid alterations in cardiorespiratory function and in human dreaming. Ordinarily, rapid eye movement (REM) sleep alternates with NREM sleep four or five times each night, and occupies 20 per cent to 25 per cent of total sleep time.

Several lines of inquiry suggest that brain monoamines play an important role in the regulation of both REM and NREM sleep[4]. Lesions which selectively destroy certain noradrenaline or serotonin-containing neurones, or drugs which influence one or more aspects of the metabolism or disposition of these monoamines, profoundly affect sleep patterns[5-8]. Such observations in laboratory animals have led to the suggestion that REM sleep may depend on catecholamine-containing neuronal systems, whereas serotonin-containing systems may be involved in the mechanism of NREM sleep[4]. This study was carried out to determine the effect of L-dopa, the immediate precursor of dopamine and noradrenaline, on human sleep. Results in seven patients indicated that the administration of L-dopa, at doses which modify extrapyramidal function, is attended by a significant reduction in REM sleep time.

Five women and two men (three with Parkinson's disease, two with Huntington's chorea, one with dystonia musculorum deformans and one with essential hypertension) were studied by continuous all-night recordings of EEG, horizontal electro-occulogram and submental electromyogram (Table 1). Each patient slept in his usual hospital room and was left undisturbed until awakened after 8 h of possible sleep. Other medication was discontinued before the study began except for patient 6, who remained on a constant amount of benztropine mesylate (Cogentin). Throughout the investigation a constant number of identical capsules containing either placebo or active drug were administered seven times each day. Three patients (2, 5 and 6) received L-dopa in combination with (+, −)-α-methyl-dopa-hydrazine (MK 485, supplied by Merck Sharp and Dohme Co., West Point, Pa.), a peripheral decarboxylase inhibitor, which does not enter the central nervous system. By blocking the conversion of exogenous dopa to dopamine outside the central nervous system, peripheral decarboxylase inhibitors make dopa available for conversion within the brain parenchyma[9]. Sleep recordings were made during a week of placebo administration and during a subsequent period of L-dopa treatment. The first two nights of sleep study were used to adapt patients to the slightly unnatural sleeping conditions and no data from these nights were included in the results. The placebo baseline for each patient was five nights. In four patients data during drug administration were taken from the five nights of highest L-dopa dosage. Because L-dopa produced insomnia at the highest dosage in three other individuals (2, 6 and 7), however, data in those patients were taken from the five nights just preceding highest dosage. Sleep records were evaluated according to standardized criteria[10] by a scorer who was unaware of whether the patients were receiving placebo or drug.

L-Dopa led to a reduction ($P < 0{\cdot}01$) in the duration of REM sleep in all seven patients (Table 1). The decrease ranged from 4 per cent to 46 per cent of baseline. Moreover, the minutes from sleep onset to the first REM period (REM latency) increased from a mean of 47 min

Table 1. EFFECT OF L-DOPA ON REM LATENCY, REM SLEEP TIME AND NREM SLEEP TIME*

Patient	Age	Diagnosis	Drug dosage L-Dopa g/24 h	Drug dosage MK 485	REM latency Placebo	REM latency L-Dopa	REM sleep time Placebo	REM sleep time L-Dopa	NREM sleep Placebo	NREM sleep L-Dopa
1	48	H	4·0–4·5	0	37±13	119+34	93± 7	57± 7	336±20	351±17
2	26	D	1·5–2·0	1	79± 9	166±65	103±11	77±11	277±40	291±13
3	69	P	1·5–3·0	0	20± 6	122±25	104± 8	56± 8	235±13	219±23
4	47	Hy	3·8	0	54±13	104±38	70± 6	53± 5	216±14	286±19
5	54	P	0·5–0·8	1	76±14	132±22	96±12	92±10	306±35	317±17
6	59	P	2·2–2·6	1	14± 4	86±22	131± 3	70±11	208±23	270±14
7	45	H	6·8–8·8	0	49±12	64± 7	94± 3	83±10	379±50	326±12
			Mean±s.e.		47±9·6	113±12‡	96± 6·9	70± 5·7†	280±24	294±16

* Results are the mean ± standard error of the mean nights and are reported in minutes.
† $P < 0.01$. ‡ $P < 0.001$ for difference between placebo and drug treatment values (two tailed t test).
H = Huntington's chorea; D = dystonia musculorum deformans; P = Parkinson's disease; Hy = hypertension.

to 113 min ($P < 0.001$). There was no change in the duration of NREM sleep. Data for patient 2 are shown in Fig. 1. REM sleep time, although quite variable, decreased significantly when the patient received L-dopa. While the duration of NREM sleep also fluctuated considerably, there was no consistent change until the last four drug nights when NREM sleep fell precipitously in association with almost total insomnia. No further decrease in REM sleep occurred at this time. Discontinuation of L-dopa was associated with a marked rebound elevation in REM sleep. In patient 5 an increase in the duration of REM sleep persisted for ten nights following withdrawal of L-dopa. The effect of L-dopa withdrawal on the duration of REM sleep was not studied in the remaining five patients.

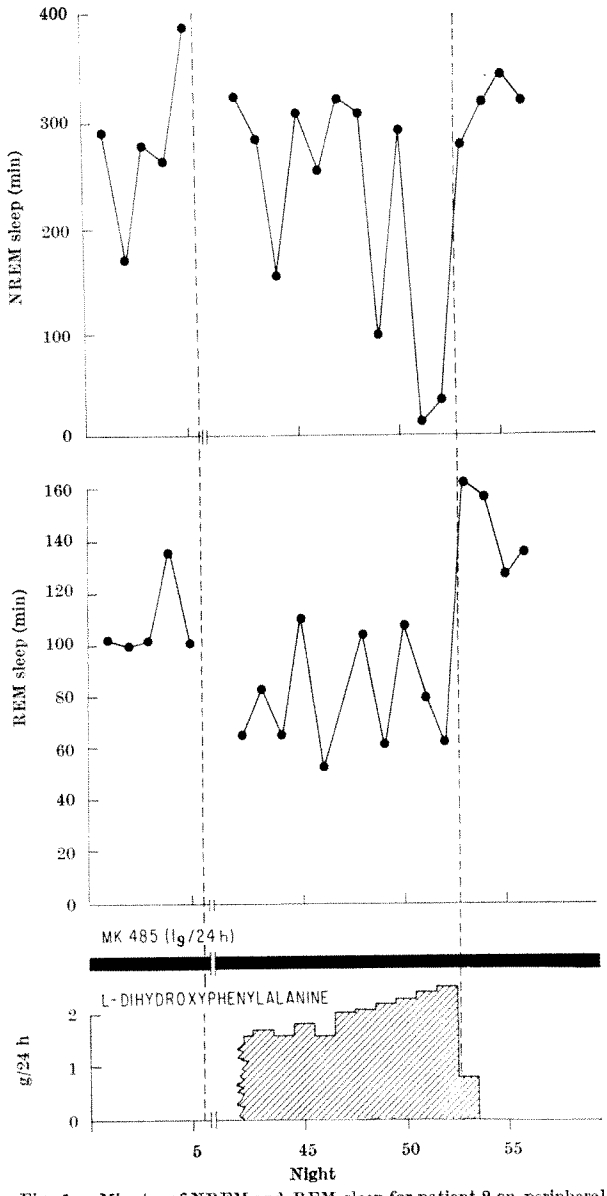


Fig. 1. Minutes of NREM and REM sleep for patient 2 on peripheral decarboxylase inhibitor (MK 485) plus L-dopa.

At submaximal L-dopa dosage several patients had apparent rebound elevations in REM sleep during the second half of the night. This effect was most marked in patient 3. While receiving 1,200 mg/day of L-dopa total REM time during the last 4 h of sleep averaged 98 min compared with 69 min during the placebo period. This observation suggests that the effect of L-dopa on REM sleep is quite transient. As a test of this possibility patients 3 and 6 were given L-dopa on a schedule which omitted any drug dosage during the 2 h preceding bedtime. In such conditions REM time did not differ appreciably from baseline levels. When the same daily dosage of L-dopa was distributed so that the last dose (500 mg of L-dopa) was given within 30 min of bedtime, REM sleep was reduced 45 per cent and 49 per cent below baseline.

The administration of L-dopa results in a substantial elevation of brain dopamine. Noradrenaline concentrations in brain are probably not increased by exogenous L-dopa, especially when given with a peripheral decarboxylase inhibitor[11]. Lumbar punctures were performed in standardized conditions[12] during the pre-drug period and the time of maximum L-dopa dosage. Assays were carried out by previously described methods[13]. The increased cerebrospinal fluid levels of homovanillic acid, the principal metabolite of dopamine, found in patients included in the present study (Table 2) suggest that central dopamine concentrations were elevated by L-dopa at a time when REM sleep was reduced.

Table 2. HOMOVANILLIC ACID LEVELS IN CEREBROSPINAL FLUID OF PATIENTS RECEIVING L-DOPA*

Patient	Drug dose (g/24 h) L-Dopa	Homovanillic acid Placebo	Homovanillic acid Treatment
1	4·0	≤4	383
2	2·5*	12	264
5	0·6*	5	40
6	2·2*	14	300
7	10·0	62	909

* L-Dopa plus 1·0 g MK 485.

We have previously observed increases in REM sleep when catecholamine synthesis was inhibited by α-methylphenylalanine or α-methyl-paratyrosine (R. J. W., T. N. C., D. J. Kupfer, J. S., F. S., and A. Sjoerdsma, unpublished). The finding that L-dopa decreases REM sleep lends further support to a relationship between catecholamines and the duration of REM sleep in humans. By contrast, studies using parachlorophenylalanine (PCPA), an inhibitor of serotonin synthesis, indicate that decreases in serotonin are associated with a reduction in REM sleep in man[14]. Administration of the serotonin precursor, 5-hydroxytryptophan, returns REM sleep to baseline in the presence of PCPA. Furthermore, 5-hydroxytryptophan has been found to increase REM sleep in normals[15,16]. Animal studies indicate that the cate-

cholamines promote REM sleep[1]; our data indicate that in man catecholamines tend to inhibit and serotonin tends to enhance REM sleep.

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Synthesis of γ-Aminobutyric Acid in Fish Erythrocytes

WE have found evidence of high activity of the enzyme glutamate decarboxylase (GAD) outside the nervous system. This enzyme catalyses the formation of γ-aminobutyric acid (GABA), which is believed to be an inhibitory neurotransmitter[1]. Recently, however, a high concentration of GABA (11 mmoles/kg wet weight) was found in flounder (*Pleuronectes flesus* L.) erythrocytes, where GABA may participate in the maintenance of a steady cell volume during changes in plasma osmolality[2,3].

The formation of GABA from glutamate was demonstrated as follows. Erythrocytes were homogenized in isotonic saline and incubated for 1 h at 10° C with 0·6 μmole/ml. homogenate of DL-[5-^{14}C]glutamate (specific activity 3·7 mCi/mmole, New England Nuclear Corp.). Amino-acids were extracted with alcohol and separated by thin-layer chromatography[3]. Spots were visualized by autoradiography and by treatment with ninhydrin reagent. The main labelled ninhydrin-positive product could not be distinguished from GABA (Fig. 1, *a*5 and *b*5). Radioactivity was also found in three other substances, one of which (Fig. 1, *b*6) was tentatively identified as α-ketoglutarate. No metabolism of GABA was detected by incubating with 5-^{14}C-GABA in similar conditions.

GAD activity was measured at 25° C by a micro method[4] depending on the production of $^{14}CO_2$ from L-[1-^{14}C]-glutamate (California Biochemical Corp.). The incubation mixture contained (final concentration): 3·5 mM sodium L-glutamate (75,000 c.p.m.), 20 mM sodium phosphate buffer (*p*H 6·5), 1 mM dithiothreitol, 0·1 mM pyridoxal phosphate and 0·25 per cent of 'Triton X-100'. In these conditions the activity of rat brain was 4·70 ± 0·25

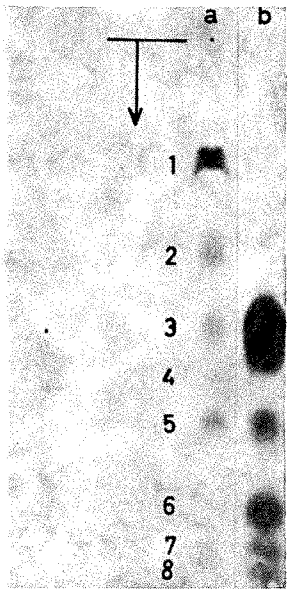


Fig. 1. *a*, Chromatogram of alcohol extract from flounder erythrocytes, showing the ninhydrin-positive spots (1–5); 3 = glutamate; 5 = GABA. *b*, Autoradiogram of the chromatogram *a*, showing the radioactive spots (3 and 5–8); 3 = glutamate; 5 = GABA; 6 = α-ketoglutarate.

μmoles/h/g wet weight (mean ± s.e., three experiments). In flounder, the following GAD activities (μmoles/h/g wet weight) were found: erythrocytes, 0·93 ± 0·08 (mean ± s.e., six animals); brain, 2·65 ± 0·27 (mean ± s.e., six animals). Other tissues contained low activities (liver, 0·06; kidney, 0·03—serum not detectable, one animal) some of which is accounted for by the presence of retained blood cells.

Like GAD of rat brain, the enzymes from flounder erythrocytes and brain were extractable with water, and were inhibited by high concentrations of Cl^- and by inactivation of pyridoxal phosphate. Thus, the erythrocyte enzyme was inhibited 77 per cent by 300 mM KCl and 69 per cent by 2 mM NaCN. The figures for the brain enzyme were 88 per cent and 72 per cent. K_m values measured at 25° C were for flounder erythrocytes 2·3 mM, for flounder brain 24 mM and for rat brain 12 mM (means of two experiments). It is interesting that flounder erythrocytes contain a low concentration of glutamate (less than 0·2 mmole/kg wet weight)[3].

GAD had been thought to occur solely in nervous tissue[1], but has recently been observed in mammalian kidney[5,6]. The kidney enzyme, however, differs from GAD of brain and of flounder erythrocytes for it is activated by Cl^- and probably does not require pyridoxal phosphate[5].

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Natural α-radioactivity Concentrations in Bone and Liver from Various Animal Species

THE radiation dose received by man as a result of exposure to naturally occurring nuclear radiation has been investigated thoroughly in recent years, and critical summaries of the data are available[1-4]. The natural radiation dose received by other animal species has received less attention. The principal source of interspecies variation in the dose is likely to be the contribution from internally deposited alpha-emitting nuclides[5]. Mayneord[6] has referred to this "almost untouched field of research", and he and his co-workers[7,8] have tabulated total alpha-activities in bone ash from about a dozen different animal species. Although data on specific isotopes in certain species have been reported[9-16], there is to our knowledge no other comparative interspecies study. We report here the preliminary results of a similar but more extensive study. The data have been obtained using the total alpha-counting technique developed by Turner *et al.*[17] and used previously in this laboratory[18-21]. We have prepared our samples for counting by cleaning, steaming, drying and grinding (bones) or by freeze-drying and grinding (soft tissues).

In Table 1 we list our data for bone samples from various species. All samples were obtained from Southern Africa, and most were of femur bone. Activities are quoted in pCi/kg wet bone, and the mean concentration factor[22] from the wet bone to the dry bone (as counted) state was 1·3. It is important to note that the total alpha-activities have been corrected, where applicable, for polonium-210 fall-off and are expressed as activities at time of collection. They also include full equilibrium contributions from radon and other radium-226 daughters; such contributions will in general not be present in full in the *in vivo* state[3]. Several of the samples did show a component of the activity which decreased with time in accordance with the 138 day half-life of polonium-210, and this is interpreted as being due to "excess", unsupported, polonium-210 alpha-activity. This interpretation was checked in several cases by alpha-spectroscopy using a large area ion-chamber of the type designed by Hill[23]. In the last column of Table 1 the percentage of the total alpha-activity due to this excess polonium-210 is given.

In man, the greater part of the total alpha-radioactivity is found to reside in the bony skeleton[2,8]. This will not necessarily be so in other species, and we have measured samples from soft tissues as well as bone. In Table 2 we list data from the "livers" of various species; these show interesting features, although they are not to be considered as representative of soft tissues in general.

Table 1. ALPHA-RADIOACTIVITY IN BONE FROM VARIOUS SPECIES

Species	No. of samples	Total alpha-activity in pCi/kg wet bone	Percentage due to excess polonium-210
Cattle	3	6,600	34±17
Sheep	2	4,000	43± 7
Horse	1	1,900	79±25
Donkey	1	1,900	44±10
Buck	1	1,600	32± 8
Fish (sea)	10	1,200	—
Dog (domestic)	4	910	N.D.
Cormorant	1	800	79±14
Jackal	1	720	N.D.
Hyena	1	680	N.D.
Dog (wild)*	2	670	N.D.
Dove	1	580	N.D.
Leopard*	1	420	N.D.
Chicken	1	400	N.D.
Baboon	1	390	N.D.
Elephant*	3	380	N.D.
Zebra*	3	320	N.D.
Lion	1	290	N.D.
Cheetah	1	270	N.D.
Seal	4	240	N.D.
Man	7	240	N.D.
Pig	7	140	N.D.

Counting statistics give an error of less than 15 per cent on all of the total alpha-activity values. Asterisks denote "old" bone samples of uncertain date of death. N.D. denotes not detected within the limits of counting statistics, and implies that the percentage of the total alpha-activity due to excess polonium-210 is less than 15 per cent. The excess polonium-210 percentage was not calculated for fish bone since it was observed to be statistically different from zero in only one out of the ten samples measured.

Table 2. ALPHA-RADIOACTIVITY IN "LIVER" FROM VARIOUS SPECIES

Species	No. of samples	Total alpha-activity in pCi/kg wet liver	Percentage due to excess polonium-210	Liver-to-bone total alpha-activity ratio
Rock lobster	9	14,800	84± 6	38*
Fish (sea)	10	1,300	71±23	1·1
Seal	4	930	96± 2	3·9
Cattle	2	160	39±19	0·02
Buck	4	150	43±33	0·09
Dog (domestic)	4	83	N.D.	0·09
Pig	9	67	N.D.	0·48
Sheep	2	66	N.D.	0·02
Man	10	26	N.D.	0·11

The asterisk for rock lobster denotes a liver-to-shell and not a liver-to-bone activity ratio. Rock lobster shell was found to have a mean total alpha-activity of 390 pCi/kg.

The results of Mayneord's group[5,7,8] provide data which can be compared most easily with our own total alpha-activity values. Our data for human bones are seen to be at a level similar to that of the Cornish bone samples[5], that is, higher than average. Both our data and those of the Mayneord group give higher bone alpha-activities than are implied in the UNSCEAR report compilation of bone radioactivity due to individual alpha-emitting nuclides. This could reflect a difference in experimental technique, but is quite possibly a variation which reflects real differences between the alpha-activities in human bone from different localities[24]. For species other than man, our results also show general agreement with earlier bone data, although here, too, wide activity variations within a single species are to be emphasized.

As tentatively suggested by Mayneord[6], man is clearly close to the bottom of the radioactive species scale. The pig is the only other species investigated by us which has a comparably low alpha-activity content in both bone and liver. Our results confirm the low values of Nelson and Rust[12] for pig bones. At the other extreme, herbivore bones and the marine animal "livers" are more radioactive than the corresponding human organs by one or two orders of magnitude.

It seems that the concentrations of radium-226, radium-228 and excess polonium-210 must be much higher in herbivore bones than in man. In marine livers, on the other hand, it is excess polonium-210 alone which is largely responsible for the high activity levels. The source of the polonium-210 activity is not hard to find: herbivores eat grass[13,25], and the first link in the marine food chain is plankton[18,20].

Although food intake seems to be the main determinant of alpha-radioactivity content of a species, other factors must be important as well. The liver-bone alpha-activity ratios in Table 2 illustrate this point. This ratio is greater than one for the marine species measured, while for herbivores it is less than 0·1. The ratios for man and pig lie in between these two values. A specific metabolic mechanism on the part of the marine "livers", probably involving direct uptake of polonium-210, is indicated. Then consider the pig. The physiological similarity between man and pig is well known[26], and it is tempting to associate the low levels of alpha-activity in both man and pig with a physiological factor. On the other hand, this similarity in activity levels could result from the similarity in human and porcine eating habits[12]. The pig samples measured in our experiments were obtained from pigs used in a feeding experiment designed for agricultural purposes. We measured the alpha-activity of the food on which these pigs had been fed, and calculated an intake of about 1,920 pCi/day per pig. Alpha-activity values for human foodstuffs as reported in the literature[5,8,27] show that any reasonable daily average for the human dietary intake is an order of magnitude lower than this figure. It seems that food intake by itself cannot explain the concentrations of the alpha-emitters in biological tissues.

The natural radiation dose from nuclear radiations must differ widely between different species. A typical

compilation of the human natural radiation dose[3] results in a dose estimate of about 150 mrem/year to human bone. Of this about 30 per cent is the result of internally deposited alpha-emitters. For either herbivore bones or marine "livers" our data predict a total natural radiation dose in excess of 1,000 mrem/year, and 90 per cent or more of this is due to alpha-radiation.

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Respiratory Mechanisms and the Metazoan Fossil Record

THE fossil record sheds little light on the problem of metazoan origins. Most of the major metazoan phyla appear in the fossil record in the Cambrian but no ancestral organisms have been found in the Precambrian, although suitable non-metamorphosed Precambrian rocks exist[1]. The oldest known metazoan fossils (latest Precambrian) are those from the Ediacara formation, Australia[2]. These fossils represent soft-bodied metazoans, some of which have been assigned to the coelenterates, some to the annelids, while others are of uncertain phyla[3]. Glaessner suggests[2] that the Ediacara animals may be significant as a stage in the evolution of small, soft-bodied animals to larger forms with the gradual acquisition of fossilizable exo-skeletons or shells.

Berkner and Marshall have proposed[4] that large scale production of oxygen by phytoplankton did not begin until there was enough oxygen in the atmosphere for the production of a protective layer of ozone, and when this had occurred (1 per cent of present level of oxygen) the metabolism of many organisms shifted from fermentation to respiration. Cloud has suggested[5] that this occurred between 1·2 and 0·6 billion years ago. Towe has proposed[6] that ancestral metazoans, operating at minimal oxygen levels, would not have possessed the oxygenases involved in collagen synthesis. With only a little collagen, these animals were soft, small and had little chance of being fossilized, but as the level of atmospheric oxygen increased near the beginning of the Cambrian, metazoans would have been released from this restriction, and the evolution of rigid structures was a selective advantage.

It does not seem necessary that ancestral metazoans were limited to conventional collagen as a primary connective material. If there had been evolutionary pressure toward a firmer body structure, it is likely that a connective material not requiring an oxygenase would have been evolved. Such a connective protein is illustrated by the collagen of *Ascaris*, which lives in an almost anaerobic environment and functions largely on glycolysis[7]. This collagen contains little hydroxyproline but large amounts of proline, and seems to lack hydroxylysine. Josse and Harrington have shown[8] that stabilization of collagen depends on its total pyrrolidine content and not on hydroxyproline alone.

We propose here that the limited oxygen of the atmosphere at the time of the evolution of early metazoans limited the absolute size attainable by a metazoan animal and thus obviated the need for a firm body. The earliest metazoans must have obtained their oxygen by diffusion. Calculations of the maximum possible sizes of diffusion animals indicate that, in modern oxygen conditions, animals with fairly high metabolic rates are limited in size to about 1 mm in thickness[7,9].

We have calculated thicknesses possible for such animals whose shape is that of a flat sheet in hypothetical primitive Earth atmosphere conditions.

The steady state equation describing such diffusion is

$$D\frac{d^2c}{dx^2} = Q = \frac{V\bar{K}c}{1+\bar{K}c} \qquad (1)$$

where c is the concentration of oxygen within the tissue sheet, x is the distance through the sheet measured from the surface, and D is the diffusion coefficient of oxygen within the tissue, Q is the rate of consumption of oxygen by the tissue, assuming an enzymic process following Michaelis–Menton kinetics where V is the maximum velocity of the consumption reaction and $\bar{K}$ is the reciprocal of the Michaelis constant (K_m).

Blum and Jenden have shown[10] that a good approximate solution to equation (1) may be obtained with a Taylor series expansion method. Applying the boundary conditions ($c=c_0$ at $x=0$ and $x=2b$, where c_0 is the concentration in the medium and $2b$ is the thickness of the tissue), the following solution is obtained[10,11]

$$c=(c_0+\bar{K}c^{*2})\left\{\cosh(\alpha^{\frac{1}{2}}x/1+\bar{K}c^*) - \left[\frac{\sinh(\alpha^{\frac{1}{2}}b/1+\bar{K}c^*)}{\cosh(\alpha^{\frac{1}{2}}b/1+\bar{K}c^*)}\right]\sinh(\alpha^{\frac{1}{2}}x/1+\bar{K}c^*)\right\} - \bar{K}c^{*2} \qquad (2)$$

where $\alpha = V\bar{K}/D$, and c^* is an "average" concentration, a constant different for each value of b and chosen to give a maximum b for each set of conditions[10].

We have determined the thickness attainable ($2b$) by a hypothetical diffusion animal for various values of c_0 by assigning an arbitrary value of c_{min} (the minimum oxygen concentration at the middle of the tissue) and solving equation (2) for $x=b$. c_{min} was chosen equal to a low concentration greater than zero ($0{\cdot}1\times Km$ for oxygen of yeast cytochrome oxidase). It was assumed that the Km of the primitive metazoan cytochrome oxidase was similar to that of modern organisms for such diverse systems as yeast and mammalian mitochondria have similar Km values[12].

The results of calculations of the maximum tissue thickness attainable by model diffusion organisms operating at various rates of oxygen consumption as a function

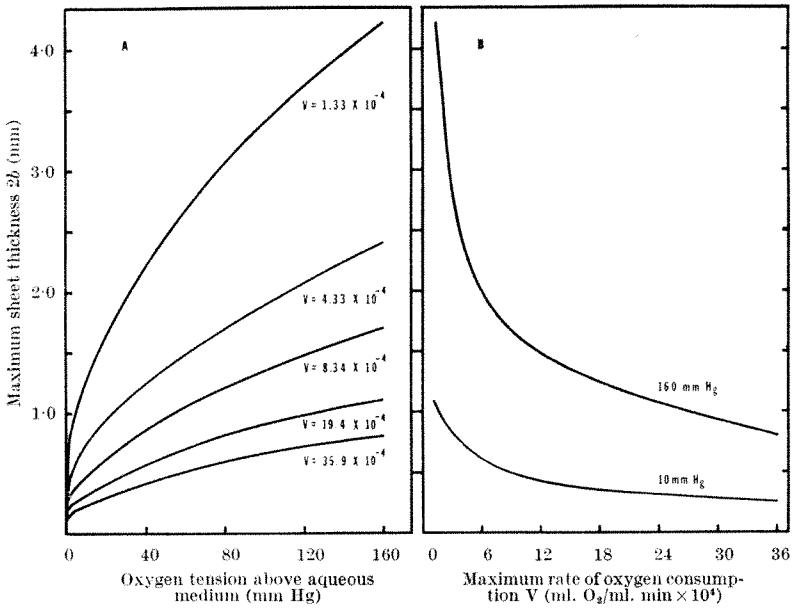


Fig. 1. *A*, Maximum thickness of tissue sheets utilizing oxygen at various rates as a function of oxygen tension above the aqueous medium. *B*, Maximum thickness of tissue sheets as a function of rate of oxygen consumption at a high and a low oxygen tension. Oxygen consumption values were chosen to cover the range of values found in modern diffusion animals[23–25]. The diffusion constant is that of oxygen in muscle at 16°–20° C (4.5×10^{-4} cm²/min)[25].

of atmospheric oxygen content are shown in Fig. 1*A*; some of the data are replotted in Fig. 1*B* to show the maximum thickness as a function of the rate of oxygen consumption. At present day oxygen level, animals of thickness greater than 2 mm are possible only when oxygen consumption rates are very low. At a primitive oxygen level of 10 mm Hg, which, as discussed below, may have been sufficient for the evolution of a more advanced respiratory system, organisms of 1 mm in thickness are possible only at the lowest rate of oxygen consumption treated. Under low oxygen tensions (2–10 mm Hg) and at consumption rates typical of many modern diffusion animals, the thickness is limited to 0·1–0·5 mm. Indeed, many modern diffusion animals (for example, planarians, polyclads and coelenterates[9,13]) are constructed as sheets with a thickness of less than 1 mm. Coelenterates are larger on account of their construction—the mass of the body is the thick non-cellular or poorly cellularized mesoglia which acts as a metabolically inert, low density skeleton on which the actively metabolizing thin sheet of tissue is spread[14,15].

Thus in conditions of low oxygen tensions, active diffusion animals with structures other than the evolutionarily limited medusoid or polypoid pattern would have been very small. The small size and soft-bodied construction of such animals explains their lack of preservation in the fossil record[16].

The ancestral metazoa, by depending on the diffusion of oxygen from the environment to their cytochrome oxidase system, were therefore necessarily limited in size. Any increase in size necessitated the evolution of a circulatory system. This could only have occurred when the atmospheric oxygen content increased to such a point that a sufficient gradient existed to allow the function of a transport pigment. Modern oxygen transport pigments capable of operating at very low oxygen tensions are known from aerobic mud-burrowing organisms existing in oxygen-poor environments[17]; for example, the polychaete annelid, *Arenicola marina*, and the sipunculid, *Siphonosoma ingens*. The concentration of dissolved oxygen in the interstitial water surrounding the burrows of *Arenicola* corresponds to an oxygen tension of 6·7 mm Hg at 15° C and, in the burrows, of 13·4 mm Hg. There is evidence that the worm can respire at this oxygen tension[18,19]. *Siphonosoma* lives in muddy sand in burrows a foot or more below the surface: these burrows are well defined, but have no detectable connexion to the surface. The organism obtains oxygen from the water in the burrows. The oxygen equilibrium curves of the respiratory pigments of these worms are plotted in Fig. 2, along with the oxygen consumption curve of yeast[20] as an example of a typical cytochrome oxidase system. These pigments operate at oxygen tensions from 3–10 mm Hg and both unload to a significant extent at oxygen tensions above the *Km* of cytochrome oxidase. We are not proposing that primitive transport pigments necessarily resemble the pigments of these worms; rather, these pigments serve as examples of transport systems which operate at low oxygen tensions. The existence of such pigments suggests, however, the possibility of the evolution of circulatory systems when the oxygen tension of the atmosphere increased to about 3–10 mm Hg (depending on the permeability characteristics of the skin of the animal).

We suggest therefore that the earliest metazoa must have been diffusion animals without circulatory systems and constructed on either the coelenterate or flatworm plan[15,21]. The flatworms were probably ancestral to the other major phyla. Only when the atmospheric oxygen tension increased to a level sufficient for the evolution of a circulatory system would it have been possible for the evolution of diverse bodily organizations, large sizes and shells or exoskeletons. When the required level of oxygen was attained is, of course, not known[1,4,22], but it must have occurred long enough before Ediacara times for the evolution of quite complex annelid worms to have taken place.

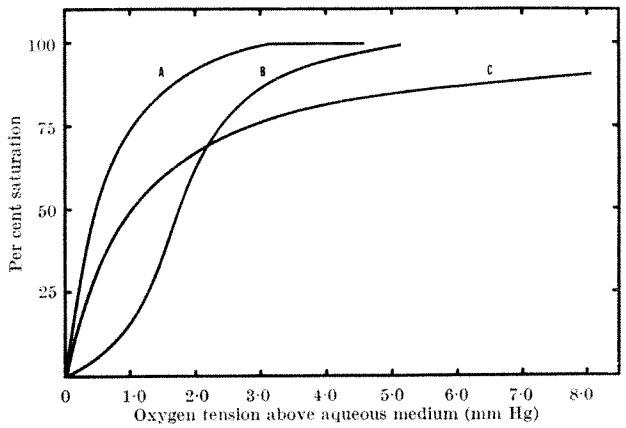


Fig. 2. Oxygen saturation curves for (*A*) yeast cytochrome oxidase[20], (*B*) *Arenicola* haemoglobin[18], and (*C*) *Siphonosoma* haemerythrin[26].

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Biosynthesis of Monoterpenes in Rose Flowers

ALTHOUGH mevalonic acid (MVA) is generally assumed to be an obligatory precursor of terpenes in higher plants, incorporation of 2-^{14}C-MVA into the principal monoterpenes of numerous species is almost invariably less than 1 per cent (ref. 1). Monoterpene biosynthesis is nevertheless assumed to occur via conversion of MVA into isopentenyl pyrophosphate (IPP) and 3,3-dimethylallylpyrophosphate (DMAPP) which condense to form, directly or in sequence, geranyl or neryl pyrophosphates (GPP and NPP), the presumed precursors of straight chain and cyclic monoterpenes respectively. More than 80 per cent of the total label from 2-^{14}C-MVA, however, has been found to pass into that part of several thujane derivatives[2], camphor[3], pulegone[4] and artemisia ketone[4], and of certain sesquiterpenes[5] also, which was derived from IPP. In only one example[6] has a monoterpene, other than a methyl cyclopentanoid monoterpene, biosynthesized from 2-^{14}C-MVA by a higher plant been shown to contain radioactivity comparably distributed between the moieties derived from IPP and DMAPP. All these experiments involved feeding leaf and stem tissue for 1–5 days, and less than 0·1 per cent of the applied tracer was incorporated during this period.

Excellent evidence for the accepted pathway has been provided by the recent discovery that within 1 h of feeding the hybrid tea rose 'Lady Seaton' with 2-^{14}C-MVA, up to 11 per cent of the total label becomes incorporated into certain monoterpenols and their β-glucosides found in the petals[7–8]. It is therefore important to determine the pattern of 2-^{14}C-MVA incorporation in these monoterpenols. Flower-heads of 'Lady Seaton' hybrid tea rose (8 days after initial splitting of the calyx; preperfect stage, see ref. 8) were incubated with the dibenzylethylenediamine salt of 2-^{14}C-MVA (5 μCi; 0·23 mg per flower head) or the potassium salt of either 2-^{14}C-(4*S*)-4-^{3}H-MVA or 2-^{14}C-(4*R*)-4-^{3}H-MVA (between 1·5 μCi and 1·0 μCi ^{3}H and 0·5 μCi ^{14}C; 0·2 mg) for 1 h after starting the MVA infusion and geraniol and nerol occurring both free and bonded as β-glucosides were isolated[8,9]. Some 2·5 to 5 per cent of the applied 2-^{14}C-MVA was incorporated into these monoterpenols. The monoterpenes were purified by preparative gas-liquid chromatography and the ^{14}C-containing material was oxidized to citral and thence ozonized to form oxalic acid, levulinic acid and acetone. Levulinic acid was converted into iodoform and succinic acid, and the latter was decarboxylated by a Curtius rearrangement[10]. Acetone was degraded by the iodoform reaction. All degradation products were isolated as solid derivatives and recrystallized. The doubly labelled monoterpenes were purified by gas-liquid and column chromatography and by complexing with calcium chloride.

Geraniol obtained from the glucoside after 2-^{14}C-MVA feeding gave citral, levulinic acid and acetone with specific activities (d.p.m./mmole) of 86,620, 44,680 and 43,940. Further degradation revealed no tracer at C1, C2, C3, C6, C7 or C9 (Fig. 1, *trans*-CH_2OH) and the tracer was equally distributed between (C4 + C5) and (C8 + C10). On biogenetic grounds the location can be assigned to C4 and C10, though isomerization of C8 and C10 is observed in some monoterpenes biosynthesized from MVA by way of geraniol[11]. Nerol from the analogous source gave citral, levulinic acid and acetone with specific activities of 17,390, 8,098 and 9,473 respectively and again it is inferred from the degradation that the tracer is equally distributed between C4 and C10 (Fig. 1, *cis*-CH_2OH).

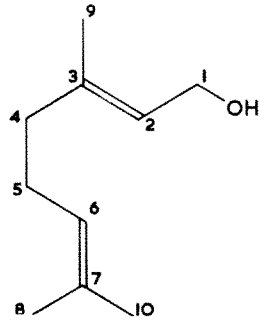


Fig. 1.

The isomer, 2-^{14}C-(4*R*)-4-^{3}H-MVA, was fed at a ^{3}H, ^{14}C ratio of 1·99 and the (4*S*)-isomer at a ratio of 1·25. The isotope ratios in free geraniol recovered after feeding the (4*R*) and (4*S*)-isomers were 1·96 and 0·04, and for geraniol from the β-glucoside were 1·97 and 0·05. The isotope ratios in nerol from the β-glucoside were 2·21 and 0·09 after feeding the (4*R*) and (4*S*)-isomers and for the free nerol from the (4*R*)-isomer was 2·20.

Three points emerge from this work. First, 2-^{14}C-MVA was incorporated into both free and bonded monoterpenes of rose petals in a highly position-specific fashion so that tracer was equally distributed between the moieties derived from IPP and DMAPP. Second, direct condensation of IPP and DMAPP to NPP (and subsequently to nerol) would lead to the loss of the (4*R*)-H of MVA, whereas condensation to GPP and isomerization to NPP would lead to loss of (4*S*)-H. This can be inferred from several studies of the incorporation of ^{3}H, ^{14}C-labelled MVA into higher terpenes[12]. Our results, the first with geraniol and nerol, the presumed precursors of all other plant monoterpenes, indicate the latter route and are consistent with parallel ^{3}H, ^{14}C studies on α-pinene from *Pinus attenuata* (unpublished results of G. Ayrey, D. V. B., D. Barnard and G. N. J. Le P.). The direct trans-cis isomerization of the 2–3 double bond of geraniol (or of a geraniol derivative) must therefore occur; such an isomerization is an uncommon biochemical reaction and further work on the mechanism of this reaction will be of interest. Third, previous conclusions[12] are confirmed, that the (4*S*)-H of MVA is lost in the isomerization of

IPP into DMAPP in higher plants, as in yeast and mammalian tissue.

The first conclusion differs from that obtained in previous investigations of monoterpene biosynthesis in leaf and stem tissue, excluding the methylcyclopentanoid monoterpenes. The existence of a metabolic pool of DMAPP, a non-MVA source for this compound, or the operation of appropriate compartmentation effects can explain the previous labelling results: presumably such factors do not operate in petals. Thus leaf and stem tissue contain discrete oil glands not seen in petal tissue.

We thank Unilever (London) Ltd for a studentship to G. N. J. Le P.

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Short Term Test for Evaluating Potential Carcinogenic Activity of Tobacco Condensates

Mouse skin has been used for many years as a convenient tissue for the assay of tumorigenic properties of tobacco condensates. This testing is prolonged and expensive, and the value of the results obtained is controversial; it is therefore desirable to find a way of shortening the experimental procedure. Sebaceous glands provide a convenient route of entry for substances painted on to the skin, and in particular for the lipid soluble carcinogenic hydrocarbons found in tobacco condensates, which can, in fact, be labelled and located in these glands. Polycyclic hydrocarbons in particular have been used to demonstrate the possibility of using destruction of sebaceous glands to indicate potential carcinogenic activity, and Bock and Mund[1,2] showed that if compounds with a benzanthracene structure are used the degree of destruction is related to tumorigenic activity. Unfortunately, some carcinogenic substances in which the benzanthracene structure is absent (for example, 7,9-dimethylbenz(*c*)-acridine) do not destroy the sebaceous glands; but this does not apply to tobacco condensates[3].

The development of image analysers[4,5] which can rapidly calculate area size of oddly shaped structures, and of reliable enzyme histological methods, have solved the main problems of analysing gland suppression data. The new technique involves the demonstration of non-specific esterase enzymes in the skin of mice painted with tobacco condensates or other carcinogenic hydrocarbons, and the evaluation of areas covered by this enzyme. Non-specific esterase demonstrated by the indoxyl-acetate method of Holt[6] seems to be confined to the sebaceous glands and a few nerve fibres.

Groups of 12 SPF mice (CFLP) each 50 days old to avoid the complication of cyclic hair growth changes were painted on three consecutive days with various doses of three different tobacco condensates and various other potentially carcinogenic substances. Four days after application of the final dose all the mice were killed and a piece of skin from the painted area was frozen in hexane pre-cooled to −75° C. Twelve 40 μm cryostat sections were cut transversely from each piece of skin, fixed in formol–calcium–gum–sucrose and non-specific esterase enzyme demonstrated as described. The total area of reaction product contained in 3,000 fields (each measuring 68,000 μm²) from each group of animals was measured with a 'Quantimet B' image analyser. The reading took about 2 h per group, all the groups being read by an operator with no prior knowledge of which compound had been painted. Results (Table 1) show a direct relationship between area of enzyme activity and known carcinogenic activity of the compound. All the tobacco condensates were ranked in dose and tumorigenic order as previously established by long term tumour production methods.

Table 1. RELATIONSHIP OF AREA OF ENZYME ACTIVITY TO KNOWN CARCINOGENIC ACTIVITY

Compound	Dose	Area (μm²) of esterase activity (mean of 3,000 fields)	Tumour yield from long term tests (80 weeks*) per cent
Tobacco condensate A	36 mg	156	38
A	60 mg	64	48
A	100 mg	36	77
B	36 mg	450	12
B	60 mg	348	32
B	100 mg	63	33
C	36 mg	522	8
C	60 mg	339	25
C	100 mg	99	27
DMBA	250 μg	51	—
Urethane	25 mg	1,392	—
Acetone (solvent) control (1)	0·33 ml.	2,796	—
Acetone (solvent) control (2)	0·33 ml.	2,549	—
Croton oil	0·1 per cent	2,067	

* Age standardized.

DMBA = 9,10-dimethyl-1,2-benzanthracene (Sigma).

This test is not entirely specific for carcinogenic activity and probably relates more to the toxicity of the painted substance to the sebaceous gland cells. It is, however, remarkable that within the range of substances tested this toxicity is directly related to carcinogenicity; and it seems therefore to be a useful rapid screen for potential carcinogenic hazards in tobacco condensates.

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Received May 27, 1970.

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Correspondence

Milk Secretion

SIR,—Professor Patton (*Nature*, **228**, 97; 1970) seems to think it absurd that we should have reported that we had detected pieces of secretory cell cytoplasm in goats' milk (Wooding, Peaker and Linzell, *Nature*, **226**, 762; 1970) because the quantity is so tiny that it could have no bearing on theories of cellular mechanisms involved in milk secretion. According to this view only the major component, water, is worthy of study! I think our finding is of biological significance because:

(1) It settled a controversy. Bargmann, one of the first to examine mammary tissue with the electron microscope, specifically rejected the old idea of classical histologists that some cytoplasm is shed (apocrine secretion) although he proposed that fat globules are pinched off with cell membrane around them. Patton published impressive chemical evidence purporting to support this, and theoretical calculations of London–van der Waals forces to show why the cell membrane only would be expected to be shed with the fat. These views were widely accepted, by myself as well, until, in reviewing the subject, we found four papers on mammary ultrastructure supporting apocrine secretion. These papers have not been quoted by Patton's group. We also were doubtful and to decide whether pieces of cytoplasm were undoubtedly detached from the cell we examined milk in the electron microscope and found a small proportion of fat globules had sizable pieces of cytoplasm attached to them ("signets"), confirming Kurosumi's view that mammary secretion is partly apocrine. However, most unexpectedly we found a very few much larger pieces of cytoplasm, which were packed with active organelles, but lacked a nucleus. These could have been produced by cell decapitation as the apocrine theory predicted. I agree that the bulk of milk is unlikely to be derived from shed cytoplasm, but Professor Patton's protest reminds me of the nurse in *Midshipman Easy* (F. Marryatt, 1836) who, excusing her illegitimate baby, said, "If you please ma'am, it was a very little one".

(2) It should change attitudes. One must now be cautious in entirely discrediting the apocrine mechanism. Although the numbers are small the "signets" can hardly be dismissed as débris, as Professor Patton does. We have now found them in the milk fat of all species examined (cow, sheep, guinea-pig, rabbit and most abundantly in the wallaby), although there were interesting variations in the number of free cytoplasmic fragments, which were frequent in the goat but few in the cow, and whole secretory cells were found in the guinea-pig. The fact that wallaby milk was rich in "signets" may suggest that in more primitive mammals the apocrine mechanism was the basic one, which was later superseded during evolution by the mechanism producing the aqueous phase of milk. The mechanism involved in milk secretion is also relevant to another controversy, whether mammary glands evolved from sweat or sebaceous glands, because apocrine processes are also involved in sweat production.

Yours faithfully,

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ARC Institute of Animal Physiology,
Babraham,
Cambridge.

Radiation from Ball Lightning

SIR,—In a recent communication (*Nature*, **228**, 545; 1970) Altschuler and his colleagues suggest that the phenomenon of ball lightning may be due to formation of the short-lived radioactive nuclides ^{15}O and ^{17}F within a localized plasma capable of yielding radioactive decay energy of the order of 10^7 J. It can readily be shown that the mean γ-ray absorbed dose-rate in a human body at 2 m distance from such a radiation source, if unshielded, would be about 175 rad s^{-1} for ^{15}O or 325 rad s^{-1} for ^{17}F. With dose-rates of this magnitude it would be surprising if there had not been reports of close observers developing signs of radiation sickness. Some may even have received lethal exposures. It is therefore suggested that medical evidence of radiation effects should be sought in persons who have witnessed ball lightning at close quarters. As an alternative approach to testing the proposed theory, however, it would seem profitable to examine, for evidence of high radiation dose, the thermoluminescent properties of any ceramic or other appropriate mineral material in the vicinity of a suspected ball lightning strike.

Yours faithfully,

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Uranium Supplies

SIR,—The figures quoted from the ENEA report on uranium resources (*Nature*, **228**, 306; 1970) are much the same as those circulated by various sources in 1967.

The need for exploration and development of new reserves was also widely stressed in 1967–68. However, the last two paragraphs of your item really underline how dated is the ENEA report.

Elliot Lake suffered several years of decline in the early 60s, when two-thirds of the houses and other buildings were empty. In 1968, however, all accommodation (except for a few houses under repair) was occupied again, and additional houses were being moved from dead areas into Elliot Lake.

Denison Mines spent some $2·9 million in 1967 on mine and mill improvements; similarly Rio Algom were reactivating old workings and developing new areas. Other operators were also preparing for additional output to meet anticipated demand.

The Canadian mining industry has been calling for years for an intelligent approach by such bodies as the USAEC and the US power authorities to the problems involved in providing a steady supply of uranium, including advance buying and long-term contracts. If the nuclear generating forecasts are accurate, there can be little doubt that there will be a shortage of uranium by 1980.

Yours faithfully,

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Definition of Intelligence

SIR,—As a user but non-worshipper of computers, I must take exception to the definition of intelligence, and to the logic employed in the letter entitled "A Definition of Intelligence" (*Nature*, **228**, 97; 1970). As to the suitability of definitions, that is a matter of opinion, but I find Webster's "the capacity to apprehend facts and propositions and their relations and to reason about them" quite satisfactory if not completely explicit. (What dictionary definition is ?)

Furthermore, you will find the authors' definition in the dictionary, under "induction". To quote Webster again, "the act, process, or result, of reasoning from a part to a whole, from particulars to generals, or from the individual to the universal". To give the letter its due, I prefer their definition of "induction" to that of Webster, but it is clear that induction is only one of several skills associated with intelligence.

What is more serious is the failure of logic, "Thus machines . . . cannot be intelligent because the machine's instruction set is a proper subset of man's instruction set".

First, even simple, admittedly non-intelligent computer programs contain instructions which man's brain does not. Second, recent computer program research has produced programming which is able to expand itself. Given rules for induction, deduction, and other "intelligent" processes, these programs expand themselves, generating further instructions on the basis of experience and observation. Third, even granting every statement in the letter, we already know from experience that one man's having a "smaller" instruction set (less intelligence) than another does not mean that such a man is incapable of innovation and intelligent behaviour. Why, then, do the authors assume that a computer has to have a larger set than man in order to innovate, improvise, discover or induce ?

While human beings are obviously more than mere biological computers, I find nothing in their argument, nor in any other I have encountered, which rules out the possibility that, some day, a computer-machine will be constructed which is able to accept a sophisticated, man-invented program, which permits this new life-form to evolve mentally through experience and learning in a manner similar to man, and do this in such a way as to satisfy any reasonable definition of intelligence. Perhaps Norbert Wiener's test of such a machine could be employed. He suggests that any computer candidate plus several men for controls be equipped with typewriters for input and output. Those who would doubt the candidate are required to interview any and all of the unlabelled teletype units by typing in questions to be answered. If the doubters do not do much better than the statistical random probability in pointing out which teletype is connected to the computer, we must conclude that the computer simulates man and displays intelligence.

Yours faithfully,

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SIR,—Fatmi and Young[1] suggest that "Intelligence is that faculty, of mind, by which order is perceived in a situation previously considered disordered". This is an attractive definition because it opens the way to measures of intelligence that are not based upon comparison with population norms.

Consider a very simple test. To create some disorder, suppose we toss a penny and represent the result as a binary number 0 or 1. Show a subject a sample of these disordered digits, then tell him that you may, or may not, change the method of selecting the digits, and ask him to tell you as soon as he knows whether you have changed it. Because the initial sample was completely disordered, the only change possible is the introduction of order, and if the subject perceives it, he has displayed intelligence by Fatmi and Young's definition.

The amount of order can be measured by the change in information or entropy of the new sample of digits compared with the old. The minimum amount that can be detected would be expected to be inversely related to the strength of the faculty that detects it. Suppose we show only 0s; the maximum amount of order (one bit's worth) has been introduced, and this should be quickly perceived by any subject. If, on the other hand, we slightly bias the penny, this would be more difficult to detect. There may be more to intelligence than the ability to perform such a task; but if it is required for it, one can set out to measure its "threshold" in the same way that one measures the threshold of an eye or ear, namely by measuring the least amount of light or sound that can be detected.

The detection of bias in a coin is a statistical problem, and one can say how many tosses are required to detect a given bias at any confidence level. Thus one could compare a subject's performance with the theoretical ideal, and if he required S tosses to achieve a performance ideally requiring I tosses, then it would be reasonable to say that his efficiency at this task was I/S. This is closely similar to the measure of quantum efficiency that is used to compare photodetectors and human vision, and it provides an absolute scale applicable in a wide variety of situations[2,3].

It is easy to see what is missing from this model; for coin tossing there is really only a single null hypothesis that we can ask a statistician to test, namely "The bias in the second sequence is the same as in the first". With many coins, the number of possible forms of order that can be introduced increases very rapidly indeed, and if the possibility of ordered sequences is introduced the number of possible null hypotheses becomes virtually unlimited.

Fatmi and Young apparently framed their definition in order to distinguish more clearly between machine and human intelligence. However, its real merit may be that it enables one aspect of the intelligence of either mind or machine to be compared with, and measured in terms of, the performance of an ideal detector of order in disorder.

Yours faithfully,

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SIR,—G. Hyde (*Nature*, **228**, 589; 1970) gives a definition of intelligence which is good but not complete, as it cannot be quantified as expressed, nor lead to such quantification.

A simpler yet more complete definition is "intelligence is the capacity to understand".

It may be possible at some time to quantify this capacity, which does not need linguistic behaviour for its definition.

Yours faithfully,

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Geological Semantics

SIR,—More than fifty years ago, Professor Crum Brown coined the word 'kerogen' (Greek = wax producer) to denote, exclusively, the oil-yielding component of oil shale. Regrettably, 'kerogen' is now being applied to the insoluble organic matter of rocks in general, and its original usefulness appears to be lost. Reference to the early literature shows also that the word 'sporopollenin' was invented to specify another indefinite mixture of insoluble matter; in this case, a major portion of exines. During a recent conference on sporopollenin, it was suggested that the meaning of this term be also widened. A research paper has now suggested that some kerogens can be identified with sporopollenin and this, in turn, may be considered as polymerized carotenoids.

Until a decade ago, one could use the word 'kerogen' with significance, although, as Carlson has stated, "one does not presume to attribute to kerogen itself any certain composition, but recognises that it is made up of a mixture of organic debris in varying proportions". Unfortunately, some contemporary research appears to be based on the imprudent assumption that, in kerogen, a singular molecular genus is involved. This, of course, is not true; kerogen, by any acceptable definition, can never be considered more than a vague mixture of partially lithified organic residuals. Simple microscopic examination will reveal this point.

An undesirable semantic trend is apparent. Initially, 'kerogen' was a term with explicit application but covering obscure organic matter in certain rocks. Today, the interpretation is equivocal but the substance has acquired a chemical identity entirely out of keeping with its actual nature and origin.

Yours faithfully,

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Theories of Electromagnetism

SIR,—The difference between Stopes-Roe (*Nature*, **228**, 193; 1970) and myself (*Nature*, **227**, 935; 1970) is less a question of fact than of what we consider has fundamental importance. We agree that the influence of a surrounding medium on the field of a magnet depends on the shape of that magnet. We also agree that for the special case of the historically important long thin magnet immersed in a magnetic medium, the Kennelly formulation is correct and the Sommerfeld formulation is incorrect.

The difference seems to be that Stopes-Roe thinks that formulae applying to long thin magnets are still fundamentally important in the theory of electromagnetism while I regard them as applying to a rather trivial special case. The inverse square law for a point pole is still important, however, provided it is not regarded as a real point pole, but as a small element in a distribution of poles. These poles may be distributed over a large surface or even a volume, but provided the distribution of poles is known, **H** can be calculated both in free space and inside the magnet itself, where it is the self-demagnetizing field. The latter is independent of the permeability of the magnet except in as far as that permeability affects the pole distribution. Alternatively one may use the inverse cube dipole law and the distribution of dipoles, and, of course, in either case the appropriate scalar potentials can be introduced with advantage.

The point is that these formulae predict the field due to a magnet of any shape or size for which the distribution of magnetization is known provided that the magnet is situated in free space. In this integral form these magnetic laws are practically useful and have some claim to be regarded as fundamental laws comparable with Biot–Savart's law for the field due to an element of an electric circuit, the usefulness of which is not that it predicts the field produced by a very small circuit, but can be integrated to predict the field due to any circuit.

Thus the purpose of my letter was to show that the classical laws for **H** due to small poles or dipoles are of fundamental importance because they can be integrated to give **H** due to a magnet of any shape or size, but if the relative permeability μ_r of a medium surrounding the magnet is introduced the integration is invalid except for certain special cases such as the long thin magnet.

The only valid solution appears to be to extend the integration to include the poles or dipoles induced in the surrounding medium. It would be premature to write further on this subject at present, and it is unlikely that it could be adequately treated in a short letter.

Yours faithfully,

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Obituaries

M. G. M. Pryor

THE scientific career of Mark Pryor, who died on October 19, 1970, was tantalizingly incomplete. A born naturalist, a more than competent botanist and knowledgeable entomologist, interested particularly in freshwater biology, his contributions to knowledge were mainly on the borderline between biophysics and biochemistry.

After gaining a first class in both parts of the natural sciences tripos at Cambridge, Pryor was a research student under A. D. Imms and produced a thesis which completely revolutionized our conception of the insect cuticle by demonstrating that the dark and horny component, "sclerotin", the nature of which had been a puzzle for a century or more, was essentially protein tanned by quinones generated *in situ* by the enzymic oxidation of diphenols. This theory, of course, was incomplete; we now know that there are other forms of polymerization going on at the same time; but it is still the basis of our understanding of the arthropod cuticle. Indeed, the same process is widespread, from the egg-shells of helminths to the capsules of skates and dogfish.

Pryor was elected to a fellowship of Trinity College in 1939. On the outbreak of war he promptly enlisted. But it was not long before he was pulled out of the army and transferred to the Timber and Adhesives Division of the Royal Aircraft Establishment at Farnborough, where his deep interest in plastics and polymers had every opportunity to develop. It was here that he conceived a highly original theory of muscle as a heat engine, according to which it was supposed that in the resting state the myosin fibres were closely aligned and stabilized by mutual attraction and that ATP acted as

a "plasticizer", weakening the attraction between the protein molecules and leaving them free to curl up under the influence of thermal agitation and so cause the muscle to contract. On his return to Cambridge to take up his fellowship after the war Pryor threw himself into the proving of this hypothesis by experiment and by thermodynamic analysis. He put in an immense amount of work, the published accounts of which are still worth reading. But in the end his theory failed to take the stage. The sliding filament model won the day.

In 1955 Mark Pryor became a tutor in his college and was later senior tutor until 1964. Then once more he became free to devote time to laboratory work. His mind was still as lively, alert and original as ever. He was determined to climb back into research. He kept before him the example of A. V. Hill, with whom he had had much friendly contact during the muscle days. And in spite of taking on heavy teaching duties for the new course of cell biology, he would surely have succeeded but for the tragic accident which brought his life to an untimely end.

Mr W. C. S. Wigley

MR W. C. S. WIGLEY died in October at the age of eighty. He made important contributions to the science of ship hydrodynamics by bringing together mathematical theory and experiment and his work is still having a profound effect on ship design.

As a scholar of Emmanuel College, Cambridge, he completed the Mathematical Tripos in 1912 with considerable distinction. After the war he joined what was known as the "Tank Department" of the National Physical Laboratory, where, for many years, he pioneered work on ship propeller design and mathematically defined hull forms for research purposes. In 1927, a paper on the mathematics of wave resistance to ships earned him a Premium of the Institution of Naval Architects and in 1941 he was awarded the Gold Medal of the institution. His work at this time led to an understanding of the action of the bulbous bow in reducing the wave resistance to a ship and to the adoption in many countries of large and strangely shaped bulb and ram bows. After 1946 he worked as a consultant to the Admiralty and the United States Navy. Many of his later studies of ship resistance and stability problems remain unpublished and are known only to those who commissioned them.

Mr Wigley became a member of the Royal Institution of Naval Architects in 1926. He was an Associate Member of the Council from 1942 and in 1967 he was elected a vice president. In the same year he was awarded the MBE.

Mr Wigley enjoyed railways and country walks and spent much time making genealogical searches of West Country church registers. He is remembered as a man of retiring disposition but of fiery and highly individual spirit too. He remained a bachelor throughout his life.

Announcements

University News

Professor J. E. Dowling, Johns Hopkins University School of Medicine, has been appointed professor of biology at **Harvard University.**

Dr A. J. Meadows has been appointed to a personal professorship of astronomy in the **University of Leicester.**

The following appointments have been made in the **University of London: Dr G. W. Bisset** to the chair of pharmacology at St Thomas's Hospital Medical School; **Dr M. Guter** to the second chair of chemical engineering at University College; **Dr N. A. Mitchison** to the Jodrell chair of zoology and comparative anatomy at University College; **Professor B. R. Rabin** to the chair of biochemistry at University College; **Dr G. F. M. Russell** to the chair of psychiatry at the Royal Free Hospital School of Medicine; **Mr. G. N. Walton** to the chair of nuclear technology at Imperial College; **Professor M. H. F. Wilkins** to the chair of biophysics at King's College; **Dr R. P. Dales** to the chair of zoology at Bedford College.

Dr J. D. M. Wright, University of Oxford, has been appointed professor of pure mathematics at the **University of Reading.**

Dr L. L. Cavalli-Sforza, director of the Institute of Genetics, University of Pavia, has been appointed professor of genetics at **Stanford University School of Medicine.**

Dr G. H. Giebisch has been appointed Sterling professor of physiology and **Dr A. J. Solnit** professor of pediatrics at **Yale University.**

Appointments

Mr C. D. Colchester has been appointed chief scientist to **Marconi Radar Systems Limited.**

Dr D. J. Littler has been appointed controller of research of the **Central Electricity Generating Board.**

Dr G. P. Murphy has been appointed director of the **Roswell Park Memorial Institute,** operated by the New York State Department of Health.

The **Atomic Energy Board of South Africa** has been reconstituted, and now includes the following members: **Mr J. W. Shilling; Dr G. S. J. Kuschke; Mr H. S. Mabin; Mr J. P. Coetzee; Mr C. J. F. Human; Dr S. M. Naudé; Dr R. L. Straszacker; Professor S. F. Dosthuizen; Mr N. J. Uys; Mr B. G. Fourie.**

Miscellaneous

Dr Pierre Deslongchamps, University of Sherbrooke, has been awarded the **E. W. R. Steacie memorial fellowship** for 1971–72 by the National Research Council of Canada.

The **US Institute of Electrical and Electronics Engineers group award** for 1970, presented in memory of Samuel A. Talbot, has been won by **Dr William S. Rhode,** University of Wisconsin.

Brigadier Ralph A. Bagnold has been awarded the **Penrose medal** by the Geological Society of America, in recognition of his work on the formation and movement of sand dunes.

Applications are invited by the council of St John's College, Cambridge, for a **Meres senior studentship** for medical research. The Meres student must be a university graduate and must carry out his research in the University of Cambridge or nearby. The studentship is tenable for between 1 and 3 years. Further information can be obtained from the master of St John's College, Cambridge CB2 1TP.

A travelling scholarship worth **£300** is offered by the **Institute of Electrical and Electronics Engineers** for visit(s) by a postgraduate student to foreign electrical or electronic research or manufacturing establishments. Candidates must submit a proposed programme of visits,

and the award will be made for the programme judged most likely to foster a closer relationship between young engineers in different countries. Further information and entry forms can be obtained from Dr J. A. Ellison, Queen Mary College, Mile End Road, London E1 4NS.

An annual prize of £250 will be awarded by the **Nutrition Society,** in memory of the late Sir **Jack** Drummond, for research in nutrition. Candidates should be less than 40 years of age and normally resident in the United Kingdom. Further information can be obtained from Dr G. L. S. Pawan, Metabolic Division, Department of Medicine, the Middlesex Hospital Medical School, London W1P 7PN. The closing date for applications is February 1, 1971.

An award of £50 will be made every two years by the board of the **British Cryogenics Council** for some outstanding contribution to cryogenics in the United Kingdom, through the discovery of a new phenomenon or the development of a new process. Applications for the period January 1969 to December 1970 should be submitted by February 20, 1971. Further information can be obtained from the honorary secretary of the British Cryogenics Council, c/o The Institution of Chemical Engineers, 16 Belgrave Square, London SW1.

The following new academicians have been elected to the **Academy of Sciences of the Soviet Union: V. S. Vladimirov** (mathematics); **B. B. Kadomtsev, Yu. B. Kobzarev, I. M. Lifshits** (general physics and astronomy); **A. N. Skrinskii, P. A. Cherenkov** (atomic physics); **L. R. Neiman, V. M. Turkevich** (technological problems of power and energy); **A. A. Voronov, N. I. Isanin, N. N. Yanenko** (mechanics and control processes); **Ya. M. Kolotyrkin, I. Ya. Postovskii** (general and technical chemistry); **A. V. Novoselova, V. D. Sadovskii** (physical chemistry and inorganic technology); **A. A. Baev, Yu. S. Ovchinnikov, A. S. Spirin, M. N. Livanov, S. S. Shvarts** (biology and biochemistry); **Yu. A. Kosygin, V. A. Kuznetsov, F. V. Chkhrov, N. A. Shilo** (geology and geophysics); **K. K. Markov, A. M. Obukhov** (oceanology, atmosphere physics and geography); **B. B. Piotrovskii** (history); **A. N. Efimov** (economics); **D. S. Likhachev** (literature and linguistics). Seventy-two corresponding members of the Academy were also elected.

ERRATUM. In the article "Selection and Covariance" by G. R. Price (*Nature*, **227**, 520; 1970), z and $\bar{z}_M$ in the last term of equation (5) should be numbers of daughters conceived by males, instead of total offspring conceived by males.

ERRATUM. In the article "The Function of Dislocations in Cell Walls and Membranes" by W. F. Harris and L. E. Scriven (*Nature*, **228**, 827; 1970), the last two sentences in column 1, page 828, should become "For positive growth, vacancies are required for the accommodation of protomers. A dislocation in a sense provides an inexhaustible supply of vacancies[12]. In negative growth, dislocation provides removable protomers without leaving vacancies in the crystal".

International Meetings

December 15, **Electrode Kinetics and Processes involving Solid Electrolytes,** London (B. Steele, Metallurgy Department, Imperial College, London SW7).

January 5–7, 1971, **The Impact of New Techniques on Palaeontology,** Belfast (Dr A. D. Wright, Department of Geology, The Queen's University, Belfast BT7 1NN, Northern Ireland).

January 24–30, 1971, **Clifford Algebra, its Generalization and Applications,** Ootacamund (Professor Allaid Ramakrishnan, Institute of Mathematical Sciences, Madras 20, India).

British Diary

Monday, December 7

Change or Decay (6.30 p.m.) Mr J. K. Wood, Institution of Electrical Engineers, at the Town Hall, Chester.

Dental Decay, Dental Care and Chemistry (6.30 p.m.) Dr G. S. Ingram, Society of Chemical Industry, at 14 Belgrave Square, London SW1.

Electrical Aspects of Spacecraft (7 p.m.) Mr H. J. Sketch, Institution of Electrical Engineers, at Grimsby College of Technology.

Invention as Part of Education (7 p.m.) Professor W. M. Thring, Institution of Electrical Engineers, at the Royal Star Hotel, Maidstone, Kent.

Some Aspects of TV Reception from Satellites (7.30 p.m.) Mr K. G. Freeman, Institution of Electrical Engineers, at BBC Training Centre, Wood Norton, Evesham.

The Michelson-Morley Experiment (5.30 p.m.) Dr E. Zahar, British Society for the Philosophy of Science, in the Joint Staff Common Room, University College London, Gower Street, London WC1.

The Report of the Marshall Committee (5.30 p.m. discussion) Institution of Civil Engineers, at Great George Street, London SW1.

The Value of Gel Permeation Chromatography for the Design of Alkyd Resins (7 p.m.) Mr H. Olley and Dr L. A. O'Neill, Oil and Colour Chemists' Association, at Hull College of Technology.

Tuesday, December 8

Digital Inverse Filters (7 p.m.) Mr J. A. Baker, Institution of Electrical Engineers, at the University, Bradford.

Flavour Analysis with Humans, Instruments and Computers (6.15 p.m.) Professor E. von Sydow, Society of Chemical Industry, Food Group, in the Wellcome Lecture Hall, Royal Society, 6 Carlton House Terrace, London SW1.

Interaction of Cardiovascular Reflexes (5.30 p.m.) Professor M. de Burgh Daly, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Fourteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

International Locust Research and Control (5.15 p.m.) Mr P. T. Haskell, Royal Society of Arts, Commonwealth Section, at John Adam Street, London WC2. (John Curtis "Woodstock" Lecture.)

Microwave Acoustics (6.30 p.m.) Professor K. W. H. Stevens, Institution of Electrical Engineers, at Farnborough Technical College, Farnborough, Hants.

Poverty in Britain (1.15 p.m.) Professor R. Titmuss, University of London, at Queen Elizabeth College, Campden Hill Road, London W8.

The Choice of Industrial Drives to 100 hp (6.15 p.m.) Mr A. F. Wilkinson, Institution of Electrical Engineers, at UMIST, Manchester.

The Significance of Bill Form in the Spoon-billed Sandpiper *(Eurynorhynchus pygmeus)*, Dr P. J. K. Burton; **Locomotion of Worms,** Dr M. K. Seymour; **The Seals of Macquarie Island** (film) (5 p.m.) Zoological Society of London, at the Zoological Gardens, Regent's Park, London NW1.

Tolerances in Building Construction (6 p.m. discussion) Institution of Mechanical Engineers, Joint Building Group, at 1 Birdcage Walk, London SW1.

X-ray Astronomy (1.20 p.m.) Professor R. L. F. Boyd, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Wednesday, December 9

Alaskan Exploration and the Development of the Prudhoe Bay Oilfield (5 p.m.) Mr R. Stoneley, Mr A. N. Thomas and Mr M. W. Clegg, Geological Society, at Burlington House, Piccadilly, London W1.

Earth Station Installation Projects (6 p.m.) Mr W. B. Hopkins and Mr N. Wheatley, Institution of Electronic and Radio Engineers, Communications Group, at 9 Bedford Square, London WC1.

Industrial Effluent (7 p.m.) Mr C. Lumb, Society for Analytical Chemistry North of England Section, jointly with the Huddersfield Section of the Royal Institute of Chemistry and the Yorkshire Section of the Society of Chemical Industry, in the Lecture Theatre, Huddersfield College of Technology, Huddersfield.

Large Scale Direct Conversion Power Generation (6.45 p.m., convention) Institution of Electrical Engineers, at UMIST, Manchester.

Marine Fouling (3 p.m.) Oil and Colour Chemists' Association, Newcastle—Student Group, at Newcastle Polytechnic, Ellison Place, Newcastle upon Tyne.

Mersenne's "Harmonie Universelle", 1636-37 (1 p.m.) Dr A. C. Crombie, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Nuclear Power Today, Tomorrow and the Day After (6.30 p.m.) Mr G. R. Bainbridge, Institution of Electrical Engineers, jointly with Chelmsford Engineering Society, at King Edward VI Grammar School, Chelmsford, Essex.

One World—The Future (5.30 p.m.) Professor B. G. Maegraith, University of London, at the London School of Hygiene and Tropical Medicine, Keppel Street, London WC1 (Last of four Heath Clark Lectures).

Specifications—Do We Need Them? (6 p.m., panel discussion) Society of Environmental Engineers, in the Mechanical Engineering Department, Imperial College, London SW7.

The Environmental Revolution (5.30 p.m.) Mr E. M. Nicholson, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

The Human Necessity for Automation (6.30 p.m.) Mr P. L. Taylor, Institution of Electrical Engineers, at the University, Sheffield.

The 2-Stage Rotary Engine as a New Concept in Diesel Power (6 p.m.) Institution of Mechanical Engineers, jointly with the Society of Automobile Engineers of America, at 1 Birdcage Walk, London SW1.

Thursday, December 10

Active Transport of Salts and Water in Living Tissues (two-day discussion meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

Addition Copolymers of the Vinyl Ester of Branched Chain Fatty Acids for Use in Electrodeposition Paints (7 p.m.) Dr W. J. van Westrenen and Mr W. H. M. Nieuwenhuis, Oil and Colour Chemists' Association, at East Ham Technical College, High Street South, London E6.

Clinical Aspects of Ultrasonic Diagnosis (10 a.m.) British Medical Ultrasonics Group, in the Barnes Hall of the Royal Society of Medicine, 1 Wimpole Street, London W1.

Development of Gloss Paints in the Twentieth Century (6 p.m.) Mr I. R. McCallum and Mr M. Devine, Oil and Colour Chemists' Association, at the St. Enoch Hotel, Glasgow.

Microelectronics (7.30 p.m.) Mr Newham, Institution of Electronic and Radio Engineers, Scottish Section, at Robert Gordon's Institute of Technology, Schoolhill, Aberdeen.

Modern Trends in Traffic Control (7.30 p.m.) Mr D. G. Hornby, Institution of Electrical Engineers, at Stevenage College of Further Education, Stevenage, Herts.

Nuclear Power Station Construction (7.45 p.m.) Mr K. J. Broome, Institution of Electrical Engineers, at the Castle Hotel, Taunton.

Recent Trends in Oscilloscope Technique (6 p.m.) Mr B. Ellison, Institution of Electronic and Radio Engineers, Instrumentation and Control Group, at 9 Bedford Square, London WC1.

Self-poisoning and Suicide (5.30 p.m.) Professor N. Kessel, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Last of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Some Applications of Medical Electronics (6.15 p.m.) Mr D. W. Hill, Institution of Electrical Engineers, at the University College of Swansea, Singleton Park, Swansea.

Studies in Somalia (7.30 p.m.) Dr K. M. Cahill, Royal Society of Tropical Medicine and Hygiene, at Manson House, 26 Portland Place, London W1.

The Design and Function of Static Seals (all-day conference) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

What is the Significance of Critical Speeds on High Speed Rotating Machines? (6 p.m., discussion) Institution of Mechanical Engineers, Steam Plant Group, at 1 Birdcage Walk, London SW1.

Friday, December 11

Chloride Titanium Dioxide Pigments—Their Value (6.30 p.m.) Oil and Colour Chemists' Association, at the Liverpool Building and Design Centre, Hope Street, Liverpool 1.

Determination of Free Ion-Pair Yields by Pulse Radiolysis (1 p.m.) Dr C. Capellos, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Extra-Galactic Nebulae, Professor J. Heidmann; **Modern Theories of Earth and Planetary Structure,** Professor S. K. Runcorn (4.30 p.m.) Royal Astronomical Society, in the Scientific Societies Lecture Theatre, 23 Savile Row, London W1.

Metamerism, Colour Constancy and Related Problems (6 p.m.) Mr R. Booth, Colour Group (Great Britain), jointly with the Society of Dyers and Colourists, in the Mechanical Engineering Department, Imperial College, London SW7.

The Computer : A Challenge to the Human Brain (9 p.m.) Mr Basil de Ferranti, Royal Institution, at 21 Albemarle Street, London W1.

The Fundamental Reference Frame and the Positions of Faint Objects (2 p.m., discussion meeting) Royal Astronomical Society, at Burlington House, Piccadilly, London W1.

Monday, December 14

Electrical Research on British Rail (7 p.m.) Mr L. L. Alston, Institution of Electrical Engineers, at the College of Further Education, Crewe.

Optical Studies of Adsorbed Layers at Interfaces (two-day symposium) Faraday Society, at the Royal Institution, 21 Albemarle Street, London W1.

Railway Breakdown Services (5.30 p.m.) Mr K. R. M. Cameron, Institution of Mechanical Engineers, Railway Division, at 1 Birdcage Walk, London SW1.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Imperial College. City and Guilds College. 1967/1970 Research Report. Pp. iii+179+16 plates. (London: Imperial College, 1970.) [2010

Marine Biological Station, University of Liverpool, Port Erin, Isle of Man. Annual Report No. 82 for 1969. Pp. 44. (Liverpool: Liverpool University Press, 1970.) [2010

Institute of Personnel Management. Annual Report 1969/1970. Pp. 48. (London: Institute of Personnel Management, 5 Winsley Street, W1, 1970.) [2110

Ministry of Transport: Road Research Laboratory. RRL Report LR 357: A Review of Road Traffic Noise—The Working Group on Research into Road Traffic Noise. Pp. vi+94. (Crowthorne, Berkshire: Road Research Laboratory, 1970.) *Gratis.* [2110

Centre for Environmental Studies. Third Annual Report, April 1969 to March 1970. Pp. 42. (London: Centre for Environmental Studies, 5 Cambridge Terrace, NW1, 1970.) [2110

Bulletin of the British Museum (Natural History). Entomology. Vol. 25, No. 5: Revisional Notes on African *Charaxes* (Lepidoptera: Nymphalidae), Part VI. By V. G. L. van Someren. Pp. 197–250+11 plates. (London: British Museum (Natural History), 1970.) 71*s.* [2110

Other Countries

Republic of South Africa: Department of Industries. Division of Sea Fisheries Investigational Reports. No. 83: Hydrological Observations in the South-East Atlantic Ocean. By J. G. Welsh and G. A. Visser. Pp. 24. No. 86: Migration of the Pilchard *Sardinops ocellata* in Southern Africa. By G. G. Newman. Pp. 6. No. 90: The Japanese Longline Fishery Off the South African Coast, 1964–1967. By C. S. de V. Nepgen. Pp. 13. (Sea Point, Cape Town: Director of Fisheries, 1970.) [1410

US Department of Commerce: Bureau of the Census. Governmental Finances in 1968–69. Pp. 56. (Washington, DC: Government Printing Office, 1970.) $0.60. [1510

The Royal Observatory at the Cape of Good Hope, 1820–1970—a Sesquicentennial Offering. Pp. 44. (Cape Town: Royal Observatory; Hailsham, Sussex: Royal Greenwich Observatory, 1970.) 35c; 4*s* 6*d*. [1610

Newsletters on Stratigraphy, Vol. 1, No. 1, 1970. Edited by Professor G. Lüttig. Pp. 1–58. (Leiden: E. J. Brill, 1970.) [1610

US Department of the Interior: Geological Survey. Water-Supply Paper 2002: Water in Urban Planning, Salt Creek Basin, Illinois. By Andrew M. Spieker. (Prepared in cooperation with the Northeastern Illinois Planning Commission.) Pp. ix+147 (Washington, DC: Government Printing Office, 1970). $1. [1910

University of Virginia. Centre for the Study of Science, Technology and Public Policy—Second Annual Report, June 30, 1970. Pp. 24. (Charlottesville, Virginia: University of Virginia, 1970.) [1910

Report of the Board of Trustees, National Art Gallery and Dominion Museum for the year ended 31 March, 1970. Pp. 38. (Wellington: Government Printer, 1970.) $0.25. [2010

Records of the Dominion Museum, Wellington. Vol. 6, No. 17 (January 26, 1970): The Stone Crab *Lithodes murrayi* Henderson, The First New Zealand Record. By J. C. Yaldwyn and E. W. Dawson. Pp. 275–284. Vol. 6, No. 18 (20 February, 1970): Classification of New Zealand Arionacea (Mollusca : Pulmonata), 3. By F. M. Climo. Pp. 285–366. (Wellington, New Zealand: Dominion Museum, 1970.) [2010

A Selected Bibliography of Library and Information Science, Spring 1970. Pp. 16. (Cleveland, Ohio: Center for Documentation and Communication Research, School of Library Science, Case Western Reserve University, 1970.) [2010

US Department of the Interior: Geological Survey. Bulletin 1261-G: Mineral Resources of the Emigrant Basin Primitive Area, California. By E. W. Tooker, H. T. Morris and Paul V. Fillo. Pp. vii+70+plate 1. Bulletin 1285: Geology of the Sycamore Flat and Paraiso Springs Quadrangles, Montery County, California. By David L. Durham. Pp. iv+34+4 plates. (Washington, DC: Government Printing Office, 1970.) [2010.

Annals of the New York Academy of Sciences, Vol. 170, Article 1: The Hepatic Circulation and Portal Hypertension. By R. C. Britton, C. M. Leevy and 59 other authors. Pp. 1–405. (New York: New York Academy of Sciences, 1970.) $23. [2010

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Fear of Enlightenment

THE view that some kinds of knowledge are too dangerous to possess is almost as old as the hills (*Genesis*, Chapter 2, verse 17) and has been suspect for the whole of that time. It is therefore something of a surprise to see that Professor Walter Bodmer and Professor Luigi Cavalli-Sforza argue in the October issue of *Scientific American* that there should be a moratorium on research intended to throw light on questions such as that of whether observed differences between American blacks and whites in IQ scores are genetic in origin. To be sure, Professors Bodmer and Cavalli-Sforza are in quite a different camp from that of the people who persecuted Galileo, for they say that even the process of trying to find out might aggravate racial tensions which already exist in the United States. Specifically, they say:

> In the present racial climate of the United States, studies on racial differences of IQ, however well-intentioned, could easily be misinterpreted as a form of racism and lead to an unnecessary accentuation of racial tensions. Since we believe that, for the present at least, no good case can be made for such studies on either scientific or practical grounds, we do not see any point in particularly encouraging the use of public funds for their support. There are many more useful biological problems for the scientist to attack.

Nobody will doubt the compassion which has impelled the authors to this conclusion, but there are good reasons for asking whether they are right.

The extent to which intelligence is inherited within homogeneous populations such as that of Britain or the white population of North America has in past decades been a similarly tendentious issue. To be sure, the way in which intelligent behaviour has become by natural selection one of the distinctive attributes of the human species implies that there must be at least some genetic components in the determination of individual intelligence. For decades, however, psychologists and their mentors have tended to shy away from attempts at quantitative assessment of the relative importance of genetic inheritance and of environment in the determination of intelligence, chiefly in the belief that studies of this kind would provide ammunition for all kinds of eccentric causes, aberrations like that of the Nazis included. Now, luckily, reticence has to some extent been discarded, with the result that there is a much fuller appreciation not merely of the ways in which intelligence (as measured by IQ) is inherited but also of the extent to which native intelligence cannot be predicted from the intelligence of the parents.

In short, such objective evidence as has now accumulated of the relationship between environmental and genetic determinants of intelligence reinforces and does not diminish the old liberal view that societies of all kinds would lose immeasurably if they did not provide good education for the children of unintelligent parents and also plentiful opportunities for upward social mobility. Nearly a century ago, honest Galton was alarmed at the possibility that a negative correlation between fertility and intelligence might undermine the viability of the human species. There are all kinds of reasons why Galton's fears were exaggerated, but one of the surest safeguards is surely to provide the fullest opportunities for social mobility. In short, the liberals of the thirties might have had the wit to recognize that an objective appreciation of the relationship between genetics and intelligence would have strengthened their case for equal opportunities and not diminished it.

Three decades later, the recognition that the spread of IQ within some social group, however narrowly defined, is likely to be greater than the mean difference of IQ between two groups is not merely a proof that social eugenics based on intelligence is bound to be an illusory business but also an argument for an egalitarian society.

So why not apply the same arguments to the comparison of different races on the grounds of intelligence? This is the first question to ask. The simplest answer is that the question has no meaning (which, fair play, Professors Bodmer and Cavalli-Sforza acknowledge). The genetic isolation between say American blacks and whites has been so complete for so long that nobody can tell whether measurements of IQ developed with some reason as a mark of intelligence within the white community should be taken seriously when applied elsewhere. The second and more interesting answer is that the wrong question has been asked. The outstanding truth about the human species is that half a dozen races have somehow

Pagination of *Nature* plus Two

Contrary to the previous announcement (*Nature*, **228**, 595; 1970), from the beginning of 1971 the pages of the three weekly issues of *Nature* will be numbered independently and not consecutively. A reference to *Nature Physical Science*, for example, would therefore be: Brown, S., and Smith, P. W., *Nature Physical Science*, **229**, 67; 1971. Volumes will continue to be numbered as at present, so that when *Nature Physical Science* and *Nature New Biology* are published on Mondays and Wednesdays next year they will begin at volume 229, which will be the same volume as the Friday issue of *Nature*.

managed to survive the climatic ups and downs of the Pleistocene. As many races and even genera may have disappeared without trace. What, after all, has become of the robust australopithecines (Leakey's *Zinganthropus*)? In circumstances in which it is plain that intelligence has been a crucial asset in survival, it is only reasonable to suppose that all of the races now extant are much of a muchness in intelligence. A demonstration that this is so would surely go a long way to damp down racial tension, as Professors Bodmer and Cavalli-Sforza acknowledge. Should they not have a little more confidence than they display that a proper investigation—including a broadening of the concept of IQ to make this applicable outside the white population for which it was first designed—would produce the result they desire? And is it not also on the cards that qualitative differences in intellectual attitudes should be investigated now, before they are buried forever by the inevitable and desirable miscegenation there is bound to be in the decades ahead?

Another reason for hoping that public funds will not be denied to proposals for making sober comparisons of the intellectual attributes of the several races is that good investigations are essential if ill-judged generalizations on these matters are to be overturned. Second-rate investigations are certain to happen and, as things are, a good deal of rampant race prejudice rests on unfounded assertions (in white communities) that blacks are intellectually less sharp than whites. A moratorium on research will not make these prejudices go away but, rather, will simply ensure that they are never challenged by objective reality. Is this really what Professors Bodmer and Cavalli-Sforza intend? Nobody will dispute the liberality of their motives in suggesting this, but is there not a serious danger that in the complicated circumstances of the modern world, liberalism may take the form of an anxiety not to rock the boat for fear of raising awkward questions? Properly stout-hearted liberalism should take quite a different tack, and should behave as if nothing is ever lost by looking the truth quite squarely in the face. In this case, to be sure, there are all kinds of semantic issues to be settled, not the least of them the definition of suitable yardsticks for the measurement of something as abstract as intelligence. It is also true that there has recently been a great deal of specious argument about the need for studies of the racial genetics of intelligence—Professor W. Shockley has been one of the most infuriating firebrands. None of this, however, amounts to a case for thinking that the whole subject should be put into cold storage. To think that is to play into the hands of the illiberals.

Weathercock Turns Round

THE British Government's decision to contribute towards the 300 GeV accelerator at CERN is at once welcome and distasteful. On balance, it is good for European science and in particular for high-energy physics that there should be a proton accelerator capable of matching to some degree the performance of the machines soon to be commissioned in the United States. The fact that the new accelerator is to be managed by the CERN consortium is also welcome, for the organization has shown itself in the past to be capable and level-headed. At the same time, however, it must be acknowledged that the way in which the 300 GeV project has hung like an albatross round the neck of European physics in the past decade is a sign that the CERN consortium is not nearly as well-knit as it has become conventional to suppose. In the years since 1964, when the project for the 300 GeV machine began to take shape, CERN has blown too noticeably hot and cold. Its first claims on public attention—and on the treasuries of European governments—were far too grandiose and inflexible. In a sense, the organization is lucky that one of its sternest critics, the British Government, is now prepared to foot the bill. It is to be hoped that the organization has learned a lesson from all this.

Complacency has been the most serious of the organization's faults. At the beginning, CERN behaved as if European governments would have to foot whatever bill was presented to them. The first design for the proton accelerator was too cumbersome. CERN nevertheless told potential contributors that there was no room for manoeuvre. If they had paid up and if it had not been for the shining example of Dr R. R. Wilson's bigger but cheaper accelerator at Batavia, Illinois, the chances are that Europe would still be lumbered with a needlessly expensive machine. In the end, only the unwillingness of governments to contribute to this first version of the big accelerator, and their scorn for the plans which accompanied it for building an even larger base of national accelerators on which to erect the 300 GeV pinnacle, persuaded CERN to trim its sails. In the past few days, there has been a great deal of talk of the organization's skill at living within an agreed budget, but this is in sharp contrast with the capacity of the organization to devise plans that are meaningful. The result, unhappily, has been to reinforce the popular but misguided impression that high energy physics is one long extravagance. It is to be hoped that the less ambitious plan to which at least the British Government is prepared to contribute will now help to restore respect for the high energy physicists. Optimism about the 300 GeV machine must naturally be to a large extent counterbalanced by doubts about the future of high energy physics programmes in the countries which contribute to CERN, notably Britain, France and West Germany. It seems to be acknowledged that the British Government will only sign the cheque expected of it if, in the course of the six or seven years during which construction at Geneva will be going on, there is progressive economy in

the cost of the high energy laboratories at Daresbury and Harwell. The larger and more influential groups of high energy physicists will be quite satisfied with this arrangement, no doubt, but less fortunate groups will be harder hit. Yet the British Government and the Science Research Council are entirely right in trying to contain the budget for high energy physics within a substantially constant sum of money. So what is to be done? In circumstances like these, it is surely high time that talk about the international character of high energy physics was lent a greater sense of reality by the setting up of some international machinery for making sure that European resources in this important field are fully pooled. The simplest way of doing this is to provide CERN not merely with the funds for building the new machine but also with responsibility for seeing that machines scattered elsewhere in Europe are made the fullest use of. This would imply that CERN would become a kind of research foundation. It would also imply that individual nations would be offended by what seem to them to be an assault on their sovereignty.

Food for the Crowds

THE latest annual report of the Food and Agriculture Organization of the United Nations (see page 1021) is a more realistic document than many previous members of the series. Sensibly enough, the report openly acknowledges that there has been a sustained increase of food production in the past few years. "At long last something of a turning point may have been reached in the difficult struggle of the developing countries to achieve a sufficiently rapid increase in their food production." In the past, the temptation to urge on readers the danger of the still growing population of the Earth has often been too strong for the authors of these reports. Now, however, it does seem clear that when seasonal ups and downs are ironed out, food production is increasing if anything more quickly than population. For the world as a whole, for example, food production per head is now about 10 per cent greater than in the early fifties and, as the annual report of the FAO makes plain, the improvement in the Far East has been particularly marked—a total increase of 14 per cent in three years. Although the new cereals are a part of the explanation, there is no doubt that much of the improvement can be attributed to the increasingly level-headed ways in which agriculture is managed in developing countries. Although the credit must largely go to the governments concerned, there is no doubt that the FAO itself deserves some share of it.

If the statistics of food production for the world as a whole are satisfactory, however, there still remains the problem that the effects of the new agricultural revolution vary enormously from one place to another. In both the Far East and the Near East, food production is gaining on population, but the balance is stagnant in Latin America while in Africa the trend is downwards, chiefly because of decreases of cereal production in north-west Africa. Elsewhere, luckily, the FAO considers that the livestock industries are growing well and that modern agricultural technology, is catching on. Obviously it is a great help to most of the developing nations of Africa that the level of economic activity is growing much more quickly than the population, so that there is cash to spare to the support of mechanization in agriculture. In the long run, this is the way in which these nations will find a way out of their immediate difficulties.

But what of the more distant future? The most obvious danger is that there may in due course be such a large increase of population that no amount of increased efficiency in agriculture will be able to provide for it. This, however, is still very much an academic matter. For one thing, the way in which the graphs of population and food production have kept more or less in step for the past thirty years suggests that demand can effectively stimulate supply without bumping up against physical limitations of an absolute character. Second, the population statistics on which these calculations are inevitably based take very little account of the way in which the birthrate has been declining in several countries in the past few years.

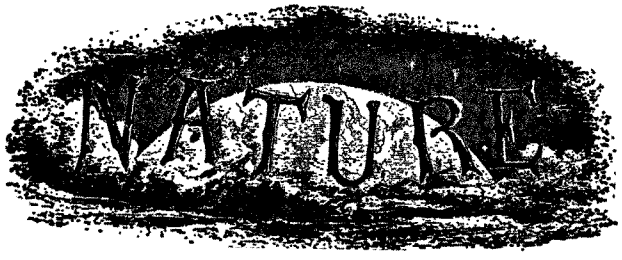
Aircraft in Trouble

THIS has been a bad week for new aircraft projects. In the United States, Congress has for the time being at least decided not to vote the funds to keep the Boeing design for a supersonic transport (the SST) alive for another year (until July). In Britain, the government has decided (see page 1019) to provide no further support for two dreams prevalent among British aircraft manufacturers for several years—the scheme for participating in an airbus designed by a European consortium and one for building an almost identical vehicle in Britain. By this act of seeming brutality, the government will have gone a long way to finish off the European airbus and at the same time to ensure that the British aircraft industry will never again become a builder of air frames in the grand manner. Almost inevitably, European airlines wishing to equip themselves with large passenger aircraft for the late seventies will have to buy instead in the United States, while the chances are high that if the European airbus does survive, this will be dependent on American and not British engines. In the United States, the boot is on the other foot, for it now seems probable that if Concorde can be made to function properly, American airlines will be going cap in hand to the British Aircraft

Corporation and to Sud Aviation for their first examples of supersonic aircraft.

Why should all this be ? With aircraft as with computers and many other modern technical developments, the scale of investment needed to bring a product to the market place is at once beyond the resources of nationally based commercial enterprises and also too great to be recouped by sales within a restricted market. Nobody should be surprised that in circumstances like these there is a fierce conflict between patriotic sentiments about the need to manufacture each product of innovation on a national basis and the need somehow to make such devices commercially viable. The trouble, of course, is that the governments which pay for these developments are also the sources of the chauvinistic zeal that serves somehow to ensure that they bring no profit. In the long run, devices must be found for making sure that governments do not behave in such a foolish way, which in turn implies that all of them will have to recognize that there is no lasting shame in having to buy capital equipment from elsewhere. With a little luck, the sober lessons of this week's decisions about new aircraft may persuade two of the governments concerned that the time has come to learn a little moderation.

100 Years Ago



AT the meeting of the Royal Geographical Society, held on Nov. 29, a paper was read "On the Geography of the Sea Bed," by Capt. Sherard Osborn, R.N. The author gave an account of our present knowledge of the configuration of the bed of the ocean, as derived from Admiralty surveys and submarine telegraph expeditions during the last fifteen years. His explanations were illustrated by a number of diagrams showing sections of the North Atlantic and other oceans. It has been definitely ascertained that the greatest depth of the ocean does not reach 3,000 fathoms in any part where telegraphic lines have been laid. The bed of the North Atlantic consists of two valleys, the eastern extending from 10° to 30°, the Western from 30° to 50° West longitude. The extreme depth of the eastern valley is under 13,000 feet, which is less than the altitude of Monte Rosa. This valley has been traced southward to the equator. It is separated from the western valley by a ridge in 30° West long., in which the average depth is only 1,600 fathoms. This ridge terminates to the north in Iceland, and southward at the Azores, so that it is volcanic in its character at both extremities. Its extreme breadth appears to be under 500 miles, and the Atlantic deepens from it on both sides. Explorations carried on in the Mediterranean, the Red Sea, and the Indian Ocean, showed similar uniformity in the level of the sea-bottom ; and the general conclusions arrived at by Capt. Osborn were that in the deep sea there is an absence of bare rock, and that there are no rough ridges, canons, or abrupt chasms.

From Nature, **3**, 133, *December* 15, 1870.

OLD WORLD

FRANCE

Space Effort Declines

AFTER ten years of expansion, the Centre National d'Etudes Spatiales—the French Space Agency—is being forced to take in its belt. Although some decrease in the agency's budget was to be expected following the devaluation of the franc in 1969, the drop of more than 20 per cent last year cannot be attributed solely to the state of the French economy. It is true that the series of satellite launchings from French Guinea with the Diamand-B rocket is keeping up to schedule and that the plans for meteorological satellites in the Éole and Météosat projects will ensure that the French space effort has a clear identity well into the nineteen seventies. But the damaging two month strike of engineers and technicians (which ended last month) at the CNES site at Brétigny, the nerve centre of the space programme, has highlighted the uncertainty surrounding the long term future of the CNES and has reinforced doubts about the correctness of the balance between national and international projects for which France has opted in its space policy.

The importance of ensuring that the French space programme fits sensibly into a wider European framework is a dominant theme in the annual report of the CNES up to July 1970. The report points out, for example, that from 1971 about 40 per cent of the cost of the launching site at Kourou in French Guinea will be met by ELDO, and it also draws attention to the agreement concluded this year to launch two models of the Franco-German telecommunications satellite, Symphonie, by the end of 1973.

Development of a series of French meteorological satellites under programmes known as Éole and Météosat is also given prominence in the report. The first satellite, Péole, is scheduled to be launched later this month into an equatorial geocentric orbit and will be followed by a second satellite, Éole, in July 1971. These will carry out preliminary studies to pave the way for a detailed survey of cloud cover in the visible and infrared wavelengths in a later project, Météosat. The Météosat satellite will also be launched into an equatorial orbit and will provide data on the western regions of Europe, on France and on Africa and Madagascar. The programme will fit into the Global Atmospheric Research Program (GARP) and it is likely to complement the European plan for putting a meteorological satellite into polar orbit.

The next satellite in the D-2 series, designed essentially for research in astronomy, is the D-2A which will be put into orbit in 1971 with five experiments to study the ultraviolet emission from the Sun and the distribution of hydrogen in the Earth's atmosphere. D-2B will follow in 1973 and will house experiments from the Space Laboratory in Marseilles and the Laboratory of Stellar and Interplanetary Physics of the CNES to study solar and stellar emissions in the far ultraviolet. The report also points out that French rocket probes should remain competitive for astronomy research for about five more years, and will be developed under the programme name FAUST.

The impending transfer of various facilities of the CNES from Brétigny near Paris to Toulouse was one of the principal bones of contention in the recent strike at Brétigny. The division of rocket probes is now being transferred and the installation of a space simulator at Toulouse is nearing completion. But the transfer of satellite work, planned for 1971–2, has now been postponed in deference to the views of personnel and there is concern at the probable lull in activity towards the end of 1971 after the D-2 and Éole satellites have been finished and before the new programme has got under way.

CERN ACCELERATOR

Green Light at Last

THE British government's decision to participate in construction of the 300 GeV proton accelerator at CERN makes it almost certain that after more than two years of oscillating fortunes, the project will finally go ahead. A formal decision must await the meeting of the CERN Council in Geneva on December 22, but the governments of the three other major contributors, West Germany, France and Italy, have already made it clear that they are ready to join the project if the British government also gives its support. The decision to participate, announced last week by Mrs Thatcher, Secretary of State for Education and Science, reverses a decision taken by the Labour government in 1968 not to back the original version of the project, which would have cost more than half as much again as the £90 million estimated for the new version. The original scheme is also now recognized to have been too inflexible to make the best use of possible developments in magnet technology during the coming decade.

The timing of Mrs Thatcher's announcement is itself significant. With more than two weeks still to go before the governments of the other eleven member countries of CERN are obliged to make their final decisions on the project, the British government has calculated that an early decision on its part would provide a firm stimulus for the other governments to add their support to the project before the meeting of the CERN Council. Officials at CERN are particularly pleased with the swiftness of the decision. The debate on the project in the Swedish parliament is also being watched with particular interest from Geneva. Although the Swedish contribution would amount to only 4·6 per cent of the total cost it is felt that the attitudes of the smaller countries are likely to be reflected in the Swedish debate.

The British government's decision will have a profound influence on nuclear physics in Britain. The £2·5 million a year which Britain will contribute to the 300 GeV accelerator will be taken from the existing nuclear physics budget of the Science Research Council, which stands at about £20 million a year. Because £7 million of this is already earmarked for the existing programme at CERN, the extra money must come from the £13 million which is shared between the Rutherford and Daresbury laboratories and the universities. The question of how best to initiate cuts in the national programme will be discussed at the next meeting of the nuclear physics board of the SRC, scheduled to take place on December 22 but now postponed because of the council meeting at CERN.

SELENOLOGY

Shedding Light on the Moon

Paris, December.

THE French laser reflector carried to the Moon by Luna 17 had its origin in a visit to the Soviet Union by General De Gaulle in 1966, when a Franco-Soviet committee was established to organize scientific cooperation. France had then just undertaken a series of laser ranging experiments with its Diadème satellite. The Russians, who were considering lunar experiments along the same lines, asked the French to participate and build a reflector. In February 1968, the arrangement was made formal by a Franco-Soviet agreement and the Centre National d'Études Spatiales (CNES), the French co-ordinating agency, awarded the contract for the construction of the unit to Sud Aviation (now known as Société National Industrielle Aerospatiale).

The list of experiments planned for these reflectors is already long and includes measurements of the distance between the Earth and the Moon, of the Moon's radius to within 50 metres (instead of the present two kilometres), of the physical librations of the Earth and Moon, a study of fluctuations in the rotation rate of the Earth, and more accurate measurements of distance between points on the Earth.

It is hoped that these last measurements will provide a direct verification of continental drift, but this calls for a series of measurements extending over several years and to a precision of better than a metre. Another objective is to define more precisely the universal gravitational constant.

Both the American and French reflectors are made up of silica cube corners, the faces of which must be ground to within two-tenths of a second of arc. The French chose a compact array of fourteen prisms, each with a base of ten centimetres, compared with the Apollo 11 reflector made up of one hundred prisms of 3·75 centimetre diameter. Additional silver plating on the reflecting faces of the trihedrals increases their reflecting power. Under comparable conditions the performance should be five times better than that of the Apollo 11 panels. The French device cannot, however, be used during the lunar day because heat expansion deforms its elements, a problem which is less severe for the Americans because of the smaller size of their elements. Thus the French reflector works better than its American counterpart during the lunar night, but cannot be used during lunar daylight. This, of course, increases the problem of pointing the laser accurately at the reflector—the reflector position has to be known with respect to points on the illuminated crescent of the Moon.

Stations involved in the Franco–Soviet experiment so far are the Crimean Observatory and the Pic du Midi Observatory in the Pyrenees. The Pic du Midi team has already experimented with the Apollo 11 reflector. A ruby laser was used to send a series of 25 Joule pulses of one millisecond through a 105 centimetre telescope which reduces beam divergence by a factor of 6 × for a 95 cm exit diameter. A 0·1 Joule/km^2 spot is produced on the Moon. Statistical analysis of 100 shots is required to verify that an echo has been received and to permit a precise measure of the time lapse between transmission and reception (about 2·5 s.) The number of acquisitions should be four times greater with the Luna 17 reflector. It should be noted that one hour is needed to verify echo reception and work is limited

to about 3 h a night, three nights a month if weather conditions are good.

The 2·5 metre telescope of the Crimean observatory can receive more reflected light and its laser is four times more powerful than that of the Pic du Midi. Unfortunately its low altitude site complicates observational work.

In the United States the Lick and McDonald observatories have measured the Earth–Moon distance to within 30 cm. The Orszag team is now building a 10 m^2 light collector which should improve the observations and will be operational by summer.

The United States and USSR are working on hollow corner reflectors which would have lower thermal capacity and thus less thermal deformation.

ROCKETRY

Comecon Lowers its Sights

from our Soviet Correspondent

FOLLOWING the success of the joint effort by the Comecon countries to produce the Interkosmos satellites, the latest Comecon space project may seem unambitious. The Soviet Union and its partners are embarking on a programme of geophysical research using instrument packages launched into ballistic trajectories by non-orbital rockets. According to its publicists, however, the main purpose of Vertikal-1, the first firing in the series which reached an apogee of 487 km before descending to 100 km where the recoverable instrument package was released, is to investigate the atmosphere between 100 and 200 km. The problem in maintaining a satellite in this region is that the atmospheric drag is still sufficiently large for the orbit to be degraded rapidly. Dr I. Zacharov, a Czech scientist writing in *Pravda*, calls this region the "ignorosphere where even the density of the air has not been studied sufficiently".

In the published programme the main purpose of Vertikal-1 was "to carry out complex investigations of the ultraviolet, X-ray and submillimetre radiation from the Sun and the absorption of this radiation in the Earth's atmosphere; determination of the electron concentration and temperature at high altitudes; and studies of meteoritic particles". The instruments include X-ray pinhole cameras and an X-ray spectroheliograph from Poland, X-ray spectrometers from the Soviet Union, equipment for the study of meteoritic particles (jointly developed by the USSR, Czechoslovakia, and Hungary), a Lyman α photometer and r.f. capacity probe from East Germany, and equipment for measuring solar submillimetre radiation and ionospheric parameters (developed by the USSR with help from Bulgaria, East Germany and Czechoslovakia). The flight was also used to measure the absorption of radio waves at frequencies of 1, 1·5 and 2 MHz, using an East German ground unit.

It is worth noting that Vertikal-1 does not seem to have been launched from Baikonur. Although the site has not been announced, it is described as being "in the territory of the European part of the USSR, at middle latitudes", perhaps in deference to the Soviet Union's Comecon partners. The rocket was assembled by a group from the USSR, East Germany, Poland, and Czechoslovakia, who were also present at the launch.

NUCLEAR ENERGY

Second Time Around

A SECOND Parliamentary bill to form two companies from the production establishments of the UK Atomic Energy Authority was published last week (*Atomic Energy Authority Bill*, HMSO, 4*s*). It is almost a carbon copy of the bill which was put forward by the Labour government last March but which failed to reach the statute book before Parliament was dissolved. The new bill proposes that nuclear fuel should be produced in the UK by British Nuclear Fuels Ltd, a company in which the government will always have a majority interest; a similar company, the Radiochemical Centre Ltd, will take over the AEA's booming, though more modest, commercial radioisotope business. It has also been suggested that private firms or individuals would eventually be encouraged to acquire a minority holding in both companies.

The Atomic Energy Authority has recognized for many years that its growing commercial activities would eventually have to be separated from its research and development functions. As a first step in that direction, a trading fund was set up in 1965 to finance the electricity generating facilities at Calder Hall and Chapelcross and the fuel production at the Springfields Works near Preston, where the formation of uranium hexafluoride from uranium ore has been improved since 1968 by the provision of a continuous fluidized bed process which cost £600,000. A large investment has also been made in production equipment for the coated pellet fuel to be used in future high temperature reactors and Springfields continues to produce the older Magnox fuel and the canned uranium oxide pellets used in advanced gas reactors. The trading fund also took over financial responsibility for the Radiochemical Centre at Amersham and it later assumed responsibility for the enrichment plant at Capenhurst, Cheshire, and the reprocessing works at Windscale, Cumberland.

The wheel has now come full circle and next April the two new companies will probably take over the facilities of the trading fund, which achieved a turnover of about £46 million last year. The companies can look forward to a world fuel market possibly worth £1,000 million a year by 1980, of which British Nuclear Fuels Ltd could expect about £200 million equally divided between home and overseas business.

As independent companies, they will be able to enter much more effectively into agreements such as those which led to the formation of the joint British–Italian fuel company Combustibili Nucleari and the collaboration with Gulf General Atomic in the US on water reactor fuels. The efforts also made along these lines by the Radiochemical Centre are highlighted by the foundation of Amersham-Searle, a radiopharmaceutical company formed in conjunction with the American company G. D. Searle. The fuel company will also be well placed to take advantage of the gas centrifuge pact agreed last March between the UK, Germany and Holland, parts of which have to be renegotiated early in 1971.

The Institution of Professional Civil Servants, which represents well over half of the 9,500 personnel involved in the change, was initially concerned at the possibility that the Conservative government would allow the fuel and radioisotope facilities to fall completely into private hands and therefore that people originally

employed by a public body would find themselves working for a private firm. The institution cautiously welcomes the bill as a whole but is continuing to negotiate with the government about some of its smaller provisions.

AIRBUSES

Veto Keeps Options Open

THE British government's decision to tighten the purse strings by refusing to inject a large sum of money into either the European Airbus or its rival, the BAC 3–11, makes the British civil aircraft market especially attractive to foreign salesmen. But the decision also gives the government considerable room to manoeuvre in its deliberations over the future of the Concorde project, and it will have wide-ranging implications for Rolls-Royce, whose future is by no means assured by the £42 million shot in the arm which it received recently from the government and the Bank of England.

Faced with a very difficult choice between the A–300B European Airbus and the BAC 3–11 (see *Nature*, **228**, 496; 1970), the government spent nearly two months deliberating before it turned its back on a large share in the business of manufacturing wide bodied civil aircraft. The BAC 3–11, on which the British Aircraft Company has already spent some £3 million, will die from lack of funds, and British European Airways, one of Europe's largest potential customers for the airbus, will have a choice between the Lockheed TriStar, the A–300B and the McDonnell Douglas Airbus.

Rolls-Royce will now be hoping that BEA will plump for the Lockheed TriStar and its RB 211–22 engines. Having lost the opportunity for selling a follow-up version of the RB 211–22 in the BAC 3–11, and with no share in the European Airbus or the McDonnell Douglas DC–10, Rolls-Royce will be looking for a long sales run with the TriStar to recoup some of the huge investment which the company and the taxpayer have sunk into the RB 211–22. BEA was known to be strongly in favour of the BAC 3–11, with the TriStar as its second choice, and it is therefore unlikely that the airline will take the DC–10. But, on the other hand, the A–300B involves substantial British participation from the Hawker Siddeley Aircraft Company, which decided to stay in the project when the British government pulled out in 1968. The airline is therefore being pulled both ways by British interests.

In making the decision not to support either airbus, the government was keeping one eye on the development of Concorde. The rapidly increasing costs of Concorde—now running at £825 million for the prototypes alone—will leave little money available for other civil aircraft, and a decision in favour of the BAC 3–11 would inevitably have made cancellation of the Concorde project more likely. Such a decision would, of course, be a singularly bad card to play while negotiations for Britain's entry into the Common Market are taking place. To help to quell such fears, however, Mr Frederick Corfield, Minister for Aviation Supply, told members of the House of Commons that he has been discussing with other governments more European participation in the manufacture of aero engines, and that he hopes such cooperation can be extended to the rest of the aviation industry.

MEGALITHS

Breton Origin for Tombs

from our Archaeology Correspondent

NEW radiocarbon determinations for megalithic tombs in Brittany substantiate the very early dating for these stone monuments and suggest that these tombs are the oldest in Europe. The unexpected possibility of a Breton origin for the custom of burial in megalithic tombs in Britain and even in the rest of north-western Europe must now be taken seriously.

The impressive megalithic collective tombs of western Europe have always seemed an astonishing achievement, somehow beyond the capabilities which could be ascribed to neolithic man. The very early carbon-14 dates produced by the French radiocarbon laboratories for the megalithic tombs of Brittany, setting them before any such tombs dated elsewhere, have been regarded suspiciously by prehistorians. For although the pros and cons of a West European rather than an East Mediterranean origin for the megaliths has long been argued, Iberia at the southern extent of their distribution and Denmark at the north have seemed the most likely regions for the development of megalithic architecture.

The very early dates in Brittany for what seemed the most sophisticated of these monuments, the corbelled passage graves, have also been doubted. The impressive burial chamber in these graves is reached by a long underground passage, and is roofed by a false vault of drystone construction. Examples of this building technique are seen in the tombs of Spain and Portugal, in Ireland (New Grange, Knowth) and in Scotland (Maes Howe) as well as in Brittany. A Breton example, Ile Carn, was dated in 1959 by the Groningen radiocarbon laboratory to 3280 BC, and other Breton megalithic tombs were dated by the French laboratories to about 3500–3000 BC. These determinations were too early for most archaeologists to accept. Unfortunately, one or two dates were more than a millennium higher—there is a determination of 6850 BC for a stone cist grave at St Michel. The necessity of explaining these as the consequence of the prehistoric use of bog oak did little to increase confidence.

Since 1959, however, further dates in the time range 3500 to 3000 BC have been accumulating, and radiocarbon determinations by Delibras, Guillier and Labeyrie (*Radiocarbon*, **12**, 421; 1970) give 3390 BC for the principal chamber at Ile Carn, and 3440 and 2890 BC for adjacent dolmens. These dates substantiate fully the previous early dates for Ile Carn and for other Breton tombs.

At present there are few carbon-14 dates for Iberia —conventionally the most favoured area for the origins of the European megaliths—and, in any case, the custom of collective burial in megalithic chambers may have developed independently in different parts of Europe. Yet, for the British Isles at least, these Breton dates may have considerable significance because the British neolithic farming economy was undoubtedly brought across the English Channel during the fourth millennium BC.

It has been considered that the unchambered long barrows found in England were a component of the first neolithic communities, with megalithic chamber tombs a later development. Recently, it has been

suggested that the timber mortuary house revealed by excavations in some British earthern long barrows (Ashbee, P., *Archaeologia*, **100**, 1; 1966; *Antiquity*, **43**, 43; 1969) may have been the prototype for the supposedly subsequent megalithic tombs of Britain (Daniel, G. E., *Antiquity*, **41**, 315; 1967). The early Breton dates now suggest a different conclusion, at which the earliest British megalith date—3160 BC for Monamore Cairn in the island of Arran (McKie, E., *Antiquity*, **38**, 52; 1964)—was just beginning to hint. It now seems possible that collective burial in megalithic chamber tombs was already a practice known to the first immigrant farmers, who reached Britain around 3500 BC. Indeed, any migrants who sailed from Breton shores after that time can scarcely have avoided a knowledge of megalithic architecture. It may be significant that the megalithic tombs of Britain lie chiefly in the west.

It remains to be seen whether a Breton origin for the British megaliths will find favour among archaeologists, at a time when migrationist explanations in prehistory seem to be going out of favour. Yet if the skills of the farmer were taken to Britain by immigrants, as undoubtedly they were, could not these first farmers have carried with them also the specialist skills of the megalithic undertaker?

TECHNOLOGICAL FORECASTING

Techniques under Fire

Paris, December

ARE the decision-making processes used in research and development an art—and a black art at that? The specialists at the Organization for Economic Cooperation and Development (OECD) are too reticent to give an explicit "yes" in reply, but at a meeting held in Paris during the last week of November they approved a report which is sharply critical of the well known decision-making techniques of which "think tanks" are at once the producers, stars and impresarios.

The report, which will be published in 1971, was prepared by Keith Pavitt (of the OECD Directorate of Scientific Affairs) and Claude Mestre (technical adviser to the French Transport Ministry). A direct outcome of an OECD conference in Paris in March 1969, the report is meant to familiarize government officials with the methods of analysis and rationalization of choice used in research and development. It bears out the suggestion that sophisticated operational research is not necessarily more productive than dead-reckoning empiricism. Born during the Second World War from defence needs, forecasting and decision-making techniques have not yet proved their effectiveness outside the area of military research, according to Pavitt and Mestre. To begin with, they explain that the arcane language of "rational" methods works against a meaningful exchange with politicians. Moreover, analysts often mix—unwittingly or not—political theory with technical hypothesis and they are always tempted in their forecasting to favour costs and advantages which can be backed up with statistics, while eliminating or underestimating elements that are sometimes very important but are not quantifiable or yet quantified. It is therefore to be feared that the fashion of highly refined technique "will lead, for purposes of prestige, to a search for very sophisticated methodology which conceals a serious ineptitude to analyse real situations".

After a general introductory statement, the authors review the principal decision-making methods which have been offered as miracle solutions to the never-ending problem of criteria of choice. Supporting its suggestions with concrete examples, the OECD report criticizes, among others, the Delphi technique (the panel of specialists used in this method are often badly selected; concern about obtaining convergent opinion tends to eliminate minority opinions and results in a triumph for conformism); simulation models (based all too often on insufficient data the applications of which are usually expensive and impractical); relevance trees ("despite their popularity, the validity of methods of this type is absolutely not proven"); the "scenarios" made famous by Herman Kahn (they are "costly and long to formulate without necessarily offering a guarantee for the proper anticipation of political, economic or social change and their influence on technological needs and trends"); the PERT method (a study of thirty-seven research and development projects in the United States showed that the use of PERT did not generate a single advance in technical performance); PPBS (planning, programming and budgeting system) on which, outside the military sector, we possess little positive or negative evidence.

On the face of things, although the authors sought to give a balanced view of the various methods now in vogue, the hundred page document is a fairly pessimistic report on the efforts of the past 20 years to create a solid foundation for technological forecasting and long range planning. In the realm of research and development, the new miracle men have yet to demonstrate their credibility.

BRITISH COUNCIL

Signs of Change

THE British Council, founded in 1934 to promote knowledge of Britain abroad and to develop closer cultural relations with foreign countries, emphasizes its dual role in the field of both science and the arts in its annual report for 1969–70. The recommendations of the Duncan Committee (*Report of the Review Committee on British Representation Overseas*, HMSO; 1969), that the council's functions in Europe should be expanded, are also given some prominence in the report. The council's proposed expenditure for 1970–71 amounts to about £14·6 million, of which about £13·5 million will be in the form of grants from the Foreign and Commonwealth Office (including contributions from the old Ministry of Overseas Development). Although this budget represents a standstill in real terms, it follows an absolute 5 per cent reduction in 1969–70.

The scientific activities of the council are concerned both with making British scientific work better known abroad and with encouraging collaboration between scientists in Britain and overseas. Visits and exchanges of personnel between British and foreign universities and research institutions are arranged or financed by the council and, during the past decade, the number of scientists involved in such activities has increased from 1,075 to 3,470. In addition, services to overseas students of all disciplines in this country are an important part of the council's work; 8,000 Commonwealth and foreign students were given assistance and advice when they arrived in Britain during 1969–70.

Future trends in the British Council have been hinted at in the Duncan Report which recognizes that the council "will become an increasingly important medium through which Britain will project her interests . . .", and it is also suggested that future reductions in expenditure would be very undesirable. The report specifically recommends that the British Council should concentrate its activities in Western Europe because of the importance of Europe in Britain's foreign policy. It also calls for more scientifically qualified British Council staff overseas. About sixty council personnel (8 per cent of the total) are scientists, and two-thirds of these serve abroad. A move to increase the proportion of scientists to about 20 per cent is suggested by the increase in scientific recruitment during 1970, which amounted to about one-third of the total.

The possibility of increased council involvement in Europe would represent a distinct reversal of the policy, suggested by the Drogheda Committee in 1954, that British Council offices in Europe should be run down so that facilities could be concentrated in the developing parts of the world. From a scientific point of view, expansion in Europe would probably mean greater emphasis on the Council's libraries and information work. But if, as the Duncan Committee envisages, the council also retains its high degree of involvement with countries outside Europe, its grants in future years will have to rise considerably.

SALARIES

Another Pay Rise for Dons

ACADEMIC staff in British universities are to receive a 10 per cent increase in their salaries, backdated to October 1, 1970. The increase is the first to be negotiated under the new negotiating machinery for university salaries, and it is the second large increase secured by the AUT within twelve months. Although the award goes only half way towards meeting the claim put in by the AUT last August, it will help to redress the imbalance in university salary scales caused by recent large increases in the salaries of clinical staff in medical schools (see *Nature*, **226**, 786; 1970).

The AUT based its claim on similar criteria to those used by the Kindersley Committee for recommending increases in doctors' salaries. University pay scales were compared with those in the civil service and other professions, and the AUT also pointed out that a change in the staff/student ratio during the past few years has increased the work load on academic staff. These sums resulted in a claim for an increase of 20 per cent, which was discussed by a committee comprised of representatives of the AUT and university authorities. This committee eventually agreed to put forward a claim of 13 per cent to the government, and this was discussed in a second committee consisting of representatives of the first committee and of the government. The final result of the deliberations was an increase of 10 per cent.

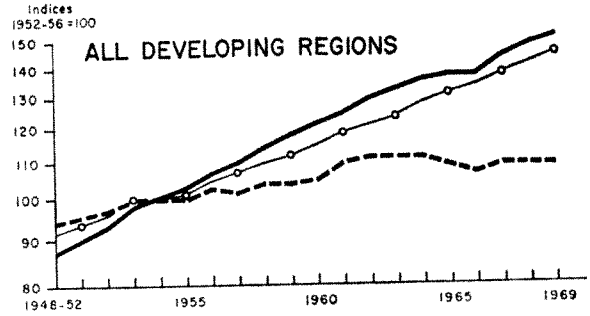
Mr Laurie Sapper, general secretary of the AUT, is not at all despondent that the final award is only half the sum he asked for. "In view of the pressures on salaries in the public sector", he said, "we consider the settlement to be the most reasonable we could hope for." The AUT is also particularly pleased that the government has given an undertaking to review university salary structures during the present academic year.

UNITED NATIONS

Food for the Future

ALTHOUGH there may have been little improvement in the standard of nutrition available to the populations of needy and developing countries over the past twenty-five years, it has nevertheless been possible to support an unprecedented increase in world population during this period without major breakdowns in food supply. This is the most telling comment which emerges from the report *The State of Food and Agriculture 1970* (available from HMSO, 60*s*) published last week by the Food and Agriculture Organization of the United Nations (FAO). Indeed, it seems that the combined effects of long term planning at government level and the remarkable and recent advances in agricultural technology may have pushed many struggling countries past the critical point in their battle to achieve and maintain a sufficiently rapid rate of food production.

The year 1970 is of peculiar significance for the FAO for it is the 25th anniversary of the organization's foundation. Furthermore, as the title page of the report rather dramatically puts it, the world is on "the threshold of the second development decade". This has provided an excellent reason for the FAO to look back briefly over the progress which has been made since 1945, and to look forward, not too gloomily, to the problems which seem likely to threaten the future progress of world agriculture.



Growth of agricultural production (—) and population (o—o) in developing countries from 1950-1969

After the second world war, improved agricultural methods brought a transient increase in food production, but this was followed by a long period of disappointingly slow progress, during which production declined but populations increased unchecked. The nadir of this depressing period was the dismal harvests of 1965 and 1966, when only massive food relief averted disaster in many countries.

Progress since 1966 has been remarkable as a consequence of the increasing interest taken by the governments of developing countries in the methods available to increase their productivity, and in the new agricultural technology which has made this possible. Four topics are highlighted by the FAO report which may offer especial advantages or present outstanding challenges in the future. The first is the hope that the high-yield dwarf cereals, developed chiefly in Mexico, may continue to provide the bulk of the carbohydrate and protein required by the developing countries.

The second faces squarely the fact that meat protein is expensive and likely to become more so; the answer may lie in increased production of fish and vegetable protein, and in intensive breeding of pigs and poultry, which have a shorter reproductive cycle than ruminants. The last points embrace more political problems. Trade balances may have to be adjusted as countries which, in the past, have imported food now find they have a surplus to dispose of. And then there is the inevitable spectre of unemployment. Social problems engendered by the use of highly developed technology in developing countries are only now becoming widely appreciated, but clearly they merit further attention.

CHEMICAL INDUSTRY

Gloom at Albright

THE gloomy prediction by Sir Richard Powell, chairman of Albright and Wilson Ltd, that a loss during the second half of 1970 will probably wipe out the profit before tax of about £1·4 million earned in the first half is symptomatic of the ill wind blowing through the whole chemical industry. The company, which manufactures a variety of products ranging from detergent chemicals to pharmaceuticals, seems to be following the trend of the other chemical giants such as ICI and Laporte, which have also recently reported disappointing results.

Not all Albright's troubles, however, can be traced back to the inclement economic atmosphere. The £20 million phosphorus plant built by the Electric Reduction Company of Canada Ltd (the ERCO division) at Long Harbour, Newfoundland, has been a source of trouble since it first started operating about two years ago. The plant was closed for several months during 1969 because of evidence of water pollution, and about £1 million was spent on measures to prevent the discharge of phosphorus into the sea. The first full shipment of elemental phosphorus by specially designed bulk tanker did not reach Britain until December 1969, although the plant was operating again in July.

It has now been announced that one of the two furnaces at Long Harbour has been shut down again because of breakage of the carbon electrodes used in the electrothermal extraction of phosphorus from the phosphate rock ore. Other modifications have also become necessary and it would seem that the target annual output of 70,000 tons is still a long way off. Although the company produces elemental phosphorus elsewhere in Canada and in Australia, the Long Harbour setback has forced it to buy phosphorus from other producers and to recommission old plant in the UK. Fortunately, many of the detergent chemicals are produced from the ore by a wet process.

Depressed markets, late delivery of equipment and shortages of sulphuric acid are responsible for troubles in the Marchon division of the company, which produces large quantities of cement and comprises several European firms, and in the Canadian associated company Belledune Fertilizers Ltd which is running at a considerable loss, principally because of the poor state of the fertilizer market. Even the Midland Silicones division, which manufactures greases, rubbers and electrical insulating material and has been a healthy member of the group in the past, has been obliged to announce harsh measures to combat rising costs and the other consequences of inflation.

Parliament in Britain

Third London Airport

THE Roskill Commission on the Third London Airport hopes to submit its report to Parliament by the end of December. Announcing this in reply to a question from Mr William Benyon, Mr John Davies, Secretary of State for Trade and Industry, also said that sufficient time will be allowed for a full debate on the recommendations of the commission, but the government feels that it must act promptly in deciding on the siting of the airport. The Roskill Commission is considering possible sites at Cublington, Foulness, Nuthampstead and Thurleigh. (Oral answer, November 30.)

Abortions

MR MICHAEL ALISON, Under Secretary of State at the Department of Health and Social Security, said in reply to a question from Mr Selwyn Gummer that 84,764 abortions took place in National Health Service hospitals between the time when the Abortion Act came into effect in November 1968 and October 28, 1970. He also said that the effect of the Act on the availability of hospital facilities varies from region to region, but no information on the amount of time spent by surgeons on terminations of pregnancy is available. Asked whether there is need for an investigation into the workings of the Abortion Act, Mr Alison said that the Secretary of State for Health and Social Security hopes soon to make a statement. (Oral answers, December 1.)

River Pollution

A SURVEY of the state of the rivers of England and Wales, being undertaken by the Department of the Environment and the Welsh Office, should be completed late next year. Mr Peter Thomas, Secretary of State for Wales, said that the report of the survey will provide an assessment of the cost of restoring polluted rivers to a satisfactory condition and of maintaining them in that condition. (Written answers, November 30.)

Bronchial Disease

IN reply to a question from Mr John Biggs-Davison, Mr Michael Alison gave the official figures for the yearly death rate in England and Wales from bronchial diseases. The figures are as follows:

Year	Asthma, emphysema, bronchitis, bronchiectasis	Malignant neoplasm of trachea, bronchus and lung
1965	692	554
1966	734	563
1967	637	585
1968	751	593
1969	772	610

Research Association

MR JOHN DAVIES, Secretary of State for Trade and Industry, said that he is reviewing the policy concerning grants to research associations for which his department retains vote responsibility. In the meantime, the department of Trade and Industry is continuing to reduce grants progressively so that industry bears an increasing proportion of the costs of the associations. (Written answer, November 30.)

NEW WORLD

Seaborg Brings Strife to AAAS

by our Washington Correspondent

A DEBATE that has long been simmering within the American Association for the Advancement of Science has been brought to a head by the election of a new president. With many of the returns from this year's postal ballot already in, Dr Glenn T. Seaborg, chairman of the Atomic Energy Commission, seems certain to be the winner. But the board of directors of the AAAS is divided both as to the terms on which Seaborg was asked to stand as president and because his tenure of an important government office is likely to inhibit the new responsiveness to social issues that some members would like the association to develop.

Issues of personality apart, the basic argument over Dr Seaborg's candidacy is on the one hand between those who believe the AAAS must preserve its scientific chastity and not fritter away its reputation by becoming a platform for the political views of particular groups, and on the other between those who wish the association to become more active as the representative of scientists on the numerous social issues that have a scientific background.

The board of directors of the AAAS voted in March this year to seek a ten-fold increase in the membership of the AAAS (the present membership is 130,000) and to become much more deeply involved in issues of social relevance. The activists in the AAAS have no personal criticism to make of Seaborg but fear his presidency would impede these plans in several important aspects.

Chief among them is that as chairman of the Atomic Energy Commission Dr Seaborg would face a conflict of interest in many of the issues with which the AAAS is involved. Senator Edmund S. Muskie, for example, has asked the AAAS to adjudicate the charges against the Atomic Energy Commission's policy on radiation safety that have been raised by two of its employees, John W. Gofman and Arthur R. Tamplin of the Lawrence Radiation Laboratory. Nor would Dr Seaborg, it is feared, find it easy to countenance studies that could turn up critical verdicts on government policy; a prime example is the study of the effects of herbicides in Vietnam recently commissioned by the AAAS—its most independent step so far and one that was taken after a difficult internal debate.

Activists in the AAAS and on its board of directors are also anxious to see the association's presidents and chairmen (after a year in office the retiring president assumes the rather more important job of chairman of the board of directors) devote more time to their offices than has been customary in the past, and more perhaps than Seaborg, with his many duties, may be able to give.

Another issue that has been raised is that Seaborg is president of Science Service Inc, publisher of the popular magazine *Science News*, which because of financial troubles was until recently seeking a merger with the AAAS. At a meeting in October this year the board of the AAAS decided against the merger but at the time Seaborg's candidacy was proposed in June there was the possibility that he would be placed in the position of heading both parties to the merger.

These misgivings are strongly denied by Dr Athelstan Spilhaus, present president of the AAAS and the original advocate of Seaborg's candidacy. On the issue of the time needed for the job Spilhaus said last week that having seen Seaborg operate as chairman of *Science Service* (of which Spilhaus also is a trustee) he knew that Seaborg was a man who "gives as much time as the job requires". Although the job of president takes up a great deal of time it can be fitted in at weekends and so forth. Of the possibility of a conflict of interest, Spilhaus says that Seaborg would of course dissociate himself from any matter concerning the AEC. As for other matters with a political content the AAAS has in the past had holders of important government offices serving as president—one former president, Dr Alan T. Waterman, was also director of the National Science Foundation. Dr Spilhaus added that during his term as president he has in any case been opposed to the AAAS getting involved in what he calls "pseudo-political science"—"Many of the people urging the herbicide study were using the AAAS as a podium to present their own views on the Vietnam war."

Another matter on which Spilhaus and his board of directors seem to have differed is the question of whether Seaborg's candidacy for president of the AAAS should have been made conditional on his being no longer chairman of the AEC. Candidates for president are chosen by the seven member Nominations and Elections Committee of the AAAS, of which Spilhaus is chairman. When the committee met in June to decide on candidates for the present elections, it seemed probable that Seaborg, whose term as chairman of the AEC had then expired, would not be reappointed. In fact Seaborg announced he had accepted a further term of 6 years shortly after being invited to stand as candidate for the AAAS presidency. Did the Nominations and Elections Committee believe that Seaborg would not be serving again as chairman of the AEC, and was this made a condition of his being asked to stand as president of the AAAS? The committee does not keep minutes of its meetings, but of the members who could be reached this week three, including Spilhaus, are certain that no conditions were placed on Seaborg's candidacy and one member, at least, believes that Seaborg's chairmanship of the AEC was not discussed at all, personal merit only being considered.

The board of directors was not informed by Spilhaus of the nominations (nor is it bound to be) until September and learnt with some disquiet both that Seaborg was a candidate and that he had agreed to stand. At a meeting held after an executive session of the board in October, several members decided unanimously to ask the board's chairman, Dr H. Bentley Glass, to invite Seaborg to reconsider his acceptance of the candidacy. Both Glass and Spilhaus and at least one other member of the board were absent from this meeting, which is not regarded by Spilhaus and the Executive Director of the AAAS, Mr William Bevan, as an official meeting of the board. Glass met with Seaborg but it seems did

not tell him that the board of the AAAS wished him to withdraw. Seaborg nevertheless called Spilhaus, apparently saying he was thinking of withdrawing his candidacy. Spilhaus said last week he urged Seaborg to stand because "I have great respect for him—he is a fine scientist, has a fine administrative ability, and I think he would make a fine president". Spilhaus explained that as chairman of the Nominations Committee, he felt it his duty to prevent the board interfering in nominations, otherwise it could perpetuate itself. As it happens the other candidate for presidency, Richard H. Bolt, chairman of the Massachusetts firm of Bolt, Beranek and Newman, is also a member of the AAAS board.

Whether or not the drawbacks of Dr Seaborg's candidacy are more real than imagined, the affair has already caused trouble on the staff of *Science*, the weekly journal published by the AAAS. The editor of *Science*, Dr Philip H. Abelson, declined to publish an article about Seaborg's candidacy written by a staff member, Philip M. Boffey, at the suggestion of Daniel S. Greenberg, the able and widely respected news editor of *Science*. As a result of Abelson's refusal Greenberg has resigned with effect from December 18.

The chief functions of the AAAS hitherto have been its publication of *Science* and its annual convention. There is a feeling in some quarters that with *Science* increasingly under challenge from young people and in congress, the AAAS should adopt a more positive role. Although precluded by its constitution from undertaking political lobbying, the association is well poised to become a more independent voice of the scientific community than, for example, the National Academy of Sciences can be. Unlike its counterpart, the British Association for the Advancement of Science, the AAAS is prestigious, well organized and financially stable. In the past, its board of directors has been divided and afflicted with what one observer calls "Prima Donnaism", but there now seems at least a measure of consensus that the association should be less timid in tackling social and political problems on which scientific knowledge can be brought to bear, even if the proper degree of involvement has yet to be worked out. Whatever difficulties Seaborg may face by virtue of his public office in coping with this issue, nobody doubts he is otherwise admirably qualified for the job of president of the AAAS.

OBSERVATORIES

Tucson Must Shed Less Light

by our Astronomy Correspondent

Tucson (Arizona), November

THIS city in southern Arizona has become the closest thing to Mecca for American astronomers, with four observatories within fifty miles—five if the microwave installation of the National Radio Astronomy Observatory on Kitt Peak is counted separately. Already there are six reflectors of between 16 and 84 inches on the mountain, run by the Association of Universities for Research in Astronomy (AURA). A 150-inch reflector is well on the way to completion and the blank for the corresponding instrument in Chile stands in the yard of the well-equipped AURA base in the city of Tucson to follow the Kitt Peak mirror on the figuring machines there. The Steward Observatory of the University of Arizona has a 36-inch and a 90-inch reflector on the mountain, and a 21-inch reflector on the campus in Tucson, and the university's lunar and planetary laboratory has five reflectors between 21 and 61 inches in the Catalina mountains. To the south, the Smithsonian Astrophysical Observatory has a 60-inch reflector on Mount Hopkins, together with smaller instruments for satellite ranging, and the 10-metre light bucket for gamma ray work.



Tucson as photographed at night from Kitt Peak, 1965.

Unfortunately for astronomy, however, the city of Tucson is growing as rapidly as the telescopes multiply, and the observatories in what once seemed to be an ideally remote area are becoming bothered by city lights. Now the directors of the observatories concerned—B. J. Bok of Steward Observatory, D. S. Heeschen of NRAO, G. P. Kuiper of the Lunar and Planetary Laboratory, N. U. Mayall of Kitt Peak National Observatory, and F. L. Whipple of the Smithsonian—have put their names to a pamphlet setting out the situation and the remedy. With the somewhat catchpenny title *Light Pollution: A Threat to Astronomy in Pima and Santa Cruz Counties, Arizona*, their paper includes what astronomers would almost regard as atrocity photographs showing the alarming view of Los Angeles from Mount Wilson as an example of the worst that could happen. The prognosis for the observatories around Tucson is luckily a little better. The people of Tucson are proud of the astronomy that goes on in their neighbourhood, and are expected to cooperate in the simple measures to keep the southern Arizona skies dark at night. What the astronomers would like to see are shields fitted to street lights to prevent light from escaping above the horizontal, roads to have a black surface (which would exclude the use of concrete), and filters fitted to lamps to cut out the violet and ultraviolet components which contribute little to the illumination but interfere with astronomical work. In particular it would be good to do away with the violet and ultraviolet emission lines of mercury vapour lamps. If anything, the pamphlet says, suitable filtered mercury lamps leaving only the green and yellow lines would be preferable to other sources of illumination which cause greater interference.

To this end the pamphlet proposes that Tucson should adopt an ordinance making the shielding and filtration of outdoor lamps mandatory. So far the only obstacles seem to be the availability of suitable

filtered lamps—the market in the Tucson area may not be large enough for manufacturers to provide what is needed—and possible objections from purveyors of food who may be afraid that the filtered light will make their wares appear unwholesome. But with Tucsonians appreciating the worth of the facilities in their area, the outlook looks favourable.

ENVIRONMENT

Senate Shoots Down SST

by our Washington Correspondent

BOTH the friends and foes of the supersonic transport were amazed by the vote last week by which the Senate resolved to cut off the funds for continuing with the aircraft. Up until the morning of the debate the $290 million appropriation for the construction of two prototypes of the Boeing Company seemed assured of a certain, if narrow, passage. Even Senator William Proxmire, the sponsor of the amendment to strike out the funds for the SST, had expected at best to get within a few votes of a draw.

Last year the Senate voted to continue with the SST by a margin of 58 votes to 22; this time the tide flowed against the aircraft by a decisive 52–41 vote. What made so many Senators change their minds? The threat of losing markets to foreign competitors, in this case the Anglo-French Concorde, is usually a persuasive argument in Congress, added to which the SST has the support of the two powerful Senators from Washington state, where the Boeing Company has its headquarters—Henry Jackson and Warren G. Magnuson, chairmen of two key committees (Interior and Commerce) and wielders of considerable power and patronage. Of the two chief arguments against the SST raised by the environmentalists, the question of climatological disaster is ominous but visibly far from proven, and the issue of the sonic boom was cunningly defused the day before the crucial debate by Senator Magnuson, who rushed through the Senate a bill prohibiting overland flights by the SST and requiring that its engine noise be no louder than that of subsonic jets in present use.

There seems little doubt that the environmental issue was decisive in swinging the vote against the SST despite all the odds in the project's favour. Although the specific arguments advanced by the environmental pressure groups may have been resistible individually, their intensive lobbying and activities in Senators' home states were not to be lightly dismissed; after the mid-term elections last month no Congressman wishes to get tagged with an anti-environment label if he can help it. As Senators on both sides of the debate pointed out, the movement against the SST draws much of its strength from wider issues, of which the plane itself was only a symbol. These are that technology must be controlled and that glamour projects such as the SST should have a lower priority than the tackling of domestic disorders. Certainly the Senate's vote will be interpreted in this sense, and will remain a decisive influence on future projects of this nature, even if the funds for the SST should be reinserted at the forthcoming conference between House and Senate. The House of Representatives voted in favour of the aircraft and as it happens the seven conferees appointed by the Senate include Senator Magnuson, and three other supporters of the SST. The Senate is unlikely to accept any conference agreement that writes back the full appropriation for the SST but a smaller sum that would still allow development to proceed could well pass.

The Senate vote on the SST cut across party lines—the 52 members who voted to delete the funds included 34 Democrats and 18 Republicans. Most of the Senate establishment, however, supported the project, twelve of the sixteen chairmen of standing committees voting in favour. The debate was notable for an unusually conspicuous piece of *force majeure*. Senator Mark Hatfield of Oregon found an item in the Transportation bill (of which the SST appropriations are a part) forbidding the extension of an airport in Oregon until further environmental studies had been undertaken. Claiming that exhaustive studies had already been made the Senator begged Senator Magnuson, the manager of the bill, to strike this clause out. Magnuson, chairman of the Commerce committee which prepared the bill, only replied "Let me say to my colleagues from Oregon that we will take this matter up after action on the Proxmire amendment (to strike out funds for the SST)". When Hatfield's name was called on the roll call he failed to vote and it was explained that he had had to catch a plane to fulfil a speaking engagement of long standing. Immediately after the SST vote Magnuson allowed the restriction on the Oregon airport to be lifted. Hatfield's vote, it turned out, was not needed; and in fact Senator Magnuson, according to one of his aides, released four votes pledged to him when he saw that the SST could not win anyway.

COMMUNICATIONS

Demise of Scientific Journals

by our Washington Correspondent

"ANOTHER damn, thick, square book—always scribble, scribble, scribble, eh, Mr Gibbon," the Duke of Gloucester is said to have observed on being presented with a new volume of the history. Not grumbling but apoplexy would be the reaction of Gibbon's patron to the literary output of today's scientific community. The average life scientist produces not one book every two decades but more than four publications a year, two of them being full length research articles. Added to which, many scientists are "notoriously poor writers", according to a report put out last week by the National Academy of Scientists.

The activities of this scribbling multitude have come under the scrutiny of the committee under Dr Philip Handler, president of the National Academy of Sciences, and are the subject of a special chapter in the report on the life sciences issued last week (see *Nature*, **228**, 902; 1970). Discussing journals as a medium of scientific communication, the committee predicts that journals will eventually be rendered obsolete by the computer console, although it may take ten years before each major research centre in the United States possesses a suitable terminal, a further decade for small groups of scientists to come to own consoles and yet another ten years to provide links with other continents.

The daunting volume of the input into the system is documented by a questionnaire sent out to biological research workers in 1967, from which it appears that each scientist contributes on average four distinct publications to the "literature" annually (see Table 1). Surprisingly, the committee reports that although few

scientists can read everything of potential interest, most consider they remain reasonably *au courant* with events on the forefront of their own disciplines. They keep up, however, not just by private reading, but by attending meetings and exchanging publications under the aegis of "invisible colleges". Some 90 per cent of life scientists polled in the survey had attended at least one meeting in 1966, 15 per cent had attended four or more, and nearly 10 per cent managed to participate in meetings outside the United States. Holders of purse strings should remain sympathetic to requests for travel grants, the committee argues.

Table 1. PUBLICATIONS REPORTED IN 1966 BY 12,364 LIFE SCIENTISTS.

Type of publication	Investigators reporting one or more	Number published	Average* no. per respondent
TOTAL, ALL TYPES	10,727	50,858	4·1
Full-length research articles	8,801	24,573	2·0
In-house publications	2,527	7,684	0·6
Books and monographs	442	489	<0·1
Chapters in books	1,710	2,416	0·2
Major reviews	887	1,134	0·1
Abstracts of original research	4,668	9,674	0·8
Other publications	2,028	4,888	0·4

*Average numbers based on 12,364 respondents to individual questionnaire.

Among the 13,000 biological journals now churning forth from the presses the academy committee finds it possible to identify—by an unstated method—about 1,000 journals in which more than 90 per cent of the truly significant work in biology appears. *Biological Abstracts* covered some 7,400 journals in 1968 but most of these are unlikely to publish anything that will materially advance the progress of science. Despite this acrid but probably accurate judgment, the committee urges federal government support for journals, some of which are being hit by rising costs and are on the way to pricing themselves out of business.

MIT

Paved with Good Intentions

by our Cambridge Correspondent

THE Commission on MIT Education has just issued a major report called "Creative Renewal in a Time of Crisis" which attempts to take stock of the role of MIT in the educational world today. There is perhaps no university anywhere that so confidently regards itself as closely associated with national goals, the quality of a technological society and the responsibility for mapping out the future of this society. However, because it takes itself so seriously, this effort at re-evaluation comes in a uniformly dull format. Too many witnesses, too many words, too many platitudes and too many authors, one is forced to admit.

The committee of 12 had submissions from about 350 witnesses. Most of these are at present associated with MIT. No one from Caltech gave evidence, and only three from Harvard, 5 minutes' bus ride away. The witnesses from all other universities numbered about 20. In view of the importance which MIT correctly attaches to itself, one would have thought that a balanced national view was every bit as important as testimony from the insiders. The report reads very much as a lowest common denominator inflated with some truly ghastly expressions of good intentions as "Such fundamental questions as the nature of truth and explanation, the sources and validity of human values, the qualitative distinctions between styles and aesthetic judgements, the role of reason and passion in individual social behaviour, ought to form the core of much of undergraduate education". Well, everyone would agree with that, all 350 of them, but such statements provide no concrete help in achieving creative renewal.

If one carps at the style of the report, it is mainly because the voluminous prose seems to cover up a genuine inability to answer very specific questions. MIT has as many graduates as undergraduates—would it make sense to become a totally graduate school? Is it likely that in ten years the physical sciences will have experienced such a severe recession that MIT ought to be looking for new fields? If the environment and urban problems are here to stay, what in MIT is peculiarly suited to solve these problems, and how are students to be attracted? How does Defense Department spending fit into MIT's future? Why are so many MIT students both outstandingly brilliant and also depressed by the place? Is it possible to convey that overall view of science which its best practitioners have at their fingertips without the drudgery of learning much by rote? The report admits that it is only dealing with general issues, and eschews practical details, but many will question whether the general issues discussed fully cover the problems facing MIT.

The chief recommendation of the report is for a new look at undergraduate education. This is sound, as undergraduate education is always worth a new look. MIT has recently been quite extensively involved in experiments to make its teaching "more meaningful". The new proposals would broaden the first two years by the creation of what is called a "First Division" in which the responsibility for education would fall on more shoulders than it does at present and an attempt would be made to give students an overview of science. Perhaps the most interesting proposal is that an experiment should be made involving the teaching of students from the age of 16. This is certainly worth serious attention in the case of students who know at that age that they are committed to a scientific career. The report pays the customary lip service to the humanities.

On graduate education, the only proposals which appear to have major significance are that more attention should be paid to training for teaching and (probably related) that credit be given in the form of a degree for those students who are unable or unwilling to pursue research but who can perform a "consolidation of knowledge" or a "scholarly review".

What future, then, for MIT education? The two commission members unhappy with the report voice concern over its inadequacy, its lack of depth and its preoccupation with minimal adjustments. They throw into the arena some more debatable topics, and because the commission has a year to run it is to be hoped that more concrete discussion will follow, if not of the generalities of the report, at least of the minority views. Ultimately, however, committees are not as effective as people at changing things. If sufficient faculty members at MIT set personal examples of high quality teaching such reports as this will prove unnecessary.

NEWS AND VIEWS

Viruses and Burkitt's Lymphoma

AMIDST the flurry of activity which currently surrounds the enzymes which synthesize DNA from RNA of RNA tumour viruses and leukaemia cells, it is as well to remember that most cancers in man and animals do not have the properties of infectious diseases. Further, the only known viruses which seem to be intimately associated with human cancers are the herpes viruses, with DNA genomes, and in particular Epstein Barr virus (EB virus) which may be causally related to Burkitt's lymphoma, a common cancer of East African children, and nasopharyngeal carcinoma, a common cancer of certain oriental populations.

It is almost ten years since Burkitt first suggested that the restricted geographical distribution of Burkitt's lymphoma and its apparent dependence on climatic factors could be accounted for if this tumour were caused by a virus disease transmitted by some insects. And since 1964, when Epstein and his colleagues detected a herpes virus, EB virus, in a line of Burkitt lymphoma cells in culture, a great deal of conflicting evidence about the significance of this virus in the aetiology of the lymphoma has accumulated. The case for asserting that any human cancer is caused by a virus must rest on a body of circumstantial evidence rather than a single piece of direct evidence. But what would constitute an overwhelming case? What circumstantial evidence is crucial? Ideally, it must be shown that infection precedes the development of the cancer and that the tumour cells contain molecules specified by the virus. Added to this, demonstrations that the virus, or viruses of the same group, can transform cells in culture, that the virus can induce tumours in animals, that vaccination against the virus reduces the incidence of the cancer, and finally that the epidemiology of the cancer is that which is expected for an infectious disease, should add up to an indisputable case.

These criteria are obviously extremely difficult to establish for any human virus, and the herpes viruses in particular have two characteristics which further compound the difficulties. First, the herpes viruses are ubiquitous and, second, they tend to persist in an infected person for their lifetime. Given these added obstacles, how strong is the case for linking EB virus with Burkitt's lymphoma or nasopharyngeal cancer? The epidemiology of the disease and of EB infections indicates that mere infection with EB virus is not sufficient to cause the cancers. If the virus has an aetiological role it must be in combination with some other environmental factor because in most regions of the world infection with EB virus either goes unnoticed or results in glandular fever but not lymphoma or carcinoma. The nature of the putative second factor is unknown, although Burkitt has suggested that for East African populations with a high incidence of Burkitt's lymphoma the second factor might be malarial infections. Attempts to vaccinate a population in a high risk area have not yet been made so there is no evidence on that score. EB virus seems to change the growth properties of lymphocytes in culture; it may therefore be capable of transformation but whether it can cause tumours in animals remains to be seen.

What evidence, then, is there to suggest that tumour cells from Burkitt's lymphoma or nasopharyngeal carcinomas contain EB virus nucleic acids, or antigens which are specific for EB virus? On pages 1053 and 1056 of this issue of *Nature* three of the leading groups, which have for several years been pursuing this problem, report the results of collaborative investigations which strengthen the argument that there is an intimate association between the genomes of EB virus and Burkitt lymphoma cells and nasopharyngeal carcinoma cells. In the first of the two articles Gunvén and Klein, the Henles and Clifford report that serological tests on ninety-six patients with Burkitt's lymphoma have revealed antibodies directed against the EB virus capsid antigen, and a virus-determined antigen which appears on the surface of infected cells. Relatives and near neighbours of these patients and fifty-three patients with tonsillitis were selected as controls and their sera contain significantly less of these antibodies. As with earlier, comparable investigations, these results indicate that patients with Burkitt's lymphoma are subject to continued antigenic stimulation from EB virus antigens.

In the second report, the same workers, together with Zur Hausen, Schulte-Holthausen and Santesson, describe the detection, by hybridization experiments, of EB virus DNA in biopsy material taken from patients with the lymphoma or carcinoma. Using the DNA to DNA hybridization technique which Zur Hausen and Schulte-Holthausen developed for detecting EB virus DNA in a line of cells derived from Burkitt tumour material but which does not yield virus (*Nature*, **227**, 245; 1970), Zur Hausen and his collaborators apparently find from one to twenty-six genome equivalents of EB virus DNA in the cancer cells of the various patients they have studied. Of course, trying to detect a small amount of viral DNA mixed with an excess of cellular DNA is fraught with technical difficulties and pushes the hybridization method to the limits of its resolution; it is therefore crucial to prove that the hybridization which is being measured is truly specific for the viral DNA. Zur Hausen and his colleagues claim that the hybridization they are measuring is specific because none could be detected between EB

virus DNA and the DNA of cells infected with a related herpes virus, cytomegalovirus, or the DNA of chick cells from Marek's disease tumours, which are caused by a herpes virus. Further, the DNA of herpes simplex virus did not hybridize with Burkitt's lymphoma or nasopharyngeal carcinoma biopsy material.

Although there was no clear correlation between the number of genome equivalents of EB virus DNA in particular biopsy specimens and the concentration of antibodies against the viral capsid antigen and the virus-determined surface antigen, the presence of at east some viral DNA correlates with the generally high concentrations of these antibodies. It seems, therefore, that as well as viral antigens the cells of Burkitt's lymphoma and nasopharyngeal carcinoma also contain EB virus DNA. But does this simply mean that these cancers become secondarily infected with a persistent, but latent, EB virus ? That remains the central and unanswered question although Zur Hausen *et al.* believe the finding that in any one patient the number of genome equivalents of EB virus DNA is constant when biopsy material is taken from a tumour at different times argues for more than an extension of a casual but persistent EB virus infection. Sceptics, however, may be less willing to persuade themselves on the strength of that evidence.

Forecasting Turbulence in the Air

CLEAR air turbulence has become a speciality of the Royal Radar Establishment, Malvern, where the high power radars which are needed to obtain reflexions from the refractive index inhomogeneities are available. Earlier this year the Meteorological Research Unit at Malvern published photographs from the radar screen showing the evolution of a patch of turbulence, the first time that the evolution has been followed from a single patch rather than by the less satisfactory expedient of piecing together observations at different times (*Nature*, **227**, 260; 1970). To round off that paper the Malvern team have now proved that the curious braided structure seen on the radar screen truly represents the turbulence that is felt by aircraft (see page 1065).

The first thing to be said, however, is that there is not a great deal that can be done about clear air turbulence apart from some fairly rudimentary predictions. To begin with, clear air turbulence is invidious because it occurs where there is no hint of trouble —in an optically clear atmosphere. In physical terms the turbulence is a form of Kelvin–Helmholtz instability which arises in the interfacial zone between two moving fluids, and can occur throughout the troposphere and into the lower stratosphere. Two favoured locations are the upper and lower boundaries of jet streams, and it is the turbulence associated with the jet stream within the westerlies flowing over England that is the object of the Malvern studies in this paper. When the radar echoes are converted into a cross-section view of the atmosphere the turbulence looks as much as anything like the breaking of waves—the braided echoes reported in the earlier paper—and this is a not over-simplistic view of the evolution of a Kelvin–Helmholtz instability in the atmosphere.

What Browning, Watkins and Starr of the Meteorological Research Unit, and McPherson of the Royal Aircraft Establishment, are reporting this week are the data from an instrumented Canberra aircraft which was in the air while the radar was recording echoes from clear air turbulence. Turbulence was recorded by the accelerometer on the aircraft when the flight path was near patches of turbulence as recognized by the radar, and comparisons between the wavelength and amplitude of the turbulence as measured by the aircraft and the radar are as close as might be expected. The radar detected all the turbulence experienced by the aircraft along the flight path which coincided with the altitude of the strongest turbulence, but there was some light turbulence at a lower altitude which went unrecorded by the radar.

It would be nice if the Malvern work meant that one day soon aircraft could be fitted with radar to detect clear air turbulence. The 10·7 cm radar transmitter operated by the Meteorological Research Unit has a peak power of the order of 1 MW and is fed into a 25 m diameter dish aerial, however, and even then is limited to a horizontal range of about 50 km and heights up to about 12 km. The alternative approach, prediction of clear air turbulence, is not well developed but it is in this direction that the studies going on at Malvern will be useful. The paper by Browning and Watkins earlier this year made a start by setting a seal on the way the evolution of clear air turbulence has been inferred from many separate observations, chiefly by the Americans. The way ahead is to find out more about the factors that control the appearance or non-appearance of the turbulence, and obviously a ground-based radar able to scan the atmosphere at frequent intervals is going to be more useful than an instrumented aircraft.

But one serious problem at present is that there can be doubt about the strength of the turbulence from the radar observations alone. Echoes from patches of light turbulence are often to all intents and purposes indistinguishable from echoes from strong turbulence, and up to now the resolution of the ambiguity has had to come from radiosonde or aircraft measurements of the strength of the wind shear. To deal with this problem from the ground the Malvern group, and others, are developing Doppler radar methods which will give direct information about the turbulent velocities. This will be the next advance on the observational side of clear air turbulence.

LIQUID THEORY

Random Configurations

from our Molecular Physics Correspondent

IF there is an orthodoxy in the theory of liquids—and recent conference proceedings suggest that there is—it has little place for notions which depart very far from the familiar, well founded and, regrettably, somewhat sterile formalism of Gibbsian statistical mechanics.

The Bernal "random geometric" approach to liquids, which is the subject of two new articles by J. L. Finney (*Proc. Roy. Soc.*, A, **319**, 479, 495; 1970), is highly unorthodox. It cuts across most of the niceties of conventional many-body theory, appealing to crystallographic rather than mechanical insight and seeking to capture the essence of the liquid in the statistical properties of nearest-neighbour configurations reduced to units of the polyhedron. Although intuitively satisfying, this primarily visual approach proves to be of some mathematical sophistication, so much so that, to the dismay of the formalists, the theory cannot be placed in any recognizable chapter of geometry, far less reduced to a single set of equations. In fact, no method has been described within the theory to relate the polyhedron statistics either to the fundamental energy surfaces in configuration space or to the practical calculation of thermodynamic entropy. Thus, not surprisingly, its advocates have so far set greater store by its descriptive than its predictive powers, being chiefly concerned to check their statistics against measurements on mechanical or computer simulated random models. But if the Bernal theory lacks the formal virtues of conventional statistical mechanics, its informal ones are none the less compelling. The original articles (*Nature*, **183**, 141; 1959: **185**, 68; 1960) can hardly be read without the conviction that the author is at least talking about liquids as we know them —". . . homogeneous, coherent, essentially irregular . . ." and abounding in entropy—rather than about some discouraging cypher of configuration integrals.

In Finney's work, realistic computer-simulated liquids similar to those which appear in recent "Monte Carlo" thermodynamic studies are compared for the first time with the random sphere packings originally constructed by Bernal. A detailed analysis of the statistical distribution of configurational polyhedra confirms that Bernal's primitive ballbearing and ball-and-spoke models gave an essentially correct picture of the instantaneous geometry of a monatomic liquid, in which the principal structure-determining factor now seems certain to be the hard, repulsive potential rather than the soft attractive part. Finney also compares his results for liquids with those for simulated solids at their melting point and he demonstrates clearly the discontinuity in the polyhedron statistics at the melting point.

Unfortunately, important as these results are, they bring us little closer to a solution of the two central problems: what is the precise relationship between the statistical geometry and the configurational energy function for N atoms, and what are the essential determinants of the statistical geometry and how are they connected with the definition of thermodynamic entropy? It is not difficult to accept that the instantaneous geometry of a large enough sample of liquid contains all the information about its wanderings in phase-space sufficient for an accurate calculation of its entropy. It is a plausible next step to maintain that this information resides in the statistics of polyhedral types related to nearest-neighbour arrangements. Beyond this, the trail disappears into the sand. Finney makes the tentative suggestion that the key to an algorithm for computing configurational entropy may lie somewhere in homology theory.

What is certain, however, is that there is little chance of an easy synthesis of Bernalian and Gibbsian statistical mechanics for, by insisting on the polyhedron as the descriptive unit, the geometrical theory seems to begin at the level of four and five-body angular correlations—several stages beyond the most refined predictinos of ordinary statistical mechanics. This is both its strength and its weakness; it justifies itself as a truly structural theory of liquids, which can hardly be said of the conventional theories, but only at the risk of being over-determined and neglecting what plausible simplifications there are. For example, it seems to waste altogether the two-body force assumption, one of the few reasonable approximations which enter nicely into the ordinary theory.

Nevertheless, as Finney is careful to point out, the search for a formal relationship between statistical geometry and entropy is worth pursuing for reasons which go beyond the microscopic theory of liquids. There are many other disordered systems in nature and technology—powders, fluidized beds, biological aggregates—whose properties might well be elucidated through a deeper and imaginative understanding of what might be called the geometry of chaos.

QUANTUM ELECTRODYNAMICS

Gravity's Role in Removing Infinities

by our Cosmology Correspondent

THE infinities which occur both in classical and quantum electrodynamics have disturbed field and particle theorists for decades. The implication that there is some kind of physical limit, or critical length, associated with fundamental particles conflicts with basic dogma, but as the work of Feynman and that of Dyson has shown there is no way to remove the self-mass and self-charge infinities of lepton electrodynamics using the conventional techniques of expanding the mass and charge distributions by a polynomial solution about a point. But by using non-polynomial Lagrangians for small distances the self-mass and self-charge can be made finite.

A group working at Imperial College, London, have been investigating this problem for some time (see, for example, *Nuovo Cim. Let.*, **4**, 101; 1970). C. J. Isham, A. Salam and J. Strathdee have attempted to find a more general solution, modifying Dirac's Lagrangian to take account of gravity as well as electrons and photons, and using a non-polynomial solution. They have now found that the very small range of gravitational forces ($\sim 2{\cdot}2 \times 10^{-22}$ m) does not, as traditionalists have assumed, make them insignificant. From the point of view of the critical length, gravity plays a vital part in quantum electrodynamics.

The crucial discovery by Isham and his colleagues is that their modification makes every Lagrangian in particle physics non-polynomial in character, with the gravitational length providing an inbuilt cutoff and preventing the occurrence of infinities. Conceivably,

the electron self-mass arises from the interaction of its electromagnetic and gravitational properties to produce the electron radius.

To a cosmologist, the most exciting implication of this work (as yet unpublished) is that the gravitational force may be repulsive over very short distances. The agreement of the predictions of this theory with those of Hoyle and Narlikar, who have approached the problem from the gravitational end rather than starting from conventional particle physics, makes both theories more attractive and their implications correspondingly more interesting. The whole question of gravitational collapse (or the lack of gravitational collapse) and the significance of this for cosmology and cosmogony seem once again to have prime importance.

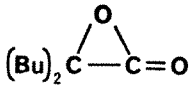
CARBONYL COMPOUNDS

More Small Rings

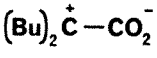
from our Organic Chemistry Correspondent

THREE-MEMBERED ring carbonyl compounds have frequently been postulated as intermediates in organic reactions. Perhaps the best known example is the intermediacy of α-lactones in solvolysis reactions of α-halocarboxylic acids. Recent attempts to isolate compounds of this type have been successful in the case of the carbocyclic cyclopropanones and with the nitrogen containing α-lactams, but all efforts to obtain an example of the analogous oxygen system, that is, an α-lactone, have been unsuccessful.

The recent report that the lactone (I) of di-t-butyl glycollic acid is stable in solution at −80° C fills this gap in the literature of small-ring compounds (Wheland and Bartlett, *J. Amer. Chem. Soc.*, **92**, 6057; 1970). As with several other small-ring carbonyl compounds, the relatively high stability of (I) is dependent on the shielding effect of the bulky t-butyl substituents. Synthesis of the α-lactone was accomplished by oxidizing di-t-butylketene with ozone, a reagent known to epoxidize highly hindered olefins. In spite of the effect of the t-butyl groups, the α-lactone is no longer stable when the solution (in 'Freon 11') is warmed to −20° C; a white precipitate of polyester $[(C_9H_{18}CO_2)_n]$ separates, the formation of which is probably initiated by equilibrium formation of the zwitterion (II). Molecular orbital calculations indicate that the two structures [(I) and (II)] have similar energies, though spectroscopic evidence clearly rules in favour of (I) as the structure of the species in solution at −80° C.



(I)



(II)

The reactions of (I) are in accord with the character ascribed to α-lactone intermediates in the solvolysis reactions already mentioned, and polymer formation parallels observations on reactions of some peroxides in which α-lactones are also believed to mediate.

Attempts to prepare the diphenyl analogue of (I) from diphenylketene were attended by polymer formation $[(Ph_2CCO_2)_n]$ even at −80° C. Presumably, the phenyl substituents tend to stabilize the zwitterionic structure relative to the covalent α-lactone, but a monomeric species was trapped by carrying out the oxidation of diphenylketene in the presence of methanol, when the product was α-methoxydiphenylacetic acid.

SEA FLOOR SPREADING

Geochemical Effects

from our Geomagnetism Correspondent

SINCE—and even before—sea floor spreading became widely accepted, there have been many attempts to relate geophysical and geochemical parameters to the distance from spreading oceanic ridges. The variation of one such property, the chemistry of the principal elements of basalts, was first reported by McBirney and Gass (*Earth Planet. Sci. Lett.*, **2**, 265; 1967), who showed that the silica saturation of basalts decreases with distance from the ridges and correlates with the decrease in heat flow. To be sure, this was not the first time that differences had been observed between ridge and non-ridge basalts; geochemists have known for some time that ridge basalts tend to be tholeiitic whereas alkali basalts are more likely to be found away from ridges. But McBirney and Gass demonstrated for the first time that variations with distance were systematic.

But do the major elements in basalts always vary with distance from the ridges? And if so, why? To answer these questions, Hart (*Earth Planet. Sci. Lett.*, **9**, 269; 1970) examined the analyses of 112 deep ocean basalts and found that the major elements do usually vary in this way. Thus, for oceanic basalts up to about 18 million years old (the limit of Hart's data), silica, calcium and magnesium decrease almost linearly with distance from the ridges, whereas potassium, iron, titanium, sodium, manganese and phosphorus increase, also approximately linearly. In addition, ferric iron replaces ferrous iron; but aluminium apparently does not change.

Several possible reasons for these variations come to mind. They could arise, for example, from progressive changes in the composition of the magma as it is extruded at the ridge crests. That such changes would be so systematic during a period of 18 million years at least is unlikely, though not impossible. Another possibility is that the basalts have been altered since they were laid down. In view of what amounts to a correlation of major element chemistry with age, this looks like a natural explanation, though deuteric alteration cannot be ruled out *a priori*.

Hart suggests that the variations are produced by alteration through weathering—a hypothesis to which he brings convincing evidence from studies of the weathering of single pillow basalts. In this context, of course, weathering means chemical exchange between the basalts and sea water, though there is still some doubt about whether pillow weathering truly results from subsequent hydration or whether it is deuteric. Insofar as the alteration in pillows is zoned, with the most heavily altered zone on the outside, Hart supports the view that pillows are chemically changed by the water. He then proceeds to compare the changes in the principal elements with those of the deep ocean basalts. The trends are very similar.

If the changes in oceanic basalts really result from long term hydration, geochemists must in future distinguish carefully between true alkali basalts and tholeiitic ridge basalts which have been weathered to

alkali composition. But there is another consequence of deep ocean basalt alteration to which Hart draws attention. This is the overall effect on the Earth's geochemical budget, especially if the extent of weathering is considered significant by virtue of its great depth within the basalt. Elements given off during the basalt weathering will, of course, increase the total supply in the sea water, though some may be taken up by the sediments. Elements absorbed, on the other hand, require a source, which must ultimately be stream runoff from land. It follows therefore that if maximum absorption is to continue the stream supply must be greater or at least equal to that required by the oceanic basalt. In this sense, the most critical elements are titanium and iron, for which the stream supply (using the best estimates at present available) is likely to be insufficient. For these elements, and to a lesser extent manganese and phosphorus, basalt weathering is likely to be the chief control on the geochemical mass balance of the oceans.

PROSTAGLANDINS

More Pharmaceuticals

from a Correspondent

UNTIL recently few people outside the medical and biological world had heard much of prostaglandins, notwithstanding some 1,500 references in the scientific literature of which three-quarters have appeared in the past three years. Then about two months ago the daily and Sunday newspapers began to carry such headlines as "A New Approach to Contraception—the Abortion Pill". The reason for the headlines was not only that prostaglandins had been shown to be usable for this purpose—the scientific fact had been known for some months—but that one large American drug firm, in particular, had announced its intention of moving from fundamental research to the large scale controlled trials needed before the "abortion pill" could be marketed.

Prostaglandins are natural substances with a wide range of biological actions, and their possible physiological, pathological and therapeutic functions are not limited to abortion. A symposium held in London by the Society for Drug Research on November 25 brought together some hundreds of university scientists and industrial pharmacologists to consider some of these possibilities. The survey by Professor J. R. Vane (London) of the fate of prostaglandins infused into the circulation showed that physiological and therapeutic functions of prostaglandins are limited or are perhaps determined by their rapid clearance from the bloodstream *in vivo*. Nevertheless, as Professor V. R. Pickles (Cardiff) showed, endogenous prostaglandins from the endometrium almost certainly act physiologically as part of the normal mechanism of menstruation, and may be involved in the causation of dysmenorrhoea or "period pains". The use of exogenous prostaglandins for the induction of labour or therapeutic abortion is perhaps derived from this physiological observation, and Professor S. M. M. Karim (Kampala) gave details of his large series of such cases.

Prostaglandins may also be involved in inflammatory reactions of various kinds, for example, in the skin, and in the eye, which is one of the sites where their occurrence was first noted. In the lungs, some members of the group cause bronchoconstriction (as in asthma), whereas others have an opposite effect which may be turned to therapeutic advantage. In various glandular tumours the occurrence of prostaglandins seems to be irregular but intriguing. Investigation is hampered by the continued absence of very potent and specific antagonists, but at least three groups of compounds show some activity of this type and further advances will doubtless be made.

It was made clear at the symposium that fundamental physiological and biochemical research is still needed and perhaps there is a place still for the small university team, though opinions differed on whether lack of gas–liquid chromatography with mass spectrometry facilities necessarily precludes all attempts to identify or assay prostaglandins in tissue extracts. There can, however, be little doubt that proper identification of prostaglandins in the sub-micromolar concentrations in which they appear in tissues poses considerable problems and many claims of identification by simple means could be checked by the more precise physico-chemical methods. Again, proposed mechanisms of prostaglandin action at the molecular level—such as the suggestion of Dr J. R. Smythies (Edinburgh) that they are involved in agonist–receptor interaction in the nervous system and elsewhere—may best be tested by using the prostaglandin analogues developed by drug firms.

REPRESSORS

Clothing the Naked Gene

from our Molecular Biology Correspondent

THE *lac* operon is now perhaps better known as a vehicle for the cultivation and public display of tender social consciences than merely an interesting topic (in this year's parlance, paradigm) in molecular biology. Among the aspects of scientific interest that still adhere to it, and one which will not, one may hope, strike terror in the populace at large, is its interaction with the repressor. The nature of this process has been studied by Gilbert and Müller-Hill, and by Riggs and his associates. Its kinetic features have now been explored by Riggs, Bourgeois and Cohn (*J. Mol. Biol.*, **53**, 401; 1970). They use a membrane-filter method for determining the instantaneous concentration of the complex, based on the observation that free but not complexed DNA passes the filter. When a large excess of unlabelled DNA is added to a sample of labelled DNA saturated with repressor, exchange occurs at a rate determined by the concentrations, and the lifetime of the complex. The rate equation for replacement of labelled by unlabelled DNA in the complex then contains two terms, one expressing the rate of dissociation, the other of association. As long as the concentration of free repressor is low, which is assured by a large overall excess of DNA, the second term can be disregarded, and the reaction follows first-order kinetics, with a rate constant of the order of $10^{-3}-10^{-4}$ s^{-1}, depending on ionic strength, the time scale of the experiment being some 30 min.

The forward reaction is measured directly, at very low DNA and repressor concentrations. The corresponding second-order rate constant is 10^{10} M^{-1} s^{-1}. Everything fits in with this straightforward kinetic scheme, and in particular the ratios of the forward and reverse rate constants under different conditions are in excellent agreement with the corresponding equilibrium con-

stants for the association, measured independently and directly.

The results have considerable bearing on the nature of DNA–protein interactions in general, and highlight the rudimentary state of knowledge concerning such processes. In the first place, the forward rate constant is extraordinarily large—greater, as the authors note, than can easily be accounted for even in terms of a diffusion-limited reaction. The electrostatic component, which the ionic strength-dependence shows to be present (even though the repressor has an overall negative charge), does not seem of overwhelming importance. It has always seemed generally most reasonable to view DNA–protein interactions of high specificity as proceeding through the single-stranded state, which becomes available in virtue of the supposed "breathing" relaxation of the double helix. Riggs *et al.* argue that in this instance such a mechanism is excluded by the reaction rate. Denatured DNA moreover does not react. The results therefore demand a re-examination of some preconceptions about the mechanism of recognition of proteins by DNA. The repressor may in this case interact with the exiguous portions of the bases exposed in the large groove.

Another example of very strong binding occurs in the colicinogenic factor of *Escherichia coli*, which is a DNA containing body, separate from the chromosome. Clewell and Helinski (*Biochemistry*, **9**, 4428; 1970) have established a number of interesting features of this material. It consists of a DNA supercoil, containing a tightly bound protein component. On treatment with detergents or proteolytic enzymes, the hydrodynamic properties change in a manner that betokens the unwinding of the supercoil. The minor fraction of uncomplexed supercoiled DNA that is present does not respond to this treatment. Two mechanisms for the action of the protein now suggest themselves: it may be a nuclease, which breaks one DNA strand, a single nick being known to be sufficient to release the tension responsible for supercoiling. Alternatively the nick may be present in the complex, but not manifested because the ends are prevented from separating by the bound protein, until this is denatured or degraded. Clewell and Helinski, however, find that if the cells are heated before lysis, intact uncomplexed supercoiled DNA is isolated, suggesting that the nick is not there all along. A further relevant finding follows from hybridization experiments, which show that the nicking reaction is specific to one of the strands. What lends this work especial interest is the current view that the introduction of a nick by an inelegantly termed "nickase" precedes the normal initiation of replication.

TUMOUR VIROLOGY

Susceptibility to Transformation

from our Cell Biology Correspondent

In 1966 and 1967, Todaro and his colleagues reported that cultivated human fibroblasts taken from patients suffering from Franconi's anaemia and Down's syndrome were more susceptible to transformation *in vitro* by simian virus 40 than corresponding cells from healthy persons, and the work of Mukerjee and his colleagues suggests that this finding also applies to cells from patients with Klinefelter's syndrome. These three diseases are associated both with a higher than usual incidence of chromosomal abnormalities and with cancer. It may well be that all the diseases involve some defect in cellular replication which results in the production of chromosome abnormalities, the increased susceptibility to cancer and the SV40 transformation. These findings have, not surprisingly, led to the suggestion that a measure of the susceptibility of a person's cells to transformation *in vitro* by SV40 might be used as an indicator of that person's relative risk of developing cancer. Clearly therefore there is more than academic interest to discovering why some human cells are more readily transformed than others.

Pursuing this question Aaronson (*J. Virol.*, **6**, 470; 1970) has repeated Todaro's experiments using naked SV40 DNA as well as intact SV40 particles as transforming agent. He finds that with SV40 DNA the differences in susceptibility to transformation between various human cell strains are eliminated; human fibroblasts which differ by a factor of a hundred in their susceptibility to transformation by SV40 particles are equally susceptible to transformation by SV40 DNA. This result implies, of course, that the relative resistance or susceptibility of a particular cell depends on some block, or lack of it, to an early event in infection.

Virus-specific T antigen appears early in infection with intact SV40, and those infected cell populations which have the highest proportion of cells containing T antigen are also the populations with the highest transformation rates. But when SV40 DNA is used, populations of various cell strains have the same fraction of cells staining for T antigen as well as the same transformation rates. This again indicates that differences in the transformation rates between susceptible and resistant human fibroblasts are the result of some failure, in the resistant cells, of an early event in infection—either a failure in adsorption, penetration or uncoating of the virus. Aaronson has now eliminated the possibility that resistant cells fail to adsorb the virus, or adsorb less of it than susceptible cells. Further, he has shown that when sensitive or resistant cells are infected with adeno-SV40 hybrid virus, which contains only part of the SV40 genome but includes the gene(s) responsible for the induction of T antigen, SV40 T antigen appears in the same fraction of populations of both sorts of cells. In other words, the block to T antigen production which is apparent when SV40 infects a resistant human fibroblast does not block the induction of the T antigen by SV40 genes enclosed in an adenovirus capsid. It seems likely therefore that the resistance or susceptibility of human cells to transformation by SV40 depends on some block, or lack of a block, to the uncoating of SV40 and, moreover, this block is specific for SV40. Clearly the correlation between susceptibility to cancer and to transformation by SV40 deserves further investigation.

Aaronson (*J. Virol.*, **6**, 393; 1970) has also been investigating the sensitivity to inactivation by ultraviolet light of three functions of the SV40 genome; its ability to transform, to induce T antigen and to induce capsid antigen. Transformation seems to be the least sensitive of the three functions. These experiments also support the suggestion that the induction of T antigen and of transformation are closely related. After initially diverging, the slopes of the survival curves for T antigen induction and transformation run parallel and all the cells transformed by the irradiated virus contain T antigen.

Season of Birth and Cognitive Development

by
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Certain groups of handicapped children clearly contain a high incidence of those born in the summer. The fault seems to lie with the British educational system.

THE relationship between human development and season of birth has been the subject of numerous enquiries—in reviewing earlier work, Fitt[1] suggested that the slight advantages in various aspects of development, including intelligence, enjoyed by children born in the summer–autumn seasons in either geographical hemisphere were a consequence of seasonal influences on conception. Later work has not always supported this conclusion. Jessup and Freedman, in a carefully analysed enquiry presented at the annual conference of the British Psychological Society in 1967, found a "trough" in the measured intelligence of children born during February–May and a less clear peak in the measured intelligence of those born during the October–November period—a finding somewhat out of phase with Fitt's conclusions. Berglund[2], working with Swedish children, found no evidence of a difference between the intelligence quotients of those born in different seasons. Ojha *et al.*[3] found no relationship between season of birth and intelligence among a sample of university entrants. Orme[4,5], working with severely subnormal adults, found a preponderance of summer-born and autumn-born individuals in the more intelligent section of his population. He considers the May–October birth season to be intellectually advantageous. Farley[6], reporting a study of a group of apprentices, concluded that there was likely to be little support for season of birth effects in normal populations. It seems to be difficult to show a clear relationship between intellectual development and season of birth except for large or special populations and even then findings are not always consistent.

There is a considerable body of evidence that in Britain there are differences of educational development between children born at different seasons of the year. Children born in the summer months undoubtedly attain lower educational standards than children born at other times of the year. This applies when chronological age is held constant, through the use of standardized attainment tests, for example. Pigeon and Dodds[7] demonstrated this with junior school children, and Jinks[8] showed how children born in summer were likely to be found more frequently in the lower streams of junior schools than would be expected on the basis of their frequency in the population under study. France and Wiseman[9] found that the performance of the children on which the eighteen tests of their educational guidance programme for primary school children were standardized varied according to the season of the year. Children born in autumn (September–December) had slightly higher scores than those born in spring (January–April), who in turn had slightly higher scores than those born in summer (May–August). This breakdown of the year into three sets of four months is an arbitrary division which mirrors the school year. It is, however, a division which has been used in previous studies, and we shall use it in this article. The differences between the performances of children born in the three periods of the year were small but significant. The findings of France and Wiseman can be regarded as a useful quantification of Jackson's observations[10] that children born in summer tend to predominate in the lower streams of streamed schools (and that the larger the school the greater the concentration of summer-born children in the lowest streams).

France and Wiseman also drew attention to the fact that the differences between children born in different seasons diminish with age. This may be one reason why investigators working with older school populations have not found the effect. Thus Armstrong[11] found no relationship between season of birth and success in the "eleven plus" examination for grammar school entry. Neither did he find any significant difference in O-level examination results for older children born at different seasons of the year.

Armstrong's criteria provide other reasons for the failure of this enquiry to agree with earlier work. Both criteria (success at eleven plus and O-levels passed) differentiate between and within groups of individuals who are of well above average performance. Similarly, Ojha's enquiry, which confirms Armstrong's findings, is concerned with a population well above average in educational development. These findings are thus not necessarily inconsistent with those noted earlier and which do exhibit differences between the performance of primary school children in accordance with season of birth.

Educationally Handicapped Children

With populations of low educational development a much clearer picture emerges. John[12] found a significantly higher proportion of summer-born children among retarded readers than would have been expected. Williams[13] and Griffiths[14] reported similar findings. Williams[13] and Bookbinder[15] demonstrated a preponderance of summer-born children in schools and classes for the educationally subnormal (ESN) or slow learners.

The effect is also clearly present in children whose handicap is psychological, rather than educational, that is when the deviance is in behaviour rather than educational development. The existence of a summer birthday effect among children referred to child guidance clinics is mentioned in Williams[13], and Lloyd[16] notes the presence of the same effect among boys at a remand home.

It is interesting to note the extent to which children born in summer predominate in the various groups of handicapped children studied. It is possible to extend the

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table reported by Williams[13] to show how more recent findings emphasize the consistency of the preponderance of children with summer birthdays in populations of handicapped children.

A child born in summer seems to have approximately a 50 per cent greater chance than a child born in autumn of some form of special treatment. It is important to try to establish the reasons for this greater risk.

Possible Causes of the Effect

Because of the inconsistent findings relating to intelligence level and season of birth we shall deal only with the clear and consistent relationship between season of birth and risk of membership of one of the groups of children with educational and behavioural handicaps described in Table 1.

Table 1. SEASONAL BIRTHDAY DISTRIBUTION OF HANDICAPPED CHILDREN

Group investigated	Ref.	Season of birth Autumn	Spring	Summer	No.
Retarded reader	12	27	32	41	1,600
	13	27	35	38	382
	14	23	33	44	128
ESN children	13	25	33	42	265
	15	26	33	42	1,289
Child guidance cases	13	28	35	37	1,511
Boys on remand	16	32	25	43	82

Figures are percentages.

The explanations suggested for the preponderance of summer-born children in these groups have been based on the organization of the school system. Two alternative explanations have been compared. One, the "term of entry effect", suggests that children born in summer are at a disadvantage because "they spend a shorter time in their infant schools than do children born at other times of the year". This was one of the reasons which led to the Plowden committee's recommendation[17] of a uniform date of entry to infant schools or first schools, so that all children spend a full three years there. The other explanation, also noted by Plowden, is the "age group effect". This suggests that the children born in summer are at a disadvantage because in the British educational system they are usually the youngest in their school age-group and may find school more of a strain than children born at other seasons.

These two effects can be separated by looking at the incidence of birth months within separate school terms. Williams[13] suggested that the age-position effect might be the more important in determining the preponderance of the summer-born in his population of ESN children and Bookbinder[15] demonstrated this clearly. The "term of entry" effect seems to be of little importance. Pigeon[18] points out that in Sweden, where children enter schools once a year and not by term, a seasonal variation in attainment still occurs and can be attributed to age group position effect. The interesting point of the Swedish situation is that their age groups are of different composition from those in England and Wales. Berglund[2] comments that the autumn-born children in Sweden are the youngest in their age group and not the oldest, as is the case in England and Wales. Thus if the age-group position effect is operating, in Sweden children born in autumn should be preponderant among those with learning difficulties. Berglund's figures relating to his population of Swedish children attending backward classes can be reanalysed to show the relative incidence of children with different seasons of birth. In accordance with prediction a high incidence of autumn-born children is found (Table 2, taken from ref. 2).

Table 2. SEASONAL BIRTHDAY DISTRIBUTION OF BACKWARD CHILDREN

	Season of birth Autumn	Spring	Summer	Total
No.	91	76	70	237
Per cent	38	32	30	100

It seems likely that age-group position effects are responsible for the high incidence of summer-born children with learning difficulties in the educational system in England and Wales. As Pigeon says[7], "While the possibility may exist that children born in the warmer summer months may obtain slightly higher scores in intelligence tests, there is little doubt that the advantages gained by these children are small compared with the disadvantages conferred on them by being born at the wrong end of the school year."

These disadvantages may also account for the high incidence of summer-born children in populations of child guidance and court cases reported in Table 1. The additional strain imposed by a difficult school situation may well be a cause of behaviour problems which lead to referral to a child guidance clinic or to a court appearance. The negative relationship between educational attainment and maladjustment, (for example ref. 19) and delinquency (for example ref. 20) is well known.

Another possible explanation relates to seasonal birth rates. The variation between birth rates at different seasons of the year is small in comparison with the differences found in the distribution of birthdays of handicapped children; most of the enquiries we have mentioned allowed for this. (Usually the birth rate during September to December is a little lower than in the other two periods: Bookbinder[15] reports ratios of 31 per cent: 34½ per cent : 34½ per cent for September–December: January–April: May–August in Bristol. Lawrence *et al.*[21] give figures for South Wales which can be evaluated as 32 per cent : 34 per cent : 34 per cent for the same periods.) It is possible, however, that there is a differential seasonal fertility between the social classes. If the lower social classes, which are more "at risk" educationally, are more likely to have summer-born children than the middle and upper social classes, this may partly or wholly account for the preponderance of summer-born children in these special groups, quite apart from any educational factors.

The Registrar General (personal communication) provided an unpublished tabulation of the breakdown of births by social class by month of birth. The figures related to a 10 per cent sample for the period July 1963 to June 1964. Table 3 is calculated from these figures.

There is thus no indication that social classes IV and V are more likely to produce children in summer than are social classes I, II and III.

Another important possibility is the existence of a congenital learning disability which is linked to season of birth. There is little evidence that congenital physical disabilities are linked to season of birth. Lawrence *et al.*[21] found that in a large population of children born with malformations of the central nervous system, conception was less frequent in April, May and June than expected. Lawrence points out that this finding is not consistent with

Table 3. SEASONAL BIRTHDAY DISTRIBUTION BY SOCIAL CLASS

Social class	Season of birth Autumn	Spring	Summer
I	30	35	35
II	32	35	34
III	31	34	35
IV	32	34	34
V	32	34	34

Figures are percentages.

Table 4. VECTOR LOADING IN CANONICAL CORRELATIONS

Solution	Canonical correlation	Vector loading of month of birth
1	0·54	−0·04
2	0·33	+0·03
3	0·31	−0·02

other work in the same area. It is, however, an interesting possibility that congenital factors affect the learning ability of children born in the summer in Britain, leading to their preponderance in special educational groups. One way of testing this is to examine the psychological development of a group of children at the time they enter school in order to see if there is any significant over-representation of summer-born children among less able individuals. If there is such an over-representation, then it is reasonable to conclude that the over-representation of summer-born children in the deviant groups of Table 1 cannot be due solely to the influence of the education systems, and that we must look for other factors such as congenital factors. These factors could of course operate as well as, or instead of, educational influences.

part of a further analysis of the data, canonical correlations were computed between the fourteen developmental variables and eight predictor variables. Seven of the predictor variables were measures of aspects of the child's home background. The month of birth was the eighth variable.

Probability tests indicated that significance could only be attached to the first three canonical correlations. The vector loadings of month of birth for each of these three solutions were as in Table 4.

Clearly the child's month of birth plays a negligible part in the solution of the interrelationships between the two sets of data.

Although the results are not positive, the data may conceal a relevant effect. So far we have considered relationships between month of birth and the developmental levels of all the 695 children. Although relationships may be negligible in the total sample, there may be an effect at the extremes of the sample. This is the point that Wiseman[22] makes when he refers to the importance of splitting the variance to examine correlations in different sections of the distribution. We illustrated the point when our review of research revealed consistent findings for groups of handicapped children—children at the extremes of the population—but inconsistent findings for the population as a whole.

Table 5. DISTRIBUTIONS OF MONTH OF BIRTH OF SAMPLE CHILDREN

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	No.
Observed in seventy weakest children	3	8	4	10	3	5	13	5	5	4	6	4	70
Observed in sample of 695	71	76	66	80	55	46	83	57	51	41	34	35	695

Data Available for our Enquiry

The Schools Council Research and Development Project in Compensatory Education is primarily concerned with the production of materials for deprived children. During this process considerable research data have accumulated. The identification techniques unit has been developing materials for the early identification of children at risk when they enter infant schools, and this work has involved the assessment of a judgment sample of 695 school entrants. Some characteristics of this sample have been described by Evans *et al.* (unpublished work). For the purposes of this article it is sufficient to say that the sample under study is drawn from two different areas, a Midland conurbation and a Welsh county. It consists of the intake in the school year 1968–1969 to twelve infant schools, eight in deprived areas, two in settled working class areas and two residential schools. The sample is likely to contain a higher proportion of slow learners than a randomly chosen sample, in view of the bias towards schools in deprived areas.

The children were all assessed with fourteen short developmental tests as part of a wider programme. The tests took about an hour to administer to each child and included measures of language development, non-verbal ability and perceptual and motor skills. The intention of assessing all children within a month of school entry was not met, but more than 94 per cent were assessed in their first term. There was nothing to suggest the existence of a relationship between birthday and late or early assessment. The relationships between the data on development and month of birth were then analysed.

First the product-moment correlation coefficients between each of the fourteen developmental variables and month of birth were noted. These ranged from a maximum of +0·06 to a minimum of −0·05. All are negligible and none is significant.

Although the straight correlation coefficients do not give positive results, it is possible that the multivariate nature of the data conceals other interesting relationships. As

The first problem was to find a satisfactory combined score for the fourteen variables on which the children had been assessed. We decided to carry out a principal components analysis of the relationships of the variables. The first principal component accounted for 54 per cent of the variance, and seemed to be a general component on which most of the variables loaded positively. The children were ranked by score on this first component, and the bottom seventy children were noted. These children could be regarded as developmentally the weakest—the least able 10 per cent of the sample of 695 entrants to infant schools.

The months of birth of these seventy children were extracted and compared with the expected distribution of month of birth, based on that of the total 695 (Table 5). Condensing the twelve months into the conventional four month periods and calculating the expected frequencies of occurrence in a sub-sample of seventy on the basis of the distribution in the 695 we produce Table 6. Clearly there is no real suggestion of any preponderance of children born at a particular time of the year among the weakest entrants to infants schools. A similar analysis for the weakest 140 children produced similar findings.

Table 6. OBSERVED AND EXPECTED DISTRIBUTIONS OF MONTH OF BIRTH OF SEVENTY WEAKEST CHILDREN IN SAMPLE

	Sept.–Dec.	Jan.–April	May–Aug.	No.
Observed	25	26	19	70
Expected	29	24	16	70

Implications for the Educational System

The findings of this study show no evidence of a "season of birth" effect among five year old children entering the British school system. It is considered not unreasonable

to have expected such an effect to have shown up in a sample of this size and composition. This result strengthens the view that the "season of birth" effect in handicapped children is likely to be a product of the educational system itself. This cannot be a conclusive finding, but we suggest that it is a probable one.

This leads to a consideration of the ways in which the education system could be modified to reduce the incidence of handicapped children among the summer-born to that existing among winter-born children. The resultant easing of pressure for places in schools for slow-learners, for the maladjusted, and so on would make a tremendous difference to the services dealing with handicapped children.

The evidence of the research findings we have discussed suggests that, contrary to widely held views, the term of entry effect is not responsible. The Plowden report, for example, recommended that there should be a uniform date of entry to infant schools, or first schools, so that all children should spend an equal amount of time there before moving on with their age group to junior or middle schools. There may be other reasons for suggesting this equal length of infant education, but research evidence does not suggest that it will remove the extra educational handicaps which summer-born children carry. It is unlikely to result in any improvement of the educational lot of the summer born. It is not more education that is needed, but different education.

We suggest that it is the age group position effect which must be the target of educational expenditure if this problem is to be eased. There are humanitarian and economic reasons for an attempt to deal with the problem. Even if we are prepared to put on one side the humanitarian pressures to reduce the number of slow learners, for example, there is still the economic argument that such a reduction might more than repay the money invested in producing it.

Any proposals which decrease the need to treat a class of children as a unit should be encouraged. The need to proceed at the pace of the class places strain on the intellectually immature. And, *ceteris paribus*, the intellectually immature are likely to be the younger children. Greater flexibility in arrangements for retaining summer-born children in classes of younger children, more use of vertical grouping, smaller classes, a wider availability of school psychological services are not new proposals. But they direct attention towards the individual and his needs and away from the group. Our principal conclusion is that resources spent in this way are likely to be much more effective in providing a fairer educational deal for the summer-born than resources spent in giving all children the same length of time in school.

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Thin Film Research Today

by
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Improved methods of study have contributed to a great advance in thin film research in the past twenty years. A brief survey shows that applications have spread to many advances of electronics and optics.

One manifestation of the growth of the importance of thin film and surface studies has been the emergence, during the past few years, of three specialist journals (*Surface Science*, *Thin Films* and *Thin Solid Films*). Until the end of the Second World War, only moderate effort was devoted to such studies and the technological importance of films was confined to a few fields such as electroplating and, in a limited sense, optics. In the past twenty years, there has been an enormous growth of activity, both in basic studies of film properties and behaviour and in the introduction of thin film technology to a host of industrial applications. In this article I shall discuss a selection of such applications.

In the past few years, methods of studying film growth and structure have improved considerably. Electron microscopy and diffraction, operated using high energy (tens of kilovolts) electrons, have been supplemented by the development of low energy electron diffraction (LEED) and Auger emission spectroscopy. At the same time the impressive advances in ultrahigh vacuum techniques have led to the situation where, for the first time, experiments can be done in known, controlled conditions. Many of the uncertainties which led to difficulties of interpretation of early measurements have been removed. In one sense, the problems associated with film studies start before the film is formed. The precise surface structure of the supporting substrate can often exert a decisive influence on thin film properties. By the use of LEED and Auger techniques, essential basic information about the nature and structure of substrate surfaces

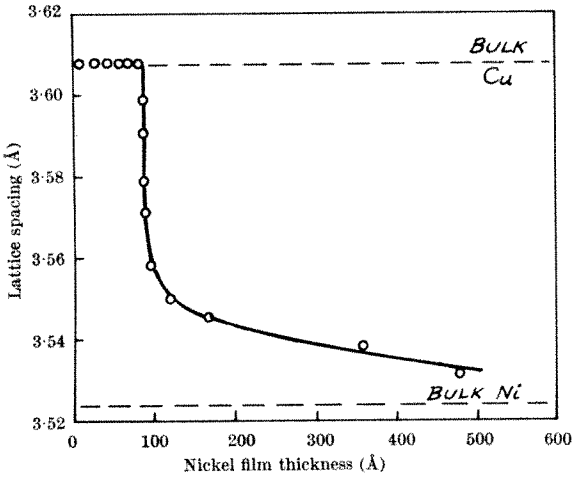


Fig. 1. Variation of lattice parameter of nickel deposited on copper substrate.

can be obtained. Moreover, the availability of ultrahigh vacuum systems makes it possible to carry out experiments without uncertainties created by the influence of residual gas in the vacuum system. Another powerful tool is the mass spectrometer, which facilitates studies of adsorption and desorption phenomena.

As was the case with the study of bulk solids, progress became very rapid when single-crystal materials were studied. Investigations of films were greatly aided by the phenomenon of epitaxy, which is a fascinating, and in some respects still perplexing, subject. For a long time it was a mystery that epitaxy could occur readily between a film and substrate even in the face of very large misfits between the lattice constants of the substrate and deposited materials. At an early stage, the idea of pseudomorphic growth was proposed, by which the lattice spacing of the deposit material was supposed to be strained to that of the substrate. Early experimental difficulties made it impossible to confirm this idea, but it has now been established that pseudomorphic growth does occur in some conditions. Fig. 1 (taken from ref. 1) shows that the lattice spacing of epitaxial nickel on a single crystal copper substrate is strained to that of the substrate for thicknesses up to 80 Å, beyond which the spacing rapidly approaches the normal value for bulk nickel.

Nucleation of Films

The very difficult problem of nucleation of films at the onset of growth has received considerable attention in recent years. The classical theory of homogeneous nucleation gives some general guidance but there are two principal difficulties. On the one hand, the quantities in terms of which the theory is expressed, such as interfacial energies, diffusion rates and surface energies, are difficult to measure and generally impossible to calculate. On the other hand, the theory shows in some cases that the critical size of nucleus (below which growth cannot proceed) contains only a few atoms. The problem thus arises of justifying the use of macroscopic thermodynamic concepts to a system containing so few atoms. Hirth, Hruska and Pound[2] have developed this ("capillarity") model to an impressive level, including the effects of desorption and surface diffusion. In their view, the theory can be expected to give reasonable results for nuclei containing more than about 100 atoms. The problem of very small critical nuclei has been tackled by Rhodin and Walton[3] in a theory of heterogeneous nucleation, which shows that the size of the critical nucleus can be obtained from the dependence of nucleation rate on temperature. Indirectly, this gives indications of the likely orientation of critical nuclei, which is important in considering epitaxial growth.

In view of the fact that surface defects serve as nucleation sites, useful information can be gained from studies of films grown on surfaces subjected to electron bombardment. Several groups are actively engaged on this problem, which is still at the stage where stimulating arguments on interpretation are the rule.

There seem to be two skeletons in the nucleation/film growth cupboard. In a cinemicrograph of a growing film, Pashley has observed that very large crystallites can be very mobile, skating rapidly across a surface to coalesce with larger, fixed ones. This is both difficult to understand and difficult to build into existing theories. The second problem arises from Barna's[4] observation that epitaxy can occur on an amorphous carbon film, deposited on a monocrystal, even for thicknesses of carbon up to 100 Å. Both these observations tend to underline the simplicity of current approaches to problems of film growth: no simple adaptation of present models seems to account for this unexpected behaviour.

Improvements in the performance of the electron microscope, which can now resolve lattice planes of simple structures, have led to rapid developments of dislocation theory, much of which has derived from the use of epitaxially grown films. The movement of dislocations has been observed by straining specimens *in situ* and considerable progress has been made in the understanding of the strength of materials. In view of the limited extent to which dislocations in a thin film can move, the mechanical strength of films would be expected to increase with decrease of thickness. This is indeed observed in measurements of the fracture strength of silver, as shown by the results in Fig. 2 (from ref. 5).

The use of low energy electrons for diffraction experiments has brought enormous advantages for surface studies. Because the penetration of slow electrons extends only to a very few atomic spacings, it is certain that the information carried by the scattered electrons refers only to the surface layers. In spite of some difficulties on the theoretical side, which limit the full exploitation of this technique, much information can be

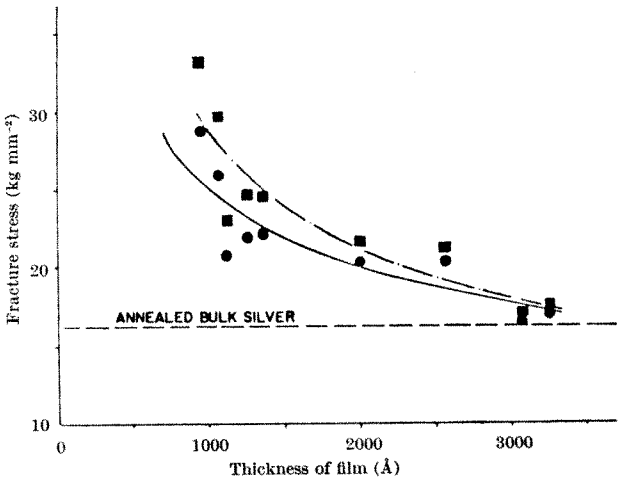


Fig. 2. Dependence on thickness of the fracture strength of silver film (reproduced by courtesy of the editor of *Thin Solid Films*). ●, As deposited; ■, corrected cross-section.

obtained about surface structures. The results have already demolished various preconceived notions on the nature of ("well understood") crystal surfaces. It should not, however, be thought that high energy diffraction has become *passée*. In addition to its continuing role in the determination of structure and orientation of individual microcrystals, it has been exploited in the study of the electronic properties of alloys. Certain alloys have superlattice structures with very long periods (for example, 40 Å for CuAu II). Study of the periodicity in such superlattices as a function of the number of free electrons and of the mass and size of constituent atoms can provide details about the electronic properties of the alloys, leading to information about the Fermi surface. For this work, many single-crystal specimens of various compositions are needed. The time and effort required to prepare bulk samples would be prodigious: the growth of films of such materials by epitaxy is a trivial problem[5].

Optical Uses

The physical properties of materials in film form which have evoked the greatest interest are optical, magnetic and electrical. Optical studies have led to the development of optical filters with remarkable characteristics: magnetic studies have produced computer memories which are much faster than existing ones, and electrical studies have led to the rapidly developing fields of thin-film microcircuitry and integrated circuit technologies.

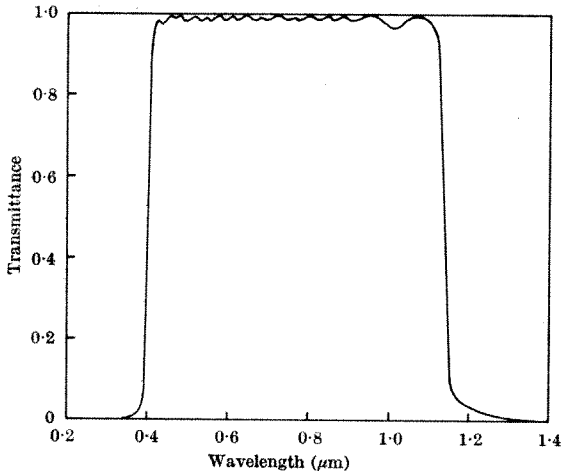


Fig. 3. Transmission curve of filter for use with solar cell.

Since the early days when the now familiar blooming layer on a lens surface was used to reduce unwanted reflexion, filters with stacks of dielectric layers have been developed to an impressive level of performance. Multilayer filters of this type have played a decisive part in the development of many instruments and devices. Thus the operation of the commonest gas lasers relies entirely on the availability of high reflecting layers which may reflect more than 99·5 per cent of the incident radiation and yet transmit a substantial part of the remaining energy. Moreover, such reflectors can be designed to have a high reflectance at one wavelength and a very high transmission at another. An important development has been the production of the second harmonic of a laser frequency by the use of a non-linear crystal. This requires a system which transmits the fundamental frequency so that the exciting radiation can get into the crystal, but which has a high reflectance at the second

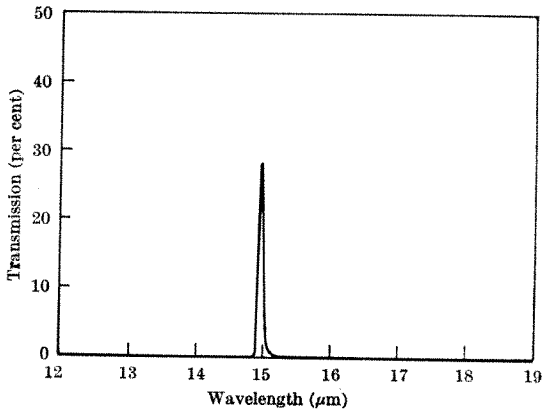


Fig. 4. An example of a narrow-band filter for the infrared (reproduced by courtesy of Grubb Parsons Ltd).

harmonic frequency. This is easily achieved with a multilayer system.

Solar Cells and Holograms

The silicon solar cell plays a vital part in providing power for artificial satellites, through the direct conversion of solar into electrical energy. Such cells have a response over a limited band (0·4 to 1·1 μm) but their efficiency decreases seriously with undue rise of temperature. Because substantial amounts of solar energy are contained in the region outside this band, filtering is essential to exclude unwanted wavelengths. A 62-layer system designed for this purpose has the characteristic shown in Fig. 3. The transmission over the band of interest exceeds 96 per cent. Multilayers have also developed impressively for use in narrow-band filters (Fig. 4). The development of instruments has benefited because a simple filter and detector can replace a more complex and costly dispersing system.

Holography provides another important optical application of thin films. Since the arrival of the laser holography has grown rapidly, with impressive results. But it has been clear for some time that, from some points of view, the photographic plate has serious limitations as a recording medium. Recently, holograms have been produced using a glass plate coated with a metal film, such as Bi, Al, Au, which is vaporized by the action of the

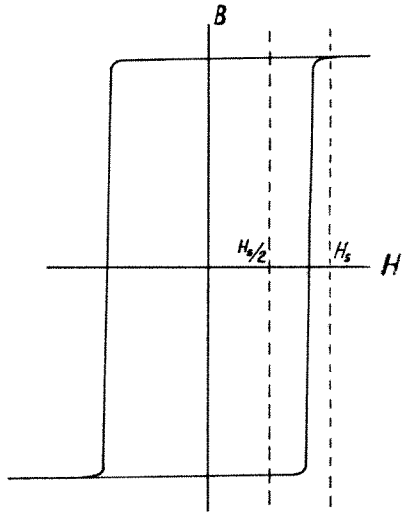


Fig. 5. Magnetization loop for a thin permalloy film.

(two) laser beams from the holography set-up. In the most intense parts of the light pattern forming the hologram, most metal is removed. Thus the transparency of the hologram varies with the illuminating beam intensity as desired. The resolution obtained is comparable with that of a photographic plate; no processing of the metallized plate is needed after exposure.

Magnetic Applications

Studies of magnetic properties of films have been important not only for fundamental theories of ferromagnetism but also as vital elements in modern methods of digital storage. Thin film elements can easily be made which display nearly rectangular hysteresis loops. Fig. 5 shows that the magnetization is unchanged by the application of a field $H_s/2$ but its direction is reversed by a field H_s. The device therefore lends itself readily to the construction of a storage array in which the state of magnetization may be read or changed by the application of current pulses to a pair of conductors which intersect at the element in question. This is essentially the technique used with the existing ferrite core of the computer store. The access time for the present ferrite core store is about 0·5 μs and it is difficult to see how any substantial reduction can be achieved. For the magnetic film store, the access time is approaching one hundred times smaller than this, thus providing potentially much faster computer systems. (So far, this very high speed cannot be utilized effectively, for it is limited by the speed of other parts of the system.) In another form of magnetic storage element, the film is deposited on a wire, which also serves as one of the access channels (Fig. 6).

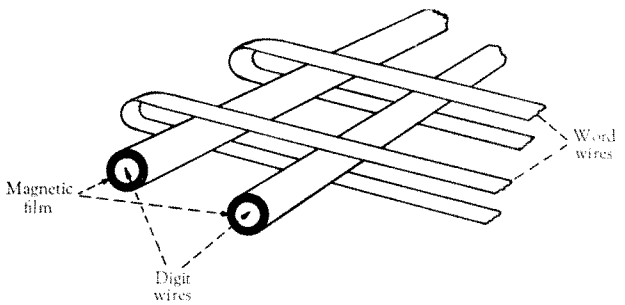


Fig. 6. Plated wire storage element.

Looking ahead, it seems likely that another magnetic effect—the magneto-optical effect—may provide a future generation of data-handling devices. Changes in magnetization of thin films of ferromagnetic materials can produce changes in the state of polarization of light transmitted or reflected by the system. Thus a laser beam can be used to scan arrays of such elements, the information being read from the state of polarization of the light leaving the specimen.

Electrical Applications

So far as electrical behaviour is concerned, the polycrystalline film, particularly of metals and semiconductors, is an extremely complicated system with various possible conduction mechanisms. Thus the ultra-thin metal film, consisting not of a parallel sided layer but of a scatter of isolated crystallites, may conduct through the effects of the substrate or through tunnelling between crystallites. The individual crystallites will not, however, behave like the bulk material because their size will generally be less than the electron mean-free path: the effects of surface scattering will therefore dominate. With the improvement in experimental techniques, understanding of the electrical behaviour of metal films is increasing rapidly although it is by no means complete. The conducting properties of semiconducting layers—once a bewildering set of results—are beginning to be understood, notably through the contribution of Hill[7], who has shown that, for many materials, the assumption that Poole–Frenkel defects dominate leads to results which agree with experimental results over eight orders of magnitude in the conductivity.

It was soon realized that thin film systems offered great promise for the construction of microcircuits. Evaporation techniques for metals and dielectrics easily lent themselves to the production of resistors and capacitors of very small bulk. Moreover, the associated large surface to volume ratio made these systems attractive from the standpoint of heat dissipation. Other techniques, such as reactive sputtering, photodissociation and photopolymerization, have also been used to produce films for electrical circuitry.

With the rise of semi-conductor technology, the emphasis on thin film circuitry has shifted from that in which individual components are produced and joined together, to that in which truly integrated circuitry is achieved. Thus the transistor, instead of being a separate, wired-in component, is made on the silicon substrate surface, with associated resistors, capacitances and connexions, by a range of techniques, including evaporation, sputtering, oxidation and heat treatment stages.

Photoresist is now widely used as a means of producing a suitably metallized pattern on the silicon surface. The surface of the silicon is coated with a film of the required material which is in turn coated with a layer of photoresist. On exposure to a light pattern, the resist can on development be selectively removed from the light parts (positive resist) or dark parts (negative resist) of the pattern. The underlying film can then be removed selectively over the areas from which the resist has been removed. By these methods, an enormous density of components can be achieved on a chip. The typical size of, for example, a transistor made by these methods is a few tens of micrometres. The advantage of this approach is not only size but also the fact that the circuits can operate at far higher frequencies than can their predecessors.

In this necessarily brief survey of interest in thin films, little more than a general indication can be given. On the more basic side, the development of new and powerful methods of studying films has led to a situation where many of the uncertainties surrounding early work can now be removed, and rapid progress is being made. Also the improved methods of control of deposition parameters and of film structures have led to the rapid exploitation of many of the important technological benefits to be had by the use of thin film systems.

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Rabbit Antibody Light Chains and Gene Evolution

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Several amino-acid positions in the light chains of rabbits are different from the same positions in the light chains of man. This observation suggests that rapid gene expansion is a fundamental mechanism in the evolution of the immune system.

INSIGHT into the mechanism responsible for antibody diversity has been provided in the past few years by the amino-acid sequence analysis of homogeneous immunoglobulins produced by plasmacytomas in man[1–5]. These studies have shown that immunoglobulins are composed of light chains (molecular weight 23,000) and heavy chains (55,000) which are linked through disulphide bonds. Man can produce two types of light chains, designated $\varkappa$ and λ, and at least ten classes and subclasses of heavy chains. The human $\varkappa$ chain has been most thoroughly studied and serves as a general model for all immunoglobulin polypeptide chains[1]. The amino terminal half of the $\varkappa$ chain (residues 1–107) is designated the variable (V) region and presumably is responsible for encoding various antibody specificities because each variable region from $\varkappa$ chains ($V_\varkappa$) studied to date has a unique amino-acid sequence. The carboxy terminal half of this chain (residues 108–214) is designated the common (C) region and is invariant in all human $\varkappa$ chains apart from a single amino-acid interchange position 191 which is inherited in a Mendelian fashion[6–9]. Because of this Mendelian behaviour the $C_\varkappa$ region is probably encoded by a single structural gene with two allelic forms[10,11]. The $V_\varkappa$ regions in man seem to be encoded by multiple genes which can be associated with either allelic form of the $C_\varkappa$ gene[4,12–14]. Thus two genes probably encode each human $\varkappa$ chain (a $V_\varkappa$ gene and a $C_\varkappa$ gene) and these genes must be joined together at some level of protein synthesis to produce a single peptide chain. These generalizations, multiple V genes and a joining mechanism to unit separate V and C genes, seem to hold for the other immunoglobulin chains[15–18].

Structural studies on the immunoglobulins from species other than man have raised important questions about the evolution of immunoglobulin genes. For example, Doolittle reported that normal rabbit light chains, derived from a heterogeneous pool, had amino terminal residue alternatives which were different from their human counterparts[19]. Such unique amino-acids have been designated species specific residues. The question then arose how multiple genes could evolve in a given evolutionary line to generate species specific residues. Obviously a detailed examination of the extent and nature of species specific residues requires rabbit immunoglobulins of restricted heterogeneity. Such immunoglobulins are now available[20–24].

We report here amino terminal sequence studies on rabbit antibody light chains which demonstrate that the

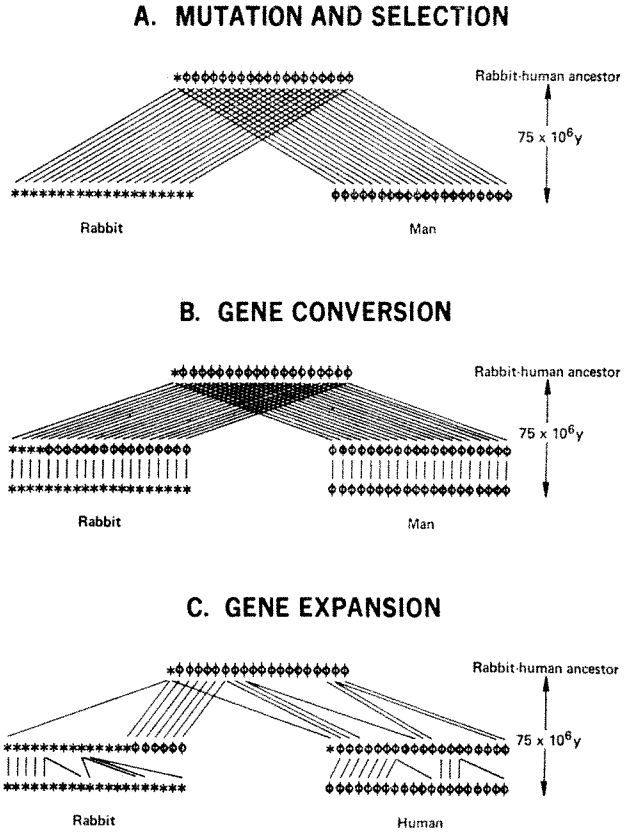


Fig. 1. Three models for the generation of species specific residues in a multigene system. Φ and * represent germ line genes differing at positions which are to become species specific. The genes present in the ancestor common to rabbit and man are represented at the top of each figure and the genes present in the contemporary individuals are at the bottom. These species diverged about seventy-five million years ago. *A*, Random mutation changes the rabbit Φ genes to * genes, and selection fixes these mutations. *B*, Gene conversion changes the neighbouring rabbit Φ genes to * genes by a process of non-homologous pairing and copy-repair[25]. *C*, The * gene is expanded by repeated duplication and integrated into the genome of the evolutionary line leading to contemporary rabbits. Later gene expansions of different individual members of the * gene family generate contemporary rabbit subgroups. The Φ gene is expanded in the human evolutionary line. Species specific residues occur because different genes are expanded in the two evolutionary lines. This diagram is not meant to indicate that there is an immediate compensatory loss of other V genes after the gene expansion. Rather, the genome probably expands and carries for a time the older functional genes as well as the newly expanded family.

V regions from these chains are encoded by multiple genes. This is suggested by their division into at least three subgroups. After appropriate alignment, the V regions from rabbit light chains are homologous to their human V_κ counterparts and presumably both were derived from a common ancestor. The existence of distinct species specific residues suggests that rapid gene expansion has occurred during the evolution of the immune system. We feel that this mechanism of rapid gene expansion must also be evoked to explain the evolution of other multigene loci such as the ribosomal system. Indeed the antibody system affords an excellent opportunity to study the general features of evolution in a multigene system. This will be discussed later.

Rabbits immunized with streptococcal vaccines may produce antibodies with restricted heterogeneity in concentrations which reach 30–50 mg/ml. of serum[20,23]. The nature of the antigen, the preparation, isolation and characterization of these antibodies and the separation of light and heavy chains have been described[25–28]. The amino-acid sequences of these light chains were determined using a Beckman model 890 protein sequencer[29] and the resulting phenylthiohydantoin derivatives were identified by gas chromatography[30] or by amino-acid analysis after hydrolysis to the free amino-acid[31,32].

Light Chain Sequences

N-terminal sequences for the light chains from eight antistreptococcal antibodies isolated from six rabbits are given in Fig. 1. Two antibody components of differing electrophoretic mobility were isolated from each of two rabbits, R24–36 and R24–61. Thus it became feasible to compare multiple antibodies of similar specificity from a single rabbit. The following points will be discussed: (1) the rabbit has three light chain V region subgroups; (2) the subgroups exist in normal rabbit light chains; (3) these subgroups are not alleles; and (4) the subgroups are apparently independent of light chain allotype.

The light chains of R27–11 and R24–36–I were examined as far as position 23 and 26 respectively. They are as homogeneous as control myeloma light chains over this portion of their sequence. These light chains, like Bence–Jones proteins, are distributed as a single component by polyacrylamide disc electrophoresis. The six other light chains had two principal residue alternatives at one or more positions. The disc electrophoresis of these light chains revealed two principal bands in each case. There is therefore an excellent correlation between the degree of heterogeneity of light chains as determined by amino-acid sequence analysis and by electrophoretic mobility[22]. On the basis of this limited sequence analysis, the restricted electrophoretic mobilities, as well as other characteristics[23], certain antistreptococcal responses seem to be primarily derived from a single antibody clone (R27–11), whereas others are derived from two (R26–90), three (R24–36), or even four (R24–61) principal clones. In each case, the heterogeneity is far less than is ordinarily present in the antibody responses to many other natural and synthetic antigens.

These light chain sequences can be aligned by homology with human κ chains (Table 1). For example, aligning the invariant Thr-Glu-Thr-Pro at positions 5–8 in both species shows that there are three discrete populations or subgroups of rabbit antibody light chains. These subgroups differ from one another in length at the amino terminus. The $V_{\kappa I}$ subgroup has an extra N-terminal residue, the $V_{\kappa III}$ subgroup has an N-terminal deletion, and the $V_{\kappa II}$ subgroup is the same size as its human counterpart. In addition to these size differences, it is also shown in Fig. 1 that certain amino-acid residues are correlated with the light chains of a given subgroup. For example, isoleucine is present at position 1 for $V_{\kappa III}$ proteins, aspartic acid at position 2 for $V_{\kappa I}$ proteins, and phenylalanine or isoleucine at position 2 for $V_{\kappa II}$ proteins. Other positions may also differentiate these subgroups (see position 13 in Table 1) but there are insufficient data to be certain.

These subgroups also seem to be present in normal rabbit light chains. Table 2 gives the residue alternatives present at the first eight positions of non-immune light chains from rabbit R14–43. Subgroup specific residues are present in these chains: $V_{\kappa III}$, position 1, Ile; $V_{\kappa II}$, position 2, Phe; $V_{\kappa I}$, position 2, Asp. Furthermore, the invariant Thr-Glu-Thr-Pro starts at three consecutive residues (4, 5, and 6) as would be expected for the three out of phase subgroups III, II, and I, respectively (see Table 2). At least 60% of the non-immune light chains in R14–43 probably fall into subgroups which are not selected by the streptococcal antigen as indicated in the legend to Table 2. Most of the non-immune chains are of the $V_{\kappa II}$ length. This can be seen at position 5 where

Table 1. RABBIT ANTIBODY LIGHT CHAIN SEQUENCES COMPARED WITH THE PROTOTYPE SUBGROUP SEQUENCES OF HUMAN κ CHAINS

				N-Terminal sequence position																	
Species	Subgroup	Antibody	Protein	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Rabbit	$V_{\kappa I}$	Group C	R27–11	Ala	Asp	Val	Val	Met	Thr	Glx	Thr	Pro	Ala	Ser	Val	(Ser)	Glu	Pro	Val	Gly	Gly
	$V_{\kappa II}$	Group C	R24–36–I	[ ]	Ala	Phe	Glx	Leu	Thr	Glx	Thr	Pro	Ser	Ser	Val	Glx	Ala	Ala	Val	Gly	Gly
		Group C	R24–61–I	[ ]	Ala	Phe	Glx	Met	Thr	Glx	Thr	Pro	Ala	Ser	Val	Thr	Ala	Pro	Val		
						Ile	Val														
	$V_{\kappa III}$	Group C	R26–90	[ ]	[ ]	Ile	Val	Met	Thr	Glx	Thr	Pro	Ser	Ser	( )	Ser	Val	Pro	Val	Gly	Gly
								Val													
		Group C	R24–36–S	[ ]	[ ]	Ile	Val	Met	Thr	Glx											
								Val													
		Group C*	R24–61–S	[ ]	[ ]	Ile	Val	Met	Thr												
								Val													
		Group C*	R24–34	[ ]	[ ]	Ile	Val	Met													
								Val													
		Group A	R22–85	[ ]	[ ]	Ile	Val	Met													
								Val													
Human	Type	Subgroup		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		17
	Kappa	$V_{\kappa I}$		[ ]	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp
		$V_{\kappa II}$		[ ]	Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser	Leu	Ser	Pro	Gly	Glu
		$V_{\kappa III}$		[ ]	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly	Glu

All rabbit chains are of the b4 allotype. All light chains are aligned to maximize their amino-acid sequence homology through the judicious use of gaps which are indicated [ ]. Certain of the rabbit light chains have two residues at a particular position (see text). ( ) indicates residues not identified. Two distinct antibody components were isolated from serum R24–36 and also from R24–61. These components had either intermediate (I) or slow (S) electrophoretic mobilities and were separated by agarose electrophoresis. The numbering is based on the human light chain length and is useful in making a comparison of proteins after they have been aligned. In discussing the sequence analysis of these subgroups in the text, the numbering will be different for $V_{\kappa I}$ and $V_{\kappa III}$ proteins. Sequence analysis starts at the amino terminus of each protein and accordingly the isoleucine in $V_{\kappa III}$ proteins is at position 1 and the aspartic acid in $V_{\kappa I}$ proteins is at position 2 (see Table 2). Group A: antibody to group A carbohydrate; group C: antibody to group C carbohydrate.

* Sequence present at the 70 per cent level.

approximately 70% of the molecules have the threonine expected of $V_{\varkappa II}$ proteins ($V_{\varkappa I}$ and $V_{\varkappa III}$ proteins have threonine at positions 6 and 4 respectively).

A somewhat arbitrary attempt has been made to construct subgroup sequences which can account for the residue alternatives observed in Table 2. The $V_{\varkappa I}$, $V_{\varkappa IIa}$ and $V_{\varkappa III}$ sequences correspond to the antibody sequences of Fig. 1. $V_{\varkappa IIb}$ and $V_{\varkappa IIc}$ are possible subgroup sequences not seen in this antibody population. Indeed, Jaton *et al.*[33] have reported an antipneumococcal antibody light chain with a sequence identical to $V_{\varkappa IIc}$. Thus the rabbit $V_{\varkappa}$ region as well as its human and mouse counterparts has multiple V region subgroups (4, 12, 14, 34). These rabbit subgroups differ, of course, from those in human and mouse light chains in that they have been derived from antibody molecules and not from myeloma proteins.

The existence of rabbit V region subgroups implies that there must be at least one gene for each subgroup. The rationale for this supposition has been thoroughly discussed elsewhere[4,5], and is summarized in Fig. 3. Furthermore, from published[35] and unpublished observations (L. H., H. L., K. E. and C. W. Todd), the light chains from thirteen normal rabbits have subgroup specific residues for each subgroup ($V_{\varkappa I}$-Asp at position 2, $V_{\varkappa IIc}$-Asp at position 1, and $V_{\varkappa III}$-Ile at position 1). Accordingly, every rabbit probably synthesizes proteins from each subgroup. The genes encoding these subgroups cannot be alleles; rather, they must be present in the germ line of each rabbit. Thus the V regions of rabbit $\varkappa$ light chains must be encoded by at least three germ line genes. (These subgroup specific residues are present in rabbits of differing light chain allotype: of the thirteen normal rabbits, nine were b4, three were b5, and one was b9. Thus the $V_{\varkappa}$ genes can be associated with any of the three light chain alleles studied.)

Table 2. AMINO TERMINAL RESIDUE ALTERNATIVES IN THE LIGHT CHAINS FROM NON-IMMUNE RABBIT R14-43 (b4 ALLOTYPE)

	Position 1	2	3	4	5	6	7	8
	Ala 78 Asp 19 Ile 3	Val 35 Asp 30 Tyr 20 Ala 5 Leu 5 Phe 5(Met)	Val 40 Ile 20 Asp 15 Leu 15(Thr) Glx 5	Val 50 Met 40 Leu 10	Thr* 70 Met 20 Glx 10	Glx Thr	Thr Glx (Pro)	Pro Ala Ser
$V_{\varkappa I}$	Ala	Asp	Val	Val	Met	Thr	Glx	Thr
$V_{\varkappa}$(a)	Ala	Phe	Glx	Met	Thr	Glx	Thr	Pro
(b)	Ala	Val	Ile	Val	Thr	Glx	Thr	Pro
(c)	Asp	Val	Val	Met	Thr	Glx	Thr	Pro
$V_{\varkappa III}$	Ile	Val	Met Val	Thr	Glx	Thr	Pro	Ser

The data are derived from amino-acid analysis of hydrolysed PTH derivatives and are expressed as the per cent of individual residues at positions 1–5. In subsequent positions only the principal residue alternatives are given. After position 1 all residue percentages are rounded off to the nearest 5%. Beneath the yield data are indicated the principal sequences which could account for the residue alternatives in the pool. The $V_{\varkappa IIb}$ and $V_{\varkappa IIc}$ sequences seem to comprise most of the normal light chain alternatives. The frequency of antibody subgroups can be estimated from the yields of subgroup specific residues (for example, $V_{\varkappa III}$-Ile at position 1, 3%; $V_{\varkappa I}$-Asp at position 2, 30%; and $V_{\varkappa II}$-Phe at position 2, 5%). ( ) indicates a minor residue (less than 5%) which is probably from the $V_{\varkappa III}$ subgroup.

* Threonine is destroyed by acid hydrolysis and was determined by gas chromatography.

Table 3. GENERATION OF RABBIT LIGHT CHAIN SUBGROUPS FROM A SINGLE "GENE"

	0	1	2	3	4	5	6
Prototype	Ala	Ala	Val	Val	Val	Thr	Glu
Subgroup	1	2	3	4	5	6	7
$V_{\varkappa I}$	Ala	[Asp]	Val	Val	[Met]	Thr	Glu
		1	2	3	4	5	6
$V_{\varkappa II}$	~~Ala~~	Ala	[Phe]	[Glu]	[Leu]	Thr	Glu
			1	2	3	4	5
$V_{\varkappa III}$	~~Ala~~	~~Ala~~	[Ile]	Val	[Met] Val	Thr	Glu

The prototype sequence is taken from the principal sequence alternatives present in normal light chains with an extra alanine at position 0 to generate the $V_{\varkappa I}$ proteins (see Table 2). To generate the three light chain subgroups from the prototype gene, certain residues must be deleted (residues crossed out) and others must be changed by mutation. Thus, a series of correlated events must occur to generate the three light chain subgroups. First, the N-terminal residue must be deleted for all proteins in the $V_{\varkappa II}$ subgroup and the N-terminal two residues deleted for all proteins in the $V_{\varkappa III}$ subgroup. Correlated with these deletions must be specific mutations at certain positions to generate the subgroup specific residues (for example, alanine to aspartic acid at position 2 and valine to methionine at position 5 in the $V_{\varkappa I}$ proteins, and so on) (see boxed residues). Thus, the generation of all rabbit light chains from a single gene seems unlikely. Rather, it is simpler to postulate that rabbits have at least three genes for light chain V regions, one gene for each subgroup.

Evolutionary Implications

The rabbit antibody light chains (b4 allotype) can readily be aligned by sequence homology against their human $\varkappa$ counterparts (Tables 1 and 4). Thus rabbit and human $V_{\varkappa}$ genes have descended from a common ancestor.

Although homologous, the $V_{\varkappa}$ regions of rabbit and man can readily be distinguished by certain species specific features. First, no set of human $V_{\varkappa}$ sequences corresponds to the rabbit $V_{\varkappa I}$ subgroup with an extra N-terminal residue, nor is there a set with the N-terminal residue deleted as in the rabbit $V_{\varkappa III}$ proteins. Second, several amino-acid positions in the light chains of rabbits are different from the same positions in the light chains of man (Table 4). The rabbit light chains in Figs. 1 and 4 have valine and glycine at positions 11 and 17, respectively, whereas most of their human and mouse counterparts have leucine and Asp/Glx, respectively, at these positions. Most normal light chains from these three species also have valine (rabbit) or leucine (man and mouse) at position 11 (L. H., M. Potter and C. W. Todd, unpublished observations).

The important new observation presented here is that species specific residues seem to be present on all light chain subgroups. If each $V_{\varkappa}$ subgroup is encoded by a different germ line gene, then multiple *V* genes in rabbit, mouse, and man must have evolved species specific residues. We shall examine models for the multigene evolution of species specificity shortly. First, it should be stressed that the number of $V_{\varkappa}$ subgroups in man (4 $V_{\varkappa}$), rabbit (3 $V_{\varkappa}$), and mouse (9 $V_{\varkappa}$) was determined from an analysis of relatively limited numbers of $\varkappa$ chains[4,34] and accordingly provides only a lower limit on the number of $V_{\varkappa}$ genes present in the germ line. Indeed, the immunoglobulin sequence data are consistent with a germ line hypothesis which suggests that a separate germ line encodes each distinct V region. Thus there may be as many as 20,000 antibody *V* genes[4]. There are, as we shall describe, precedents for such a multigene system. Moreover, these other multigene systems can also evolve in a species specific fashion.

Britten and his co-workers have demonstrated by DNA hybridization techniques that families of repeated DNA sequences are present in all higher organisms[36,37]. These repeated sequences comprise from 20 to 80 per cent of the total nuclear DNA. There are 50–2,000,000 related sequences per family. Some of the families are ancient (several hundred million years old) and individual members have diverged from one another significantly, while other families are recent and have diverged very little. RNA complementary to some repeated DNA sequences has been observed in every cell type studied. Thus certain of these repeated sequences must be transcribed and perform some function in eukarocytes.

Two of these multigene families have known functions. The tRNAs genes of higher organisms are probably present in multiple copies. For example, at least fifteen copies of each of the sixty tRNAs are present in *Drosophila*[38]. The number of ribosomal genes has been estimated to range from 100 to 400 in a haploid set of chromosomes in species as varied as yeast[39], *Drosophila*[40], frogs[41], pea seedlings[42], and man[43]. The ribosomal system has been studied in detail and has the following general properties: (1) the multiple ribosomal genes are all clustered at a single complex locus[44]; (2) the ribosomal genes of a single species are very similar[45] (little divergence has occurred); and (3) the ribosomal genes from

Table 4. A COMPARISON OF LIGHT CHAIN RESIDUE ALTERNATIVES IN MOUSE, MAN, AND RABBIT κ CHAINS AFTER ALIGNMENT TO MAXIMIZE SEQUENCES HOMOLOGY

	Position															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Rabbit κ (9)*	[Ala]	[Ala]	Ile Val Phe	Val Glx	Met Val Leu	Thr	Glx	Thr	Pro	Ala Ser	Ser	[Val]	Ser Thr Glx	Glx Ala Val	Pro Ala	Val	Gly	[Gly]
Human κ (41)*	Glu	Asp Glu Lys	Asp Ile Val Met	Gln Val Leu Ile	Met Leu	Thr	Gln	Ser Thr	Pro	Ser Ala Leu Gly Thr Asx	Ser Thr Phe	Leu	Ser [Pro]	Ala Leu Val Met	Ser Thr	Val Pro Leu	Gly Arg	Asp Glu
Mouse κ (21)*		Asp Glu Glp	Ile Val Thr	Val Gln Thr Leu	Met Val Ile Leu	Thr	Gln	Ser Thr Asx	Pro Thr Glx	Ala Ser Thr Leu Asx	Ser Thr Phe Tyr	Leu	Ser [Ala] Pro	Val Ala Met	Ala Ser Thr	Ala Leu Ile Val Ser Pro	Gly Ser	Glu Lys Asp Gln

The rabbit light chain alternatives are taken from the sequences presented here. The human κ chains are taken from ref. 4 and the mouse κ chains from ref. 34. The boxed residues are those which are unique to a single species (species specific residues) and present in three or more chains in that species. The alanine at positions 1 and 2 in rabbit chains is also boxed because it is seen at a significant level in normal rabbit light chains (see Table 2).
* These numbers indicate the number of myeloma or antibody light chains from which this information was obtained.

species widely separated on the phylogenetic tree can differ markedly in size[46], GC content[47], and base sequence[45]. Thus the ribosomal system is a clustered, multigene system with a specific set of functions which can evolve in a species specific fashion. In these characteristics the ribosomal system appears similar to the *V* genes of the immunoglobulin system. The ribosomal genes are different in that they appear to be much more highly conserved, as might be expected from their more limited range of functions.

Other multigene families, however, can diverge rapidly from one another, more in line with the rate of divergence of immunoglobulins. For example, Hennig and Walker have compared the DNA from sixteen members of two rodent families and conclude that even in closely related families, the banding profiles in CsCl and the reassociation rates of the DNA exhibit significant differences[48]. Thus individual families of genes must be able to change rapidly, such that, for example, those of the rat (*Rattus norvegicus*) can be readily distinguished from those of the mouse (*Mus musculus*). How is this species specificity generated ?

Two general mechanisms could generate species specificity in a multigene system. The co-evolution model suggests that multiple genes evolve in parallel in a given evolutionary line. Presumably different evolutionary lines would follow independent paths and accordingly species specificity will result (Fig. 1*A*, *B*). Three such examples will be considered. The gene expansion model postulates that in a given evolutionary line, a single gene is copied many times over, integrated into the genome and subsequent divergence generates a family of genes which deviate about an average sequence which corresponds to the gene originally expanded (Fig. 1*C*). If different genes are expanded in different evolutionary lines, species specificity will result.

Co-evolution: Mutation and Selection

Contemporary genes may have evolved in a direct linear fashion from their ancestral counterparts with species specificity being generated by random mutation and selection (see Fig. 1*A*). Such a model requires that identical (parallel) mutations occur in each gene and that these multiple mutations become fixed in the population. It is difficult to imagine selective pressures which could cause, for example, 500 ribosomal genes to evolve in parallel, particularly in view of the fact that frogs lacking half of their rRNA genes are quite viable[49]. This model seems very unlikely.

Co-evolution: Gene Conversion

Contemporary genes may be changed through a correction process involving other genes (Fig. 1*B*). Two such models have been proposed. Callan has suggested a master-slave model in which multiple gene copies (slaves) are corrected against a single copy (master) at each cell division[50]. Thus, all the slaves would appear to evolve as a single gene because they are all made identical to the single master gene at each cell division. This "effective" single gene system could explain species specificity in that a single gene in each species (master) directs the evolution of the entire multigene system. The master genes in rabbit and man might diverge just as have their single cytochrome genes and these divergences would be reflected in their respective families of slave genes. Obviously this mechanism cannot apply to antibody V regions as they are not all identical.

Gally and Edelman have proposed that favourable mutations could spread through a population of similar genes by "democractic gene conversion"[51,52]. In this model (Fig. 1*B*) any one of the multiple genes could undergo a favourable mutation and this mutation could spread to neighbouring genes. Again it is difficult to imagine what selective pressures could ensure that favourable mutations spread to each member of a large multigene family (for example, ribosomal genes). Democratic gene conversion postulates that gene conversion can occur among the antibody genes of a given V region subgroup, but not among genes of differing subgroups (otherwise it would be impossible to maintain distinct subgroups in a given species). Accordingly, this model fails to account for species specific residues which are present in multiple light chain subgroups (see positions 11 and 17 in the rabbit, Table 4). To explain these residues, the democratic gene conversion model would require independent mutation and selection for each species specific residue in each subgroup. This requirement is similar to that for the model in Fig. 1*A* and is accordingly unattractive. A more plausible explanation comes from a consideration of gene expansion.

Gene Expansion

Families of repeated nucleotide sequences may originate in rather sudden events of excessive replication of particular sequences. Such gene expansion has been designated saltatory replication[36] and is illustrated in Fig. 1*C*. A single gene in the rabbit–human ancestor may undergo one or more such expansions in the line that is to become the contemporary rabbit. Thus most of the contemporary rabbit antibody genes may have evolved from a single gene in the rabbit–human ancestor and likewise the contemporary human antibody genes may have evolved from one or a few different genes in this same ancestor (Fig. 1*C*). If the primitive rabbit gene differed at positions 11 and 17 from the primitive human gene, species specific residues would result. Later gene expansions could

generate the V region subgroups. The gene expansion model has three requirements. First, many gene copies must be made in a short period of time. Second, these gene copies must be integrated into the genome so they can be transmitted to progeny. Finally, the resulting gene family must determine or be associated with a favourable phenotype.

Strong support for sudden gene expansion (saltatory replication) comes from the mouse satellite DNA example[53]. The mouse (*Mus musculus*) genome has a satellite DNA which can be isolated from chromosomal DNA after shearing because of its unusual GC content. This satellite comprises 10% of the total DNA and represents approximately one million nearly identical copies of a nucleotide sequence about 300 residues in length. The guinea-pig (*Cavia porcellus*) also has a satellite DNA, but hybridization studies have demonstrated that these two satellite DNAs have little, if any, sequence homology[53]. Indeed, sequences homologous with the mouse satellite cannot be found in the genomes of guinea-pig (*Cavia porcellus*), rat (*Rattus norvegicus*), or the British wood mouse (*Apodemus sylvaticus*)[53]. Two conclusions can be drawn: first, mouse satellite DNA was generated after the divergence of mice and other rodents, and second, the mouse satellite gene copies are so similar they must have been generated recently (little divergence has occurred) and in a short period of time. This latter point suggests that more conventional genetic mechanisms of gene expansion such as non-homologous crossing over may occur too infrequently to explain the rapid generation of a million very similar gene copies. Because Hennig and Walker have found extensive divergences in the multigene families of rodents, rapid gene expansion must also occur in these other systems. Gene expansion therefore seems to be a general mechanism in the evolution of eukaryotes.

Is there a mechanism for eliminating multigene families which have become useless? It seems that there are controls on the levels of genomic DNA in higher creatures. For example, different mammalian species have amounts of nuclear DNA which vary by no more than 10%[54]. The same is true of reptiles and of birds[54]. Thus the net gain (or loss) from DNA expansion (and contraction) must be similar in various mammalian species. Although non-homologous recombination could eliminate large blocks of useless genes, obviously little is known about the specific mechanisms which regulate the mammalian genome size.

The nature of multigene systems has been studied at the nucleotide level primarily using unfractionated DNAs from different species. The antibody system affords the first opportunity to examine individual gene products and to characterize at the protein level the evolution of a multigene system. Further characterization of immunoglobulins from different species should therefore place additional constraints on the mechanism responsible for the evolution of multigene systems.

Specific antibodies with molecular uniformity, generated in certain rabbits in response to immunization with groups A and C streptococci, are especially useful proteins for studying rabbit immunoglobulin structure, genetics, and evolution. The work reported here indicates that rabbit light chains can be divided into at least three discrete subgroups which differ in size at their amino terminus. It seems likely that each subgroup is encoded by at least one germ line gene. These rabbit light chains (b4 allotype) are clearly of the $\varkappa$ type and have evolved species specific residues in the intervening 75 million years since man and rabbit diverged. A mechanism of gene expansion seems to be necessary to account for this species specificity. Indeed, gene expansion seems to be generally required of many systems in eukaryotes (for example, ribosomal genes and mouse satellite DNA), and may be a fundamental mechanism of gene evolution in higher organisms.

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Variability in the Lambda Light Chain Sequences of Mouse Antibody

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The simple pattern of variability in mouse lambda chains suggests that diversity is generated by somatic spontaneous mutation and by sequential selection by antigen of single step mutants.

THE ratio of $\varkappa$ to λ light chains in serum immunoglobulin varies considerably among species[1]. For example, in cow[1] the ratio is about 0·1; in man[2] the ratio is 2, and in the BALB/c mouse it is between 10 and 20 (unpublished data of M. G. W. and W. Geckeler). The same ratios are found in the plasmacytomas which arise in man and mouse (ref. 2 and unpublished data of M. G. W., M. C. and J. Hirst), showing that individual cells of the normal plasmacyte population are transformed, at random, to the neoplastic state.

The expression of the λ class within a species might be the consequence of its potential to contribute to immunoglobulin diversity relative to that of the $\varkappa$ class. In our hypothesis the serum ratio of $\varkappa/\lambda$ is an approximate reflexion of the germ-line variable gene ratio, $V_\varkappa/V_\lambda$. But if there is a special mechanism for generating diversity, it might be postulated to be defective in diversifying $\varkappa$ in cow, to operate equally well on both $\varkappa$ and λ in man and to be defective in diversifying λ in mouse, and so on. Sequence comparisons of the variable part of the λ chain in mouse could tell us if such considerations were relevant. Some preliminary data on the similarity of two λ chains produced by plasmacytomas of BALB/c mice[3,4] encouraged us to carry out this detailed study which shows that λ chains do vary in sequence but, as expected, the pattern of variability is much simpler than that found in humans.

Results of the sequence analysis are shown in Fig. 1 and the methods are described in the legend. Six of the ten λ chains seem to be identical as far as residue 113—the length of the variable region; two differ from these by one residue (one base change); one by two residues (two base changes); and one by three residues (four base changes). These differences can be seen in Fig. 2. We maintain that the overlapping group of six λ chains is coded for by a unique germ line gene which is varied in a step-wise fashion because each single point mutation is acted on by antigenic selection.

There are three regions of high variability in human light chains. These regions are probably involved in determining the contribution of the light chain to the antibody combining site[5]. These three "specificity" regions, it should be emphasized, cover only about 20 per cent of the length of the variable region. The striking finding is that all the amino-acid replacements that we observed fall precisely into these specificity regions (Fig. 2), providing strong evidence that antigenic selection acts principally on point mutations in these three regions. The fact that one λ chain (S178) differs by three amino-acids (four mutational steps) from the overlap sequence suggests that this selection is sequential on single step mutants because the four mutations are also all in the specificity regions.

This result allows another point to be made, namely that the four variant λ sequences are derived from one germ line gene coding for the six overlap sequences. We postulate, on the basis of a germ line model, that the mouse has a family of duplicated V_λ genes of which 60 per cent are identical and 40 per cent are varied. This family must have arisen recently by extensive duplication of one V_λ gene (the overlap) followed by divergence and antigenic selection. It might also be argued that the 60 : 40 ratio is a reflexion not of the germ line gene ratio but of strong antigenic selection, were it not for the fact that the overlap λ chains are associated with immunoglobulins with the different specificities of their heavy chains (unpublished results of M. G. C., M. W. and J. Hirst). The problem of the germ line model is to account for a selection for replacements in the specificity regions without accumulating any replacements elsewhere. From the totality of human sequences roughly 20 per cent of the residues are invariant, 20 per cent are in specificity regions and 60 per cent are neutral or modulating. Invariant residues are preserved because mutations affecting them destroy antibody structure. The replacements in specificity regions are strongly selected for by antigen. This accounts for their high frequency. Replacements occurring elsewhere, however, must be unselected or weakly selected because, in part, they are neutral or effective only in concert with previous mutations in specificity regions. These neutral or modulating mutations outside of the specificity regions should be at least twice as frequent as those in specificity regions. In a germ line model, in which there are about 100 V_λ genes, the four mutations leading the three amino-acid replacements in the specificity regions of the S178 λ-chain would have had to be selected without any one of an expected eight or more neutral mutations occurring elsewhere in the postulated S178 germ line gene coding for the V region. This is unlikely. In a somatic model with sequential selection on single step mutants from one germ line V_λ gene, neutral mutations would be seen only very rarely in the initial stages of selection. This is why, for example, the constant region coded for by one germ line gene seems to be constant. Consequently, we take the overlap sequences to be coded for by a single V_λ germ line gene and only a somatic model will be considered from now on.

Our findings merit a comparison of the diversities within human and mouse V subgroups and a discussion of the distribution of the total subgroups between $\varkappa$ and λ in these two species. Human light chains within a subgroup show an average of thirteen amino-acid replacements in any two proteins compared with one in mouse λ. On purely *a priori* grounds a greater degree of diversity would be expected for humans than for inbred BALB/c mice. Aside from the problem of polymorphism, the human light chains are derived from individuals with a history of approximately 50 years of somatic diversification whereas the mouse light chains are derived from individuals of 0·5 years of somatic diversification. If we could study the amino-acid replacements in human light chains during the initial stages of diversification, the same

Tumour	1 5 10 15 20
S104	PCA-ALA(VAL,VAL,THR,GLX,GLX,SER,ALA,LEU)(THR,THR,SER,PRO,GLY,THR,GLX,VAL,THR,LEU)(THR,CYS)
XP8	PCA-ALA(VAL,VAL,THR,GLX,GLX,SER,ALA,LEU)(THR,THR,SER,PRO,GLY,THR,GLX,VAL,THR,LEU)(THR,CYS)
J698	PCA-ALA(VAL,VAL,THR,GLX,GLX,SER,ALA,LEU)(THR,THR,SER,PRO,GLY,THR,GLX,VAL,THR,LEU)(THR,CYS)
H2061	PCA-ALA(VAL,VAL,THR,GLX,GLX,SER,ALA,LEU)(THR,THR,SER,PRO,GLY,THR,GLX,VAL,THR,LEU)(THR,CYS)
J558	PCA-ALA(VAL,VAL,THR,GLX,GLX,SER,ALA,LEU)(THR,THR,SER,PRO,GLY,THR,GLX,VAL,THR,LEU)(THR,CYS)
HOPC-1	PCA-ALA(VAL,VAL,THR,GLX,GLX,SER,ALA,LEU)(THR,THR,SER,PRO,GLY,THR,GLX,VAL,THR,LEU)(THR,CYS)
RPC-20	PCA-ALA(VAL,VAL,THR,GLX,GLX,SER,ALA,LEU)(THR,THR,SER,PRO,GLY,THR,GLX,VAL,THR,LEU)(THR,CYS)
S176	PCA-ALA(VAL,VAL,THR,GLX,GLX,SER,ALA,LEU)(THR,THR,SER,PRO,GLY,THR,GLX,VAL,THR,LEU)(THR,CYS)
H2020	PCA-ALA(VAL,VAL,THR,GLX,GLX,SER,ALA,LEU)(THR,THR,SER,PRO,GLY,THR,GLX,VAL,THR,LEU)(THR,CYS)
S178	PCA-ALA(VAL,VAL,THR,GLX,GLX,SER,ALA,LEU)(THR,THR,SER,PRO,GLY,THR,GLX,VAL,THR,LEU)(THR,CYS)
	25 30 35 40
S104	ARG-SER-SER(THR,GLY,ALA,VAL,THR,THR,SER,ASX,TYR)(ALA,ASX,TRP)(VAL,GLX,GLX,LYS,PRO,ASX,HIS,
XP8	ARG-SER-SER(THR,GLY,ALA,VAL,THR,THR,SER,ASX,TYR)(ALA,ASX,TRP)(VAL,GLX,GLX,LYS,PRO,ASX,HIS,
J698	ARG-SER-SER(THR,GLY,ALA,VAL,THR,THR,SER,ASX,TYR)(ALA,ASX,TRP)(VAL,GLX,GLX,LYS,PRO,ASX,HIS,
H2061	ARG-SER-SER(THR,GLY,ALA,VAL,THR,THR,SER,ASX,TYR)(ALA,ASX,TRP)(VAL,GLX,GLX,LYS,PRO,ASX,HIS,
J558	ARG-SER-SER(THR,GLY,ALA,VAL,THR,THR,SER,ASX,TYR)(ALA,ASX,TRP)(VAL,GLX,GLX,LYS,PRO,ASX,HIS,
HOPC-1	ARG-SER-SER(THR,GLY,ALA,VAL,THR,THR,SER,ASX,TYR)(ALA,ASX,TRP)(VAL,GLX,GLX,LYS,PRO,ASX,HIS,
RPC-20	ARG-SER-SER(THR,GLY,ALA,VAL,THR,THR,SER,ASX,TYR)(ALA,ASX,TRP)(VAL,GLX,GLX,LYS,PRO,ASX,HIS,
S176	ARG-SER-ASN(THR,GLY,ALA,VAL,THR,THR,SER,ASX,TYR)(ALA,ASX,TRP)(VAL,GLX,GLX,LYS,PRO,ASX,HIS,
H2020	ARG-SER-THR(THR,GLY,ALA,VAL,THR,THR,GLY,ASX,TYR)(ALA,ASX,TRP)(VAL,GLX,GLX,LYS,PRO,ASX,HIS,
S178	ARG-SER-ASN-THR-GLY-ALA-VAL-THR-THR-SER-ASX-TYR (ALA,ASX,TRP)(VAL,GLX,GLX,LYS,PRO,ASX,HIS,
	45 50 55 60 65
S104	LEU,PHE)(THR,GLY,LEU,ILE,GLY,GLY,THR,ASX,ASX)ARG(ALA,PRO,GLY,VAL,PRO,ALA)ARG(PHE,SER,GLY,
XP8	LEU,PHE)(THR,GLY,LEU,ILE,GLY,GLY,THR,ASX,ASX)ARG(ALA,PRO,GLY,VAL,PRO,ALA)ARG(PHE,SER,GLY,
J698	LEU,PHE)(THR,GLY,LEU,ILE,GLY,GLY,THR,ASX,ASX)ARG(ALA,PRO,GLY,VAL,PRO,ALA)ARG(PHE,SER,GLY,
H2061	LEU,PHE)(THR,GLY,LEU,ILE,GLY,GLY,THR,ASX,ASX)ARG(ALA,PRO,GLY,VAL,PRO,ALA)ARG(PHE,SER,GLY,
J558	LEU,PHE)(THR,GLY,LEU,ILE,GLY,GLY,THR,ASX,ASX)ARG(ALA,PRO,GLY,VAL,PRO,ALA)ARG(PHE,SER,GLY,
HOPC-1	LEU,PHE)(THR,GLY,LEU,ILE,GLY,GLY,THR,ASX,ASX)ARG(ALA,PRO,GLY,VAL,PRO,ALA)ARG(PHE,SER,GLY,
RPC-20	LEU,PHE) THR-GLY-LEU-LEU(GLY,GLY,THR,ASX,ASX)ARG(ALA,PRO,GLY,VAL,PRO,ALA)ARG(PHE,SER,GLY,
S176	LEU,PHE)(THR,GLY,LEU,ILE,GLY,GLY,THR,ASX,ASX)ARG(ALA,PRO,GLY,VAL,PRO,ALA)ARG(PHE,SER,GLY,
H2020	LEU,PHE)(THR,GLY,LEU,ILE,GLY,GLY,THR,ASX,ASX)ARG(ALA,PRO,GLY,VAL,PRO,ALA)ARG(PHE,SER,GLY,
S178	LEU,PHE) THR-GLY-LEU-ILE-GLY-ASN-THR-ASX-ASX-ARG(ALA,PRO,GLY,VAL,PRO,ALA)ARG(PHE,SER,GLY,
	70 75 80 85
S104	SER,LEU,ILE,GLY,ASX)LYS(ALA,ALA,LEU)(THR,ILE,THR,GLY,ALA,GLX,THR,GLX,ASX,GLX,ALA,ILE,TYR)
XP8	SER,LEU,ILE,GLY,ASX)LYS(ALA,ALA,LEU)(THR,ILE,THR,GLY,ALA,GLX,THR,GLX,ASX,GLX,ALA,ILE,TYR)
J698	SER,LEU,ILE,GLY,ASX)LYS(ALA,ALA,LEU)(THR,ILE,THR,GLY,ALA,GLX,THR,GLX,ASX,GLX,ALA,ILE,TYR)
H2061	SER,LEU,ILE,GLY,ASX)LYS(ALA,ALA,LEU)(THR,ILE,THR,GLY,ALA,GLX,THR,GLX,ASX,GLX,ALA,ILE,TYR)
J558	SER,LEU,ILE,GLY,ASX)LYS(ALA,ALA,LEU)(THR,ILE,THR,GLY,ALA,GLX,THR,GLX,ASX,GLX,ALA,ILE,TYR)
HOPC-1	SER,LEU,ILE,GLY,ASX)LYS(ALA,ALA,LEU)(THR,ILE,THR,GLY,ALA,GLX,THR,GLX,ASX,GLX,ALA,ILE,TYR)
RPC-20	SER,LEU,ILE,GLY,ASX)LYS(ALA,ALA,LEU)(THR,ILE,THR,GLY,ALA,GLX,THR,GLX,ASX,GLX,ALA,ILE,TYR)
S176	SER,LEU,ILE,GLY,ASX)LYS(ALA,ALA,LEU)(THR,ILE,THR,GLY,ALA,GLX,THR,GLX,ASX,GLX,ALA,ILE,TYR)
H2020	SER,LEU,ILE,GLY,ASX)LYS(ALA,ALA,LEU)(THR,ILE,THR,GLY,ALA,GLX,THR,GLX,ASX,GLX,ALA,ILE,TYR)
S178	SER,LEU,ILE,GLY,ASX)LYS(ALA,ALA,LEU)(THR,ILE,THR,GLY,ALA,GLX,THR,GLX,ASX,GLX,ALA,ILE,TYR)
	90 95 100 105 110
S104	PHE-CYS(ALA,LEU,TRP)(TYR,SER,ASX,HIS,TRP)(VAL,PHE,GLY,GLY,GLY,THR)LYS(LEU,THR,VAL,LEU)(GLY,
XP8	PHE-CYS(ALA,LEU,TRP)(TYR,SER,ASX,HIS,TRP)(VAL,PHE,GLY,GLY,GLY,THR)LYS(LEU,THR,VAL,LEU)(GLY,
J698	PHE-CYS(ALA,LEU,TRP)(TYR,SER,ASX,HIS,TRP)(VAL,PHE,GLY,GLY,GLY,THR)LYS(LEU,THR,VAL,LEU)(GLY,
H2061	PHE-CYS(ALA,LEU,TRP)(TYR,SER,ASX,HIS,TRP)(VAL,PHE,GLY,GLY,GLY,THR)LYS(LEU,THR,VAL,LEU)(GLY,
J558	PHE-CYS(ALA,LEU,TRP)(TYR,SER,ASX,HIS,TRP)(VAL,PHE,GLY,GLY,GLY,THR)LYS(LEU,THR,VAL,LEU)(GLY,
HOPC-1	PHE-CYS(ALA,LEU,TRP)(TYR,SER,ASX,HIS,TRP)(VAL,PHE,GLY,GLY,GLY,THR)LYS(LEU,THR,VAL,LEU)(GLY,
RPC-20	PHE-CYS(ALA,LEU,TRP)(TYR,SER,ASX,HIS,TRP)(VAL,PHE,GLY,GLY,GLY,THR)LYS(LEU,THR,VAL,LEU)(GLY,
S176	PHE-CYS(ALA,LEU,TRP)(TYR,SER,ASX,HIS,TRP)(VAL,PHE,GLY,GLY,GLY,THR)LYS(LEU,THR,VAL,LEU)(GLY,
H2020	PHE-CYS(ALA,LEU,TRP)(TYR,SER,ASX,HIS,TRP)(VAL,PHE,GLY,GLY,GLY,THR)LYS(LEU,THR,VAL,LEU)(GLY,
S178	PHE-CYS(ALA,LEU,TRP)(TYR,SER,ASX)ARG-TRP (VAL,PHE,GLY,GLY,GLY,THR)LYS(LEU,THR,VAL,LEU)(GLY,

S104	GLX,PRO)LYS
XP8	GLX,PRO)LYS
J698	GLX,PRO)LYS
H2061	GLX,PRO)LYS
J558	GLX,PRO)LYS
HOPC-1	GLX,PRO)LYS
RPC-20	GLX,PRO)LYS
S176	GLX,PRO)LYS
H2020	GLX,PRO)LYS
S178	GLX,PRO)LYS

Fig. 1. Partial sequence of mouse λ chains. Two of the light chains in this comparison are produced by tumours donated by Drs M. Potter and K. R. McIntire, NIH, and designated RPC-20, HOPC-1, and MOPC-104E. Tumour XP8 is a variant of tumour MOPC-104E (ref. 8). S104, S178, J698, H2020, H2061, J558 and S176 are λ chain producing tumours induced in our laboratory. Tumours J698, H2020, H2061 and J558 produce myeloma proteins of the γA type. These proteins were isolated from the sera of tumour-bearing mice[9], and the light chains were isolated from these proteins[10]. RPC-20 and S178 secrete exclusively λ chain which was isolated from the urine of female BALB/c mice bearing these tumours[9]. Tumours S104, HOPC-1 and XP8 produce myeloma proteins of the γA, γG2a, and γM classes respectively as well as urinary λ chains. In these cases light chain was isolated from the urine. The purified light chains were aminoethylated[11] and oxidized with performic acid[12]. The tryptic peptides from the first 113 residues were isolated and further digested with either thermolysin or chymotrypsin. These peptides were separated and analysed as previously described[13] with the modification that peptides were eluted and hydrolysed in 6 M HCl in 4 per cent thioglycolic acid[14]. Certain of the peptides from each protein resulting from these digestions are shown. For certain of these peptides part of the sequence of amino-acids has been determined by the subtractive Edman procedure[15] after elution from the fingerprint paper with 50 per cent pyridine. The positions of these peptides in the sequence were determined by their homology with the peptides of the S178 protein for which the order of peptides has been determined, and the amino-acids in each peptide were aligned by homology with what we believe to be the sequence of the S178 protein, with the exception of the sequence between residues 3–10 and 41–43 (I. C., and M. G. W., manuscript in preparation). The sequence 3–10 is aligned according to ref. 4; 41–43 is still in doubt. Portions for which the exact sequence has been determined by the subtractive Edman procedure or is inferred from the known action of the enzymes used for digestion of the S-aminoethylated protein are placed outside the brackets. The position of the tryptic peptide comprising the first twenty-three residues of these proteins was identical in fingerprints of tryptic digests of performic acid oxidized protein. Because this peptide did not react with the ninhydrin reagent, it was detected using an arginine specific stain[16]. The peptide could be located on one dimensional electrophoresis by staining guide strips for arginine containing peptides[16]. The peptide was eluted from the unstained portion of the paper and analysed for amino-acid composition or digested with thermolysin. The peptides resulting from thermolysin digestion were separated by one dimensional electrophoresis and guide strips were stained with ninhydrin and by chlorination[17]. One ninhydrin-negative peptide, detected by chlorination, had the composition Glu-Ala. It was assumed that this peptide represented the first two residues of the λ chains, and that the first residue of the λ chains was pyrrolidone carboxylic acid. The compositions of the peptides were determined after acid hydrolysis, so that it is not possible to distinguish between glutamine and glutamic acid or asparagine and aspartic acid. But because all the peptides had identical electrophoretic mobilities it is unlikely that there are differences involving these amino-acids. It is inferred that the residue at position 25 and 52 in the S178 protein and at 25 in the S176 protein is asparagine rather than aspartic acid, for the electrophoretic mobility of the peptides containing this residue is identical to the corresponding peptides from other proteins in which either a glycine or serine replacement occurs.

characteristically simple pattern seen for mice should emerge. But because the available human light chains are the products of genes which have undergone many sequential steps of somatic antigenic selection, the simple pattern is camouflaged. It can be revealed, however, by statistical analyses of the kind illustrated in Fig. 2 (ref. 5). Extensive sequential selection leads eventually to a steady state of diversification in which the rate at which new mutations creating additional specificities are fixed by antigenic selection equals the rate at which already acquired specificities are lost. This steady state population can accumulate both neutral and modulating replacements outside the critical regions. The steady state in humans seems to occur when the average number of amino-acid differences between two random chains in a subgroup is about thirteen. This implies an average of six mutational steps from the germ line gene. The BALB/c mouse is far from this steady state level although in principle, given a long enough life span during which antigenic selection could act, it too would approach this value of six. At the extreme we have seen that BALB/c mice can express variant λ chains such as S178 which are four mutational steps away from the germ-line gene, a value close to the average for humans. We conclude that the rate at which diversity is being generated in mouse λ is not particularly low.

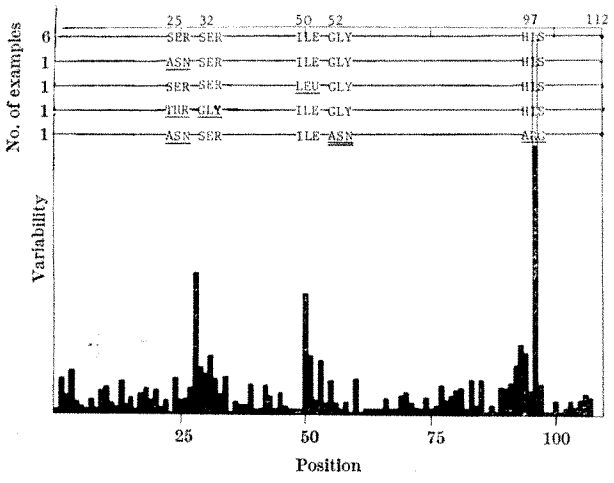


Fig. 2. Diagram of mouse λ sequences superimposed on a plot of the extent of variability versus position in the variable region sequences of human light chains, κ and λ. The portions of the mouse λ proteins in which no differences could be detected are indicated by a line. The positions at which differences were found are indicated by the residues involved. The underlining indicates the number of base changes needed to get the replacement. The plot is taken from ref. 5.

Now we shall look at the distribution of V subgroups in man and mouse. In man four or five V_κ and five V_λ subgroups are known[6] so that the ratio of germ line genes for V_κ/V_λ is one. In the BALB/c mouse there are (by our interpretation of the data) about twenty V_κ subgroups[7]. The V_κ/V_λ ratio is therefore twenty, for only one subgroup is found to be associated with the BALB/c λ chain which is defined by its C terminal peptide . . . Ala-Asp-Cys-Ser (ref. 3 and unpublished data of M. G. W. and W. Geckeler).

It seems likely to us that the wide range in serum ratios of κ/λ in different species is a reflexion of the germ line gene ratios V_κ/V_λ. Were selection by the random population of antigens to act equally well on all V subgroup genes then the serum ratio of κ/λ in man should be one and in mouse twenty, a value close to that found. If the κ/λ ratios in serum were explicable in terms of a special mechanism operating with differential effectiveness on V_κ and V_λ genes, in man V_κ and V_λ would be diversified with equal effectiveness whereas in mouse only V_κ would be diversified. The variation that we have observed in mouse V_λ would be due to spontaneous mutation. In the mouse the germ line V gene ratio reflects the serum ratio of κ/λ, so we conclude that the postulated special mechanism is no more effective than spontaneous mutation in generating diversity. Consequently, postulates of special mechanism for diversification by hypermutation hyperrecombination are gratuitous. Clearly, it is more reasonable to suppose that the total of about ten to twenty V subgroups seen for mouse or human light chains is the minimum needed to prime the process of diversification rapidly. This suggests that the variation within a mouse κ chain subgroup will also follow a pattern of variability as simple as that found in mouse λ chains.

Let us assume that the distribution of mutations which are fixed by antigenic selection in λ chains is random and follows a Poisson distribution. From the fact that six out of ten λ chains are the expression of a germ line gene, we can calculate that there is an average of 0·5 mutation per chain. The distribution, therefore, is 60 per cent with no mutations, 30 per cent with one mutation, 8 per cent with two mutations, 1 per cent with three mutations and 0·2 per cent with four mutations. Because a mutation that is fixed by antigenic selection implies an amino-acid replacement, 40 per cent of the total λ chains will be variant of which 75 per cent (30/40) will have only one replacement. Single replacements therefore account for most of the diversity. Because these antigenically selected single replacements are limited to the specificity regions, there will be in a mouse only about fifty different functional λ chains. If every germ line V gene for the light and heavy chain is diversified to the same extent and if there are a total of twenty germ line V genes for light and five for heavy chain then the 0·5 year old mouse (the age at which plasmacytomas were obtained) can make 10^5 different antibodies solely as a consequence of single mutations. If the rate of increase in diversity were linear (the crudest assumption), then a 1 week old mouse could express 5,000 different antibodies. This would tax the immunologist's ability to detect any missing specificities.

Consequently, our interpretation of these data is that the six identical λ chains are coded by one germ line V_λ gene which is diversified somatically by spontaneous mutation to yield the four variants. The first single step mutants selected for by antigen are those which occur in the three critical regions responsible for the specificity. The process of selection is sequential on each additional mutation conferring a new specificity.

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Reactivity of Inbred Mouse Sera with Antisera to the Variable Region of Light Chains

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The presence of common antigenic determinants in a panel of human and mouse Bence–Jones proteins and a human myeloma protein has facilitated a study of the genetic control of immunoglobulin synthesis in mice.

LIGHT chains of immunoglobulins have a variable and a common region, corresponding respectively to the N-terminal and C-terminal half of the amino-acid sequence[1]. Subdivision of light chains into K type and L type is based on the sequence of the common region[1]. One structural gene codes for the common region of K type proteins in man, whereas two genes code for the common region of L type proteins[2–4]. The number of genes that code for the variable regions of light chains in the genome of each individual has not yet been determined.

In human Bence–Jones proteins there is a relatively small number of basic sequences for the variable region of light chains[5,6]. The other known sequences can be derived from the basic sequences by substitution of a few amino-acids[5,6]. Light chains related to the same basic sequence are thought to belong to a subgroup, three of which have so far been identified for K type proteins and four or possibly five for L type proteins[1]. Subgroups have also been identified for K type mouse Bence–Jones proteins[7,8].

The genetic control of the structure of light chains has not been explained. It is not known whether there is a single structural gene, coding for each basic sequence and subject to somatic variability generating processes, or whether there are as many structural genes as there are variable sequences.

We report here a study on the genetic control of the variable region of light chains in inbred mouse strains. Because it is impossible to isolate single molecular species from the immunoglobulins of an individual, unless the individual has been immunized in such a way as to produce a monoclonal antibody, a method was devised to identify and estimate antigenic determinants of immunoglobulins by means of serological reactions carried out with unfractionated serum. The method used to estimate an antigenic determinant was the inhibition of precipitation of an iodinated antigen[9]. For example, in the precipitin reaction defining determinant *a* we used an antiserum prepared against the fragment[10] of human Bence–Jones protein 27 (BJ 27), which corresponds to the N-terminal half[11]. (Human Bence–Jones proteins and protein fragments were obtained from sources reported in ref. 10.) To increase its specificity, we absorbed the antiserum (anti-BJ 27f) with a different Bence–Jones protein (BJ 79) conjugated to bromoacetyl-cellulose[12] and tested sera or immunoglobulins for inhibition of the precipitin reaction anti-BJ 27f/[^{125}I]BJ 27, as described in detail previously[9]. Antisera prepared in different ways were used to define two other antigenic determinants of light chains, called *b* and *c*[9]. Details of the experimental conditions are given in the legend to Table 1.

The presence of antigenic determinants *a*, *b* and *c* in a panel of human and mouse Bence–Jones proteins and one mouse myeloma protein has been investigated[9]. Some proteins inhibited the precipitin reactions, whereas others of the same type did not. This was taken as an indication that the antigenic determinants were present in the variable region of cross-reactive human and mouse light chains (see ref. 9). These antigenic determinants were not species-specific but were present in the light chains of man, mice, rat, pig, horse, sheep and cattle; they were not present in the heavy chains of these species nor in chicken immunoglobulins[9].

Table 1. DISTRIBUTION OF DETERMINANTS *a*, *b* AND *c* IN A GROUP OF HUMAN AND MOUSE BENCE–JONES PROTEINS AND IN A MOUSE MYELOMA IMMUNOGLOBULIN

	Determinants			
Human Bence–Jones proteins	*a*	*b*	*c*	Type
BJ 27	+	+	−	L
BJ 27f	+	+	−	L
BJ 79	−	+	−	L
BJ 99	−	+	+	L
BJ 10	−	+	−	L
BJ 1	+	+	+	L
BJ 98	−	+	−	L
Mouse Bence–Jones proteins				
MOPC 41 A	+	+	+	K
MOPC 46 B	−	−	−	K
MOPC 29 A	−	−	−	K
MOPC 197 A	−	−	−	K
MOPC 30 A	−	−	−	K
RPC 5 (IgG)	+	+	+	K
MOPC 20	−	−	−	L

The antigenic determinants *a*, *b* and *c* are defined by precipitin reactions of antisera directed against the variable region of human L type Bence–Jones proteins with BJ 27, BJ 79 and BJ 99 respectively. The preparation of the antisera and their fractionation have been described[9]. The antisera were fractionated so as to render them specific for a single antigenic determinant (or a group of determinants present together on the same protein). Ten μg/ml. of each iodinated Bence–Jones protein used as antigen were incubated for 1 h at 37° C with the corresponding antiserum at equivalence in the presence of 5 to 100 μg/ml. of proteins tested as inhibitors. The equivalence zone was determined for each precipitin reaction as previously described[9]. The incubation mixtures were then placed for 2 days at 4° C to ensure maximal precipitation and then filtered on Whatman GF glass filters and counted as previously described[9]. The table summarizes the results of inhibition of precipitation reactions: + indicates an inhibition of more than 80%; −, an inhibition of less than 20%. Controls were run by substituting each antiserum with serum of a non-immunized rabbit.

To study the genetic control of variable regions of light chains, we have analysed pooled sera of inbred mice and sera obtained from individual mice. Pooled sera and mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. To establish that determinant *a* could be identified in serum immunoglobulins and that a reliable estimate of the fraction of light chains carrying determinant *a* could be obtained, the following preliminary experiments were carried out. Mouse Bence–Jones protein[10] MOPC 41, IgG myeloma protein MOPC 5, pooled IgG (purchased from Pentex), light chains prepared from pooled IgG[13], and pooled serum of non-inbred Swiss mice were used as inhibitors of the precipitin reaction defining determinant *a*. It was found that the cross-reacting mouse Bence–Jones protein MOPC 41 competed with BJ 27 with similar affinity for the antiserum. From the inhibition curves reproduced in Fig. 1 it was

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calculated that 5·5 μg of MOPC 41 gave 50% inhibition of precipitation in the presence of 5 μg of [^{125}I]BJ 27.

Light chains in IgG molecules inhibited the precipitin reaction with approximately the same efficiency as free light chains; 14 μg of the cross-reacting myeloma immunoglobulin RPC 5 gave 50% inhibition of the precipitin reaction defining determinant *a*. Similarly the amount of pooled light chains and pooled IgG required to obtain 50% inhibition of the precipitin reaction were determined to be respectively 62 and 220 μg. In these reactions the amount of [^{125}I]BJ 27 present in the incubation mixture was 10 μg (see legend to Table 1), so the calculated fraction of light chains carrying determinant *a* represented approximately 14% of the pooled light chains. An independent estimate of the amount of light chains carrying determinant *a* was obtained by analysing mouse serum in the same way. The pooled serum of non-inbred Swiss mice used in this experiment contained 6 mg/ml. of immunoglobulin. From the inhibition curve it was calculated that 36 μl. of this serum, containing 216 μg of immunoglobulin, were required for 50% inhibition of the precipitin reaction. This was very close to the amount of pooled IgG required for 50% inhibition, 220 μg.

The results of the analysis of sera of inbred mice are reported in Table 2. The number of μl. of serum required to obtain a 50% inhibition of the precipitin reaction identifying determinant *a* were calculated from the inhibition curves obtained in three independent experiments; the values obtained were within a ±10% range. Very similar values were obtained for eighteen of the nineteen sera examined. The amount of serum of ST/bJ mice required for 50% inhibition was about seven times lower than that of other mice, indicating that a much larger fraction of the light chains of ST/bJ mice carried determinant *a*.

Differences in the inhibitory titre of serum of mouse strains could be the result of different levels of serum immunoglobulins. To test this, the immunoglobulin level in sera of inbred mouse strains was determined by measuring the inhibition of precipitation of [^{125}I]IgG with anti-mouse IgG. Inhibition curves were obtained for each serum as already described[9] and the amount of serum required for 50% inhibition of the precipitin reaction was calculated (Table 2): the differences in IgG level were relatively small. Taking into account these differences, it was calculated that for fifteen of the sixteen strains examined, the fraction of light chains carrying determinant *a* varied between 11% (SWR/J) and 15% (BALB/cJ). In ST/bJ mice this fraction of light chains represented 80% of the total light chains.

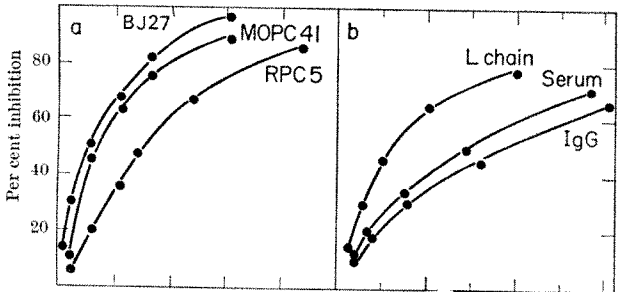


Fig. 1. Inhibition curves of the precipitin reaction anti-BJ 27f/[^{125}I] BJ 27. (This precipitin reaction defines determinant *a*).

a, Inhibition curves of BJ 27, a human Bence–Jones protein, of MOPC 41, a mouse Bence–Jones protein, and of RFC 5, a mouse IgG myeloma protein[10]. The conditions of the experiment are as described in the legend to Table 1. *b*, Inhibition curves of mouse pooled IgG (Pentex), mouse pooled light chains, and mouse pooled serum. The conditions of the experiment are as described, but antigen was used at a concentration of 200 μg/ml. and the antiserum was undiluted. The concentration of immunoglobulins used as inhibitors is indicated; for the experiment in which serum was used, the number of μg of immunoglobulin present in serum are indicated.

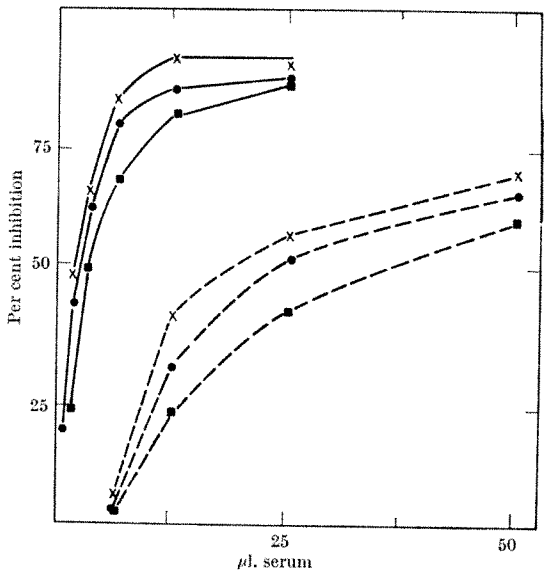


Fig. 2. Inhibition curves of the precipitin reactions defining determinant *a*. Pooled sera of ST/bJ and C57BL/6J mice were used as inhibitors of these reactions. The conditions of the experiment are those described in Table 1. The antisera were used undiluted. The concentrations of the iodinated antigens were; for the reaction defining determinant *a* 100 μg/ml.; for the reaction defining determinant *b* 150 μg/ml., and for the reaction defining determinant *c* 200 μg/ml. ———, ST/bJ serum; - - - -, C57BL/6J serum; ×, inhibition curves of the reaction defining determinant *a*; ●, *b*; and ■, *c*.

The sera of twenty-one C57BL/6J mice and of seven ST/bJ mice were individually examined for inhibition of the precipitin reaction defining determinant *a*. The number of μl. of serum required for 50% inhibition was calculated as described; they were found to range between 16 and 27 for C57BL/6J mice (average value 22·61) and between 1·4 and 2·5 for ST/bJ mice (average value 2·25). Thus all the sera examined contained a fraction of immunoglobulins carrying determinant *a* and this fraction is remarkably constant in individuals of the same inbred strain.

We have previously shown that determinants *a*, *b* and *c* are present together in the mouse Bence–Jones

Table 2. DISTRIBUTION OF ANTIGENIC DETERMINANT *a* AND QUANTITATIVE ESTIMATION OF IgG IN SERA OF INBRED MOUSE STRAINS

Strain	μl. of serum required for 50% inhibition of precipitin reaction	
	Determinant *a*	IgG
DBA/1J	24	21
DBA/2J	21	20
CBA/cdJ	21	21
BALB/cJ	24	33
C3H/HeJ	24	22
C3HBe/FeJ	21	26
C57BL/6J	22	23
C57BL/10Sn	24	20
C57BL/KsJ	22	25
LP/J	25	32
CE/J	23	30
RF/J	24	20
129/J	23	24
SM/J	24	29
SWR/J	19	20
ST/bJ	2, 2	15

The data were obtained from inhibition of precipitation of the reactions anti-BJ 27f/[^{125}I]BJ 27 and anti-Ig$_{mouse}$/[^{125}I]IgG$_{mouse}$. Mouse sera of the strains listed were used as inhibitors of these reactions. [^{125}I]BJ 27 was used at a concentration of 100 μg/ml., and dissolved in borate–saline buffer (*p*H 8·4). 50 μl. of this solution were added to 50 μl. of anti-BJ 27f diluted 1 : 2. These concentrations were found to correspond to equivalence for the precipitin reaction. After incubation for 1 h at 37° C 50 μl. of mouse serum diluted at different concentrations with borate–saline buffer were added and the incubation at 37° C was continued for one additional hour. The samples were then placed at 4° C for 24 h and filtered afterwards through Whatman GF/A filters[9]. The radioactivity retained on filters was then determined as previously described[9]. Inhibition curves were drawn for each experiment and the amount of serum required to obtain 50% inhibition of precipitation determined from these curves. The values reported are the averages of three independent determinations. The values obtained were in all cases within a ±10% range. [^{125}I]IgG$_{mouse}$ was used at a concentration of 2·5 mg/ml. and dissolved in borate–saline buffer (*p*H 8·4). 50 μl. of this solution were added to 50 μl. of undiluted anti-IgG$_{mouse}$ obtained from Pentex. The inhibition reaction was performed as described.

proteins or myeloma proteins examined and are also very frequently present together in the same molecules of pooled light chains[9]. The reasons for this behaviour of these antigenic determinants are not clear, but we have suggested that they are in some way related[9]. To confirm the results obtained with determinant *a*, pooled sera of ST/bJ and C57BL/6J mice were examined for inhibition of the precipitin reactions defining determinants *b* and *c*. The antisera and antigens used in these precipitin reactions have been described[9]. The inhibition curves obtained are shown in Fig. 2. The amount of serum required for 50% inhibition of the precipitin reactions defining determinants *b* and *c* were 2·3 and 3·3 μl. respectively for ST/bJ serum and 25 and 38 μl. respectively for C57BL/6J serum. This confirmed that these antigenic determinants are present in proportionally similar amounts in different sera and are thus presumably present in the same light chain molecules.

Our findings indicate that the relative amount of light chains carrying determinant *a* is remarkably constant in different inbred strains of mice. Only in ST/bJ mice is the fraction of light chains carrying determinant *a* much greater than in the other strains: this is difficult to interpret without further information on the structure of light chains in ST/bJ and in the other mouse strains.

It seems likely, however, that the relative proportion of light chains carrying determinant *a* is under genetic control. Crosses of ST/bJ mice with other inbred mice and a study of the offspring might help to establish this point. We hope that it will be possible in this way to gain information which will support or disprove some of the theories that have been suggested to explain the variability in amino-acid sequence of antibody peptide chains. Among the mechanisms that have been suggested are recombination between a small number of genes coding for the variable region[14] and somatic hypermutation of a small number of structural genes[15]. Both mechanisms presuppose the existence of a small number of genes only, coding for the variable region of light chains; it may well be possible that by studying inbred mouse strains we could establish the existence and learn about the inheritance of these hypothetical genes.

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DNA Polymerase II

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A membrane associated DNA polymerizing enzyme has been solubilized, partially isolated and characterized.

In a cell free extract of *Escherichia coli pol A*⁻ (DNA polymerase negative) mutant cells[1] most of the residual DNA synthesizing activity is associated with a fast sedimenting cellular component, most probably the cell membrane[2]. This complex can perform several functions characteristic of DNA replication and produces new DNA semiconservatively at a polymerization rate close to that observed for DNA replication *in vivo*[3]. A bothersome feature of the membrane system, however, is its brief active period; DNA is replicated for only a few minutes. I shall describe briefly in this article a procedure to solubilize and characterize an enzyme associated with this system. This released enzyme will be called DNA polymerase II, and the enzyme described by A. Kornberg[4], DNA polymerase I.

In a series of pilot experiments I found that non-ionic detergents solubilize an active deoxynucleoside triphosphate polymerizing activity from the fast sedimenting cellular structure. Fig. 1*a* shows that the membrane structure identified by the radioactive label in its lipid component (extractable by chloroform–ether) bands as a rather diffuse peak in the sucrose gradient and carries nearly all the DNA synthesizing activity. After treatment with the detergent most of the DNA synthesizing activity is found floating on top of the gradient, probably in a molecular form too small to sediment far during the relatively short centrifugation period. This and similar experiments encouraged me to try the isolation of the solubilized incorporating activity. Table 1 shows the procedure used. Other detergents like sodium deoxycholate were also successfully used in removing an active DNA synthesizing activity from the membrane; a prolonged incubation in 0·01 M EDTA was less effective.

Properties of the Solubilized Enzyme

The molecular weight of DNA polymerase II was estimated to be in the range of 60,000—90,000[7] by gel filtration on 'Sephadex G-200'; this shows that the enzyme is indeed released from larger cellular structural components. The optimal conditions for enzyme activity are *p*H 7·6 (*cf.* the membrane system's *p*H optimum is *p*H 7·0), 0·008 M $MgSO_4$, 0·05 M KCl. The incorporation of 3H-TTP into acid precipitable deoxyribonuclease sensitive material (Fig. 2) proceeds linearly for at least 90 min in marked contrast to the enzyme *in situ* where deoxynucleoside triphosphates are polymerized for only a few minutes.

Table 2 summarizes further properties of the enzyme. Because an excess of unlabelled TTP rather than TDP (or TMP) depresses incorporation of radioactive ^{3}H-TTP

into acid precipitable material, I conclude that the triphosphates are the precursors for the polynucleotide synthesized. The enzyme is strongly inhibited by mercuri-compounds, like the intact membrane system but unlike DNA polymerase I. The enzyme activity is resistant to preincubation with an antiserum against DNA polymerase (prepared by Dr T. Jovin and made available to me by Dr H. Schaller); the activity of DNA polymerase I, incubated under the same conditions with the antiserum, decreases to 10 per cent of a control value.

Table 3 shows that the activity depends on an external DNA primer (unlike the membrane system which seems to use exclusively the cell DNA attached to it). Best primers are ultrasonically sheared DNA pieces with molecular weights around 1–2 million. Fragments of *E. coli* DNA, calf thymus DNA and T7-DNA, when offered in equal amounts, possess the same priming activity.

Single stranded DNA, extracted from phages fd or φX 174, or produced by heat denaturation of *E. coli* and calf thymus DNA fragments, are not accepted as primers for DNA polymerase II. Supercoiled φX-RF (the double stranded form of circular φX DNA) also does not prime the DNA polymerase II reaction; introduction of several endonucleolytic nicks (by pancreatic deoxyribonuclease) converted the φX-RF into a good primer for the DNA polymerase I but not for the DNA polymerase II. High molecular weight *E. coli* DNA can be converted into an effective primer of the DNA polymerase II by pretreatment with exonuclease III[8], an enzyme which attacks double stranded DNA at its 3′-ends. The ^{3}H-labelled DNA synthesized on such a primer DNA is double stranded as judged from its buoyant density in CsCl equilibrium gradients and its inability to adsorb to nitrocellulose filters at high ionic strength. Such a pretreated DNA

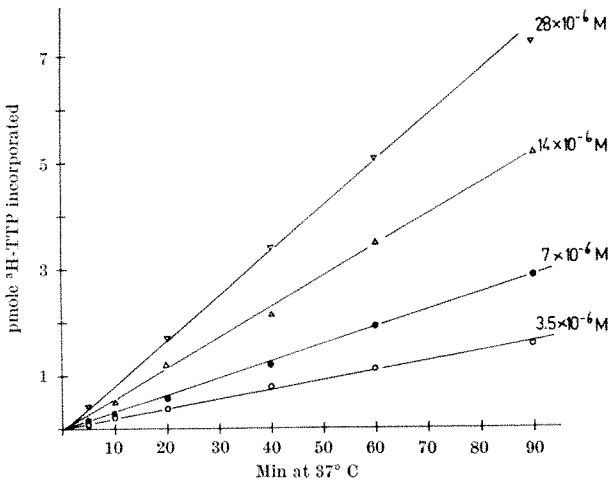


Fig. 2. Kinetics of incorporation. Reaction mixtures: the buffer was 0·08 M KCl–0·05 M Tris-HCl (*p*H 7·6)–0·007 M $MgCl_2$–0·005 M mercapto-ethanol; 12 μg sheared calf thymus DNA; about 0·9 μg of the enzyme preparation; the four dNTPs in concentrations given on the graph; specific activity of ^{3}H-TTP is 10 μCi/μmole. The final volume was 0·7 ml. Samples (0·1 ml.) were withdrawn from the reaction mixture and diluted into cold 0·9 ml. 0·01 M EDTA. The DNA was then precipitated with trichloroacetic acid on 'Millipore' filters and radioactivity was counted.

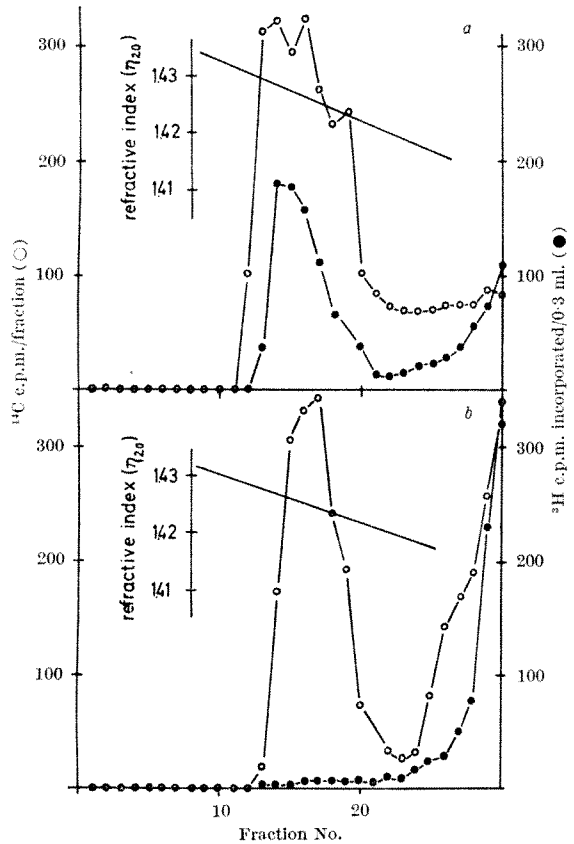


Fig. 1. Effect of detergents on *E. coli* membrane preparations. An oleic acid requiring derivative of the strain *E. coli* H 560 (DNA polymerase negative, endonuclease I negative; constructed by Dr Hoffmann-Berling by a cross between the Cairns–de Lucia mutant and an endonuclease I⁻-donor strain) was grown in a synthetic medium[5] supplemented with 0·1 μg/ml. ^{14}C-oleic acid (0·5 μCi/μmole). The harvested cells were treated with lysozyme in a 20 per cent sucrose solution buffered by 0·05 M MOPS (morpholinopropane sulphonic acid, adjusted to *p*H 7·2)–0·005 M EGTA and lysed with the nonionic detergent 'Brij 58'[9]. The resulting viscous cell lysate was briefly sonicated and the membrane fraction prepared by sedimentation through a sucrose gradient as described[2], treated for 2–3 s with a sonifier and dialysed. One part (*b*) was treated with 'Triton X-100' (an anionic detergent: alkylphenoxy-polyethoxyethanol which is currently used in many laboratories to solubilize bound proteins[14,15]) at an approximate detergent : protein ratio of 0·5; the other part served as control. Both lysates were then centrifuged through 26 ml. preformed linear 60–40 per cent sucrose gradients buffered in 0·1 M Tris-HCl (*p*H 7·6)–0·005 M mercaptoethanol–20 per cent glycerol. The 'SW 27' rotor of the Spinco centrifuge was used for 16 h at 25,000 r.p.m. and 6° C. 0·3 ml. samples were removed from each fraction, supplemented with $MgSO_4$ (final concentration 0·005 M), with the four deoxynucleoside triphosphates (2×10^{-5} M each; ^{3}H-TTP 50 μCi/μmole) and 4 μg ultrasonically sheared calf thymus DNA. Incubation was at 37° C for 30 min. The refractive index indicates the density increment in the gradients. *a*, Untreated control sample; (*b*) 'Triton X-100' treated membrane preparation.

Table 1. THE ISOLATION PROCEDURE

	Vol. (ml.)	Protein conc. (mg/ml.)	Enzyme units/ml.	Specific activity (units/mg protein)	Total activity
Crude extract	300	11	(3·1)	(0·28)	(930)
"Membrane fraction"	68	10·5	(10·4)	(1·0)	(710)
'Triton X-100' supernatant	86	2·1	7·6	3·6	655
DEAE-cellulose eluate	53	0·2	5·7	27·5	302
DNA column eluate	12	0·06	8·1	135·0	98

About 20 g *E. coli* H 560 cells were washed twice and resuspended in 150 ml. of a 20 per cent sucrose solution buffered by 0·05 M MOPS–0·005 M EGTA (*p*H 7·2). Lysozyme (30 mg) was then added, and the cell suspension stirred overnight in the cold room. 'Brij 58' (2 per cent in 150 ml. water) was added together with $MgSO_4$ (final concentration: 0·002 M). The resulting viscous lysate was treated for 10–20 s with a sonifier. The cells' débris was sedimented at 30,000*g* for 20 min, resuspended in 50 ml. of the MOPS-EGTA buffer and again pelleted. The washed débris was then homogenized in 50 ml. standard buffer (0·05 M Tris–HCl–0·001 M EDTA–0·01 M mercapto-ethanol, *p*H 7·6). This suspension was slowly stirred in the cold room after addition of enough 'Triton X-100' to give a detergent/protein ratio of about 0·25. After 40–60 min the insoluble material was removed by centrifugation (100,000*g*; 30 min). Most of the DNA polymerizing activity (70–90 per cent) was found in the supernatant. This fraction was applied to a DEAE-cellulose column (5 cm × 10 cm) previously equilibrated to standard buffer. The column was washed with 0·25 M NaCl in standard buffer and the enzymatic activity eluted with 0·35 M NaCl. The most active fractions of the DEAE-cellulose eluate were combined, dialysed against standard buffer and adsorbed to a DNA column. In this column circular single stranded phage fd DNA is immobilized in agar beads (1 mg fd DNA/g wet agar; unpublished results of Bonhoeffer and Schaller). More than 95 per cent of the protein applied to the column were not adsorbed or can be washed off with standard buffer. The remaining proteins including DNA polymerase II were eluted by a salt gradient. For unknown reasons 60–70 per cent of the enzymatic activity applied to the column are not recovered in the eluate. Experiments with the DEAE-cellulose enzyme fraction gave essentially the same results as those reported below for the DNA column enzyme fraction.

Enzyme assay. Total volume of 0·2 ml. contained: 0·05 M Tris-HCl (*p*H 7·6), 0·005 M mercaptoethanol, 0·008 $MgSO_4$, 2×10^{-5} M each of dATP, dGTP, dCTP and ^{3}H-dTTP (100 μCi/μmole). The mixture was incubated at 37° C for 30 min. Because the membrane associated enzyme is active for only a few minutes, no precise incorporating activities can be given for the crude extract and the membrane fraction. The data shown are based on the total incorporated radioactivity during the lifetime of the membrane system. One enzyme unit is defined as the activity which incorporates 1 nmole ^{3}H-TTP into acid precipitable material (in 30 min at 37° C).

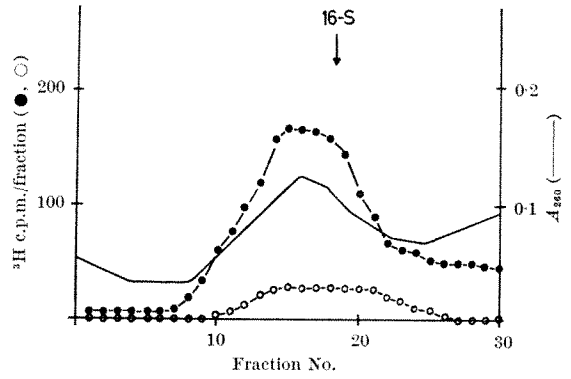


Fig. 3. Alkaline sucrose gradient analysis. About 0·8 mg *E. coli* DNA were treated with 10 units[8] exonuclease III for 45 min at 37° C. The DNA solution was then mixed with an equal volume of water saturated phenol and precipitated with isopropanol in the cold. Portions of 20 μg *E. coli* DNA were then added to reaction mixtures of the composition described in Table 2. NaOH (final conc. 0·2 M) was added to stop the reaction. Alkaline sucrose gradients: linear gradients from 20 per cent to 5 per cent sucrose–0·5 M NaCl–0·2 M NaOH (*p*H 12·5). Sedimentation: 14 h at 25 r.p.m. in the 'SW 27' rotor of the Spinco centrifuge. Sedimentation is from right to the left. The sedimentation marker is circular ^{32}P-labelled single stranded φX-DNA[10]. ○—○, DNA, labelled during a 2 min incubation period; ●—●, DNA, labelled during a 40 min incubation period.

was also used as primer for the experiment shown in Fig. 3. Even after short incubation periods (2 min) the radioactive (that is, *in vitro* synthesized) DNA sediments in an alkaline sucrose gradient with the bulk of the primer DNA, suggesting that the new DNA is covalently linked to pre-existing DNA strands. In another experiment the same exonuclease III treated DNA preparation was used for an experiment in which bromodeoxyuridine triphosphate replaced TTP and ^{3}H-dCTP replaced ^{3}H-TTP in the reaction mixture of Table 2. After incubation for 160 min, enough CsCl was added to the reaction mixture to give a density of 1·72 g/cm^3. The sample was then centrifuged near equilibrium. The result (Fig. 4) shows that the radioactive DNA appears at positions in the gradient where DNA with long segments of bromouracil containing polynucleotides is expected to band.

Table 2. SYNTHESIS OF DNA BY DNA POLYMERASE II

	pmole ^{3}H-TTP incorporated
Complete system	21·7
+ EDTA (0·01 M)	<1
+ EGTA (0·01 M)	19·3
+ rATP (0·005 M)	22·4
− dATP	3·4
− dATP, dGTP, dCTP	1·8
+ dTDP (4×10^{-4} M)	18·1
+ dTTP (4×10^{-4} M)	5·9
+ dATP (4×10^{-4} M)	22·7
− mercaptoethanol	19·2
+ *p*-chloromercuribenzoate (− mercaptoethanol) (5×10^{-4} M)	<1
+ Pancreatic deoxyribonuclease (10 μg/ml.)	<1
+ Pancreatic ribonuclease (10 μg/ml.)	22·1

Complete system: 2×10^{-5} M each of dATP, dGTP, dCTP and ^{3}H-TTP (50 μCi/μmole) in 0·08 M KCl–0·045 M Tris-HCl (*p*H 7·6)–0·005 M $MgCl_2$–0·005 M mercaptoethanol in a total volume of 0·3 ml. plus 4 μg sheared calf thymus DNA and 0·2 μg of an enzyme preparation eluted from the DNA column. The mixture was incubated at 37° C for 30 min.

DNA Polymerase II in *pol A*$^+$ Cells

The sensitivity of DNA polymerase II towards *p*-chloromercuribenzoate can be used to screen extracts of bacterial cells for the presence of the enzyme; the difference between the incorporating activity in the presence and in the absence of the mercuri compound should correspond to the activity of DNA polymerase II. The data of Table 4 show that in a total extract of an endonuclease I$^-$, *pol A*$^+$ strain such a difference is not detectable because of the high level of DNA polymerase I. In the membrane fraction, after three cycles of washings with the MOPS-EGTA buffered sucrose solution, a significant fraction of the incorporating activity can be inhibited by *p*-chloromercuribenzoate. Table 4 shows that the activity of DNA polymerase II amounts to 1–2 per cent of the total DNA polymerizing activity of a *pol A*$^+$–*E. coli* cell extract.

Table 3. PRIME DEPENDENCE OF DNA POLYMERASE II

DNA	Approximate molecular weight	Treatment	^{3}H (c.p.m.) incorporated DNA polymerase I	II
E. coli DNA	30×10^6	—	8,772	51
E. coli DNA	30×10^6	Pancreatic deoxyribonuclease	10,587	151
E. coli DNA	30×10^6	Micrococcal deoxyribonuclease	408	<10
		Exonuclease III	n.d.	1,906
E. coli DNA	$1–2\times10^6$	Ultrasonics	9,021	2,160
E. coli DNA	$1–2\times10^6$	Heat denatured	3,560	101
φX single strand	$1·7\times10^6$	—	102	<10
φX-RF (supercoil)	$3·4\times10^6$	—	109	<10
φX-RF	$3·4\times10^5$	Pancreatic deoxyribonuclease	3,311	75
No	—	—	67	<10

The enzymes are tested in the conditions described in the legend to Table 2. 8 μg of the various DNA species are added to the reaction mixture.

Table 4. RELATIVE ACTIVITY OF DNA POLYMERASE II

	Active enzyme units (total activity) No pCMB	10^{-3} M pCMB
Total cell lysate	93,950	93,910
MOPS–EGTA washed débris	3,680	2,235

10 g *E. coli* H 512 cells (*endo I*$^-$, *pol A*$^+$) were lysed with lysozyme and 'Brij' as described. The débris was washed three times with MOPS–EGTA buffered 20 per cent sucrose solution. Enzyme activity was tested as described in Table 2.

DNA polymerase II does attach to single stranded DNA (making the fd DNA column useful in the isolation procedure) and to double stranded DNA (the enzyme also binds to large molecular weight thymus DNA immobilized on a cellulose matrix)[11]. But neither single stranded DNA (linear or circular) or high molecular weight DNA is an effective primer of the polymerization catalysed by DNA polymerase II. Exonuclease III, on the other hand, converts an otherwise poor primer DNA into an effective template for the enzyme; it seems therefore that DNA polymerase II (like all the other known DNA polymerases) needs an end (presumably the 3′-OH end) of a polynucleotide strand as well as a complementary template strand for its function. Endonucleolytically nicked DNA duplexes do not prime the polymerization reaction of DNA polymerase II, probably because this enzyme lacks the exonucleolytic activity essential for the "nick translation" of DNA polymerase I (see ref. 4). From the limited data collected so far it seems that the DNA polymerase II resembles the T4 DNA polymerase described by Goulian, Lucas and Kornberg[12], an enzyme which has been shown by genetic analysis to be the "replicase" for phage T4 DNA[13]. DNA polymerase II is the only DNA synthesizing activity in *Pol A*$^-$ strains which can be

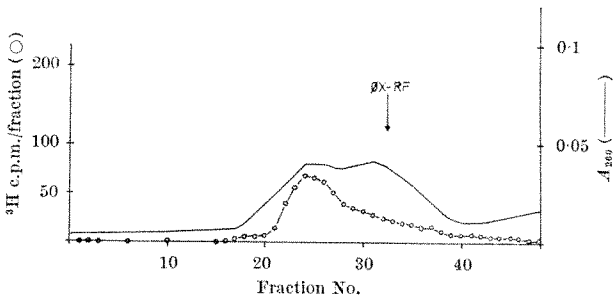


Fig. 4. CsCl equilibrium analysis. The radioactive bromodeoxyuridine labelled *E. coli* DNA in a final volume of 3·2 ml. (initial CsCl density: 1·72 g/cm^3) was centrifuged for 44 h at 35,000 r.p.m. in the 'SW 65-rotor' of the Spinco centrifuge. Density marker is ^{14}C labelled φX-RF DNA ($\varrho = 1·705$ g/cm^3)[10].

released in an active form from the membrane structure. Whether it is the only polymerizing enzyme on the replication site cannot be decided yet. It is feasible that other enzymes lose their activity during the biochemical manipulations.

Dr H. Hoffmann-Berling provided the bacterial strains used in this work. I thank Mrs M. Kurth for technical assistance, Dr H. Schaller for gifts of exonuclease III and bromodeoxyuridine triphosphate, and my colleagues at the Friedrich-Miescher-Laboratorium for comments on the manuscript.

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Epstein–Barr Virus in Burkitt's Lymphoma and Nasopharyngeal Carcinoma

Serological tests on 96 Burkitt lymphoma patients reveal antibody activity against virus-determined membrane antigen and viral capsid antigen while nucleic acid hybridization results on Burkitt's lymphoma and nasopharyngeal carcinoma add confirmatory evidence of an intimate association between the viral genome and the two diseases.

Antibodies to EBV associated Membrane and Viral Capsid Antigens in Burkitt Lymphoma Patients

THE sera of Burkitt lymphoma (BL) patients contain antibodies to Epstein–Barr virus (EBV) associated membrane antigens which may be revealed by membrane immunofluorescence tests[1–3]. Because the virus is ubiquitous and appropriate control subjects have not been studied, it has not been known whether this serological activity is particularly associated with the BL disease. We have investigated the serological reactivity against the EBV-associated membrane antigens and the EB viral capsid antigens (VCA) in all the BL patients seen at the Kenyatta National Hospital between 1966 and 1969, in comparison with various controls.

Sera which react with living biopsy BL cells are obtained more frequently from BL patients in long-term remission after chemotherapy than from donors with growing lymphomas[1–3]. Unless the patients have received many transfusions, positive sera do not react with red cells or normal bone marrow cells of the BL biopsy donors, or with normal lymph node or leukaemic cells of other subjects. Positive sera are also encountered at a low frequency among Africans with other neoplastic diseases and among healthy Africans. Established lymphoblastoid cell lines, derived from BL biopsies, provided more readily available and more regularly reactive target cells than biopsies[4,5], particularly if kept in culture conditions favouring membrane antigen expression[6]. Because the antibody activity was found in 7S IgG[4,7], this fraction was isolated from isoantibody-free BL sera and coupled to fluorescein isothiocyanate (FITC), thus converting the previous indirect test into a direct reaction[8,9]. Sera could now be screened for cross-reactivity in blocking tests with the labelled antibodies in the reference conjugates. This reaction was specific: it was blocked by unconjugated BL sera, but not by membrane-reactive, anti-human species specific antibodies[10] or poly-specific HL-A antibodies from frequently transfused donors[9]. The membrane sites reacting with anti-HL-A and anti-species antibodies are therefore distinct from the sites that reacted with the BL conjugates.

A comparison[5] between the cell membrane reactivity of different BL-derived cell lines, and the frequency of cells containing EB-viral capsid antigens (VCA), as defined by the Henle test[11,12], revealed that only lines with more than 1 per cent VCA-positive cells showed significant numbers of membrane antigen (MA) positive cells. Commonly there were approximately ten times more MA than VCA positive cells. This suggested that MA is determined by EBV, but indicated also that anti-MA and anti-VCA antibodies were distinct. This was further shown by absorption experiments and by the occurrence of "discordant" sera with predominantly one type of reactivity[13]. More recently, MA was induced by infecting MA-negative cells with EBV concentrates[14,15] or by exposure to lethally X-irradiated, EBV-carrying cultures[4]. Because the virus neutralizing titres of "discordant" sera paralleled their anti-MA and not their anti-VCA reactivities[16], it was suggested that MA represents viral envelope components in the cell membrane, in analogy with the herpes simplex system[17].

The blocking of direct membrane fluorescence has not been extensively evaluated with regard to disease-related patterns. In a preliminary study[9] eighteen of twenty African BL sera showed a high blocking activity against the FITC-conjugated IgG fraction from a BL patient (F-Mutua). The two sera with low activity came from a single patient, one taken in long term remission shortly before tumour recurrence, and the other when the patient was moribund. All 20 sera had high anti-VCA titres. Sera from unmatched control subjects with few exceptions had low MA-blocking activity. Nasopharyngeal carcinoma (NPC) was the most important exception. African and Chinese NPC patients showed regularly high anti-MA reactivity[9,18]. This corresponds with the regularly high anti-VCA titres found in NPC patients[19] and their fre-

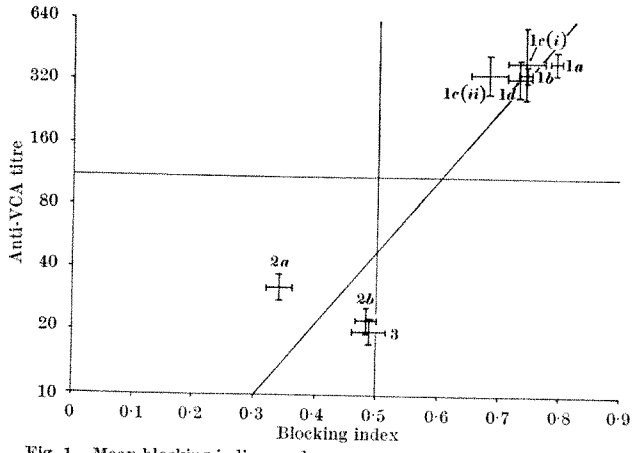


Fig. 1. Mean blocking indices and mean anti-VCA titres, with standard errors, for all groups involved in this study. An arithmetic mean of the blocking indices of all sera was calculated for each serum group, together with the standard error of the mean, assuming normal distribution of the observations. VCA titres were transformed into logarithmic units. The arithmetic mean and standard error of the logarithmic values were calculated, assuming normal distribution as above. The geometric mean titre obtained by conversion of the log titres was used for the comparison between the groups. To calculate regression and correlation between the MA and VCA tests, MA (BI) values were entered on a linear abscissa and VCA titres on a logarithmic ordinate. 1*a*, BL, pretreatment sera; 1*b*, BL, randomly selected sera; 1c (i), BL, tumour-free for 6 months; 1c (ii), BL, tumour-free for 1 yr; 1*d*, BL, tumour-bearing for at least 3 months; 2*a*, BL patients' relatives, age unknown; 2*b*, BL patients' relatives, age known; 3, tonsillitis cases.

quent reactivity in immunoprecipitation tests against an EBV-associated soluble antigen[20,21].

Our study was undertaken to assess the anti-MA and anti-VCA levels in BL patients in different disease stages, in comparison with their healthy blood relatives and Africans with chronic tonsillitis.

Selection of Sera

Ninety-seven histologically confirmed BL cases were seen at the Department of Head and Neck Surgery, Kenyatta National Hospital, Nairobi, from February 1966 to December 1969. Sera were available from ninety-six of the ninety-seven patients. They were grouped as follows:

Group 1*a:* Sera of fifty-six patients taken immediately after admission, before treatment.

Group 1*b:* One randomly picked serum from each of the ninety-six patients, assembled by a table of random numbers, applied to lists of available sera from each individual.

Group 1c (i)*:* Sera of nine patients without clinically detectable tumour for 6 months at the time of bleeding.

Group 1c (ii)*:* Sera of ten patients without detectable tumour for 1 year at the time of serum sampling. One patient here is represented twice; his tumour recurred 4 years after the initial treatment and regressed again after chemotherapy.

Group 1*d:* Sera of eighteen patients with persisting finally lethal tumours, taken at least 2 months before death, and at least 3 months after first admission.

Groups 2*a and* 2*b:* Sera from the families of BL patients, mostly siblings and parents, but also some neighbours. No donor is represented by more than one serum. The age and sex distribution of sixty-three relatives (group 2*b*) is known. For another group of sixty-three donors, designated 2*a*, this information is not available.

Group 3*:* Sera from fifty-three tonsillitis cases seen at the Kenyatta National Hospital during the same period as the BL patients. These were not all tonsillitis cases seen, for only occasional patients were bled, on request from Stockholm.

Further information on the groups is given in Table 1. Because of insufficient quantities of serum, ten patients in group 1*a*, six in 1*b*, two in 1c (i), two in 1c (ii), two in 1*d*, eight in 2*a* and *b* and eight in group 3 were not tested for anti-VCA titres.

Test Procedures

In the test for antibodies to MA, EBV-carrying lymphoblastoid cell lines established from BL biopsies[22] were used as target cells in the direct membrane immunofluorescence reaction[8,9]. IgG, conjugated with fluorescein isothiocyanate (FITC), from the BL patient Mutua (Kenya Cancer Council registry number 454) served as reference reagent. Mutua has been in total tumour regression since 1965 and his serum is free of detectable isoantibodies[4]. It reacts with at least two antigenic receptors on the surface of BL cells in EBV-carrying cultures[23].

Cultures containing 40–80 per cent membrane reactive cells were selected by a pretesting procedure[9], and incubated with a non-blocking reference serum, a known blocking serum (the unconjugated serum of Mutua), and with the test sera. After incubation and washing, the cells were exposed to the conjugate as described[9]. A blocking index (BI) was calculated by subtracting the proportion of positive cells in the test-serum exposed sample from the proportion of positives in the sample exposed to the conjugate alone, and dividing the difference by the latter figure. The negative and positive reference sera served as controls for the technical quality of the test.

Each serum was tested in at least three independent tests. The reactions were classified according to criteria described previously[9]. For each serum, an arithmetic mean BI was calculated.

The procedure for testing anti-VCA antibody involved the preparation of acetone-fixed smears from the EBV-carrying E3 Bline (BL), maintained for 3 days on arginine-free medium, and the titration of antibodies in indirect immunofluorescence tests as described elsewhere[11,12].

Elevated EBV-associated Antibody Levels in BL Patients

The arithmetic mean BI and the geometric mean anti-VCA values of the various groups of sera are presented in Fig. 1. The two tests correlate strongly ($r = 0{\cdot}97$; regression equation $\log_2(\text{VCA}/10) = 11{\cdot}8\ \text{BI} - 3{\cdot}6$). All BL groups gave high mean values in both tests, differing significantly from the two BR (Burkitt patients' relative) groups and from the tonsillitis group. The patients entering group 1*a* were subdivided according to sex and age and comparisons were made between sera stored for different times. No significant differences were found. The BL patient categories gave similar results with regard to mean BI or mean VCA titre. The differences between the control groups were also insignificant. By contrast, the differences between any of the large BL groups (1*a* or 1*b*) and any of the control groups were highly significant.

Fig. 2 presents in a scatter diagram the relation between BI and anti-VCA values for individual sera. As in previous studies[9,18], the two serological reactions correlate nicely ($r = 0{\cdot}79$ for 253 sera; regression equation of $\log_2(\text{VCA}/10) = 9\ \text{BI} - 2{\cdot}5$).

For a schematic comparison with earlier data, the "four quadrant" diagram has been used as previously[18], with an arbitrary subdivision between high and low blocking indices (0·5 borderline) and anti-VCA titres (low ≤ 80, high ≥ 160) (Table 2).

Table 1. AGE AND SEX DISTRIBUTION OF SERUM DONOR GROUPS

Group	Number of serum donors	Male/female ratio	Per cent of patients in the age range (years) 0–2	3–4	5–6	7–8	9–10	11–12	13–14	15–16	17–18	19–20	> 20
1*a* (untreated BL)	56	1·24	2	16	38	25	13	5	0	2			
1*b* (randomly assembled BL sera)	96	1·53	2	11	38	22	16	5	1	3	1		
1c (i) (BL in regression for about 6 months)	9	3·5		11	33	45	11						
1c (ii) (BL in regression for about 1 year)	10	4·0		10	30	40	20						
1*d* (BL with persisting tumour)	18	2·6		11	33	28	16	6		6			
2*a* (relatives of Burkitt patients, first group)	63	Unknown			Unknown					Unknown			
2*b* (relatives of BL patients, second group)	63	0·75	3	15	6	11	14	11	2			3	35
3 (African tonsillitis patients)	53	0·90		6	16	19	12	35	10	2			

Groups 2*a* and *b* were site-matched with some of the BL cases and district-matched to the entire BL population. Group 3 came from other areas.

Possible Biological Interpretations of Serological Data

Although the antibodies measured by the MA and VCA tests are directed against different antigens[13,23,25], there is a good correlation between the two antibody levels in most human sera[9,18,24]. Discordant minorities exist[13], however, and their study is important because they may contribute to a better understanding of virus–host relationships.

Table 2. DISTRIBUTION OF ANTI-VCA TITRES AND BLOCKING INDICES COMPARED WITH PREVIOUSLY PUBLISHED DATA

(*a*) Present material (BL: Burkitt group 1*b*; BR: Burkitt relatives, groups 2*b*+2*a*; T: tonsillitis, group 3).

Blocking index	VCA titre: High (≥160) BL	BR	T	Total per cent	Low 0≤80) BL	BR	T	Total per cent
High (≥0·5)	78	8	1	87 (34 per cent)	8	29	20	57 (23 per cent)
Low (<0·5)	2	3	0	5 (2 per cent)	2	78	24	104 (41 per cent)

(*b*) Previously published comparisons of the two tests (per cent values).

	High (≥160) 9*	23†	17‡	Our material	Low (≤80) 9*	23†	17‡	Our material
Ref.:								
High (≥0·5)	37	40	41	34	8	8	10	23
Low (<0·5)	11	10	6	2	44	32	43	41

* 279 sera, including African and non-African donors with Burkitt's lymphoma, nasopharyngeal carcinoma, infectious mononucleosis, and other malignant and non-malignant diseases.

† 151 sera from African and non-African cases of Burkitt lymphoma, nasopharyngeal carcinoma, other head and neck tumours, infectious mononucleosis and normal African and non-African controls.

‡ 191 sera from African and Chinese nasopharyngeal carcinomas, Indian oro- and hypopharyngeal carcinomas, and African controls with malignant and non-malignant diseases.

In this series, 75 per cent of all sera gave concordant results in the two tests (Table 2) whereas 25 per cent were discordant according to previously defined criteria. In earlier tests, the frequency of concordant sera was 81 per cent[9], 82 per cent[24] and 84 per cent[18] respectively. The somewhat lower degree of concordance observed in our series reflects different compositions of the serum collections. This series, which is the first all-African collection analysed, contains an unusually large number of sera with low anti-VCA titres and high blocking indices (fifty-seven sera, 23 per cent). Of these fifty-seven sera, twenty-nine were from Burkitt patients' relatives and twenty from tonsillitis cases. A prevalence of low anti-VCA and high anti-MA levels was previously noted[13] in a small series of sera from Burkitt patients' relatives. The presence of several such sera in the tonsillitis group suggests that this pattern is not restricted only to the relatives. The opposite discordance, high anti-VCA titres and low anti-MA values, was frequently seen in patients with large nasopharyngeal carcinomas[18], presumably because of adsorption of anti-MA antibodies to the tumour. The absence of NPC patients from this material may explain the small number of such sera (five sera, 2 per cent).

A comparison of BL patients and various African controls has previously shown[19] that the geometric mean anti-VCA titre of the patients was eight times higher than that of the controls. A difference of the same order was also found in this study. Moreover, a significant elevation of the anti-MA antibody level was noted. Whereas the mean BI values of all BL groups differed significantly from the controls, there were only slight differences between the different BL groups. The slightly lower mean BI in patients in remission for 1 year may reflect a decline in antigenic stimulation. The temporary rise of anti-MA levels after local radiotherapy[26] suggests that continued antigenic stimulation may be important for the maintenance of high anti-MA levels.

Although differences in mean BI obtained with undiluted sera are no doubt real, high blocking activities observed in these conditions do not necessarily denote equal levels of antibodies. Studies in progress (P. G., G. K., P. C., G. H., and W. H., in preparation) have shown that

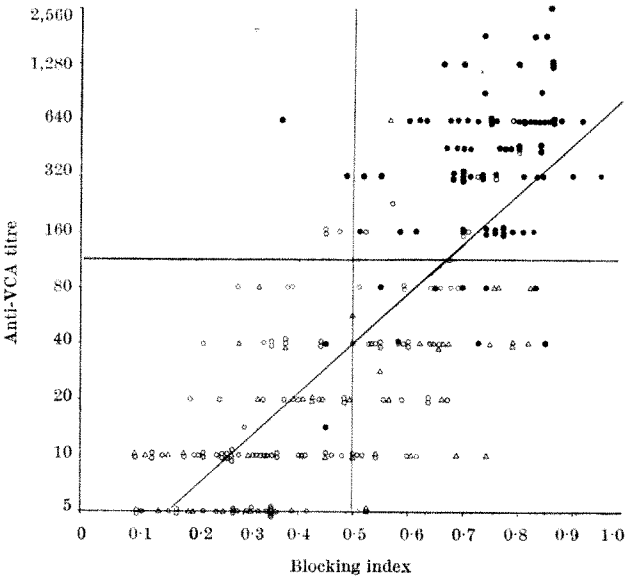


Fig. 2. Scatter diagram of BI and anti-VCA values obtained with BL group 1*b*, the two groups of Burkitt patients' relatives (2*a* and *b*) and African tonsillitis patients (group 3). Each point represents an individual serum. All sera were obtained from different donors. To avoid double representation of BL donors that entered more than one BL category, only the large, randomly assembled group 1*b* is shown together with the three control groups (2*a*, 2*b* and 3). ●; BL 1*b*; ○, BL relatives 2*a*+2*b*; △, tonsillitis patients.

undiluted BL sera with high blocking indices may yield blocking titres ranging from 1 : 2 to 1 : 400 upon dilution, taking a BI of 0·50 as the titration endpoint. The relationship between blocking titres and clinical history will be described elsewhere.

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EBV DNA in Biopsies of Burkitt Tumours and Anaplastic Carcinomas of the Nasopharynx

A TEST system involving DNA–DNA hybridization has recently been described which makes possible the detection of Epstein–Barr virus (EBV) nucleic acid in tumour cells[1]. This method has been used to demonstrate EB viral genome equivalents in the "virus-free" Raji line of Burkitt tumour origin[1]. The presence of viral nucleic acid in these non-virus producing cells revealed that EBV can persist in a masked form, thus exhibiting characteristic features of well known members of the DNA tumour virus group.

Several observations have suggested a close association of EBV with Burkitt's lymphoma (BL) of African children. A high proportion of cells from biopsies was shown to exhibit EBV-determined cell membrane antigens[2,3] unless these were blocked by the patient's antibodies. While EB viral antigens were rarely detectable in biopsy cells, they appeared regularly in a proportion of the cells placed in culture, often within a few days[4]. Clones cultured in the presence of antibodies to EBV from singly picked cells of established continuous BL cultures all revealed the presence of EBV in a small fraction of the cells[5]. All African BL patients have shown antibodies to EBV[2,6,7] usually at high titres, whereas among African control children antibodies are present generally at low titres, or, in a few, absent altogether. High levels of EBV-related antibodies are also found in most patients with anaplastic nasopharyngeal carcinomas (NPC) but not with other carcinomas of the head and neck[8–10].

To elucidate further the association of EBV with BL and NPC, we tried to detect and quantify EB viral DNA in tumour cells by nucleic acid hybridization. We found that DNA from BL and NPC biopsies always annealed with EBV DNA to a greater extent than DNA from other malignant tumours from control individuals with antibodies to EBV-related antigens.

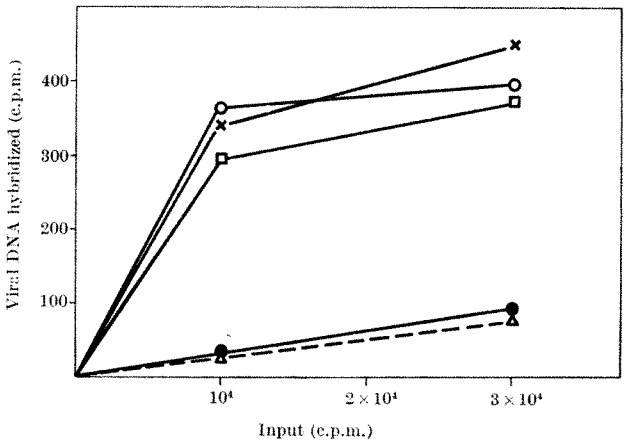


Fig. 1. Hybridization of EBV ^{3}H-DNA with DNA from biopsies of KCC 1140 (×—×), KCC 1124 (○—○), KCC 1097 (□—□), SÖD (△ - - - △), and with DNA from KB cells (●—●).

Tumour Cells and DNA Preparations

Biopsies were kept frozen from the time of surgical removal until DNA extraction. Biopsies from "control tumours" were kindly provided by Dr Gerlach, Würzburg (SAU), Dr Waubke, Giessen (SCH and BRÜ), Dr Johansson, Stockholm (SÖD), and Dr von Bülow, Tübingen (Marek's disease). All other biopsies were collected at the Kenyatta National Hospital, Nairobi. The diagnosis of every tumour was confirmed by histological examination. Sera from all but two patients were tested for antibodies to EB viral capsid antigens[7,8] and to EBV-induced early antigens[11] by the indirect immunofluorescence technique, and for antibodies to EBV-determined cell membrane antigens[3] by blocking of direct immunofluorescence[12].

For DNA extraction about 0·3–0·5 g of tumour material was thawed and ground with purified sea sand (Merck, Darmstadt). Thereafter 10 ml. of 0·01 M *tris*(hydroxymethyl)-aminomethane chloride (Tris), *p*H 8·1, containing 1 per cent sodium dodecyl sulphate was added[13]. This was followed by digestion with 1 mg/ml. of pronase P (Serva-Chemie, Heidelberg) for 60 min at 37° C. The nucleic acids were extracted by three consecutive phenol treatments[13]. Extensive dialysis against 0·1 × SSC (0·015 M NaCl – 0·0015 M citrate) was followed by treatment with ribonuclease (20 μg/ml., heated for 10 min to 80° C before use). The samples were subjected to another phenol extraction and dialysed against 0·1 × SSC. ^{3}H-labelled EBV DNA was prepared and purified by sucrose velocity sedimentation as described[1,14]. The dialysed fractions were lyophilized, redissolved in 0·1 × SSC, again dialysed against 0·1 × SSC, and examined for absorbance between 220 and 300 nm.

^{3}H-labelled herpes simplex virus (HSV) DNA was extracted from partially purified HSV by the same procedures as reported for EBV[14]. The infected cells were exposed to 1 μCi/ml. of ^{3}H-thymidine (specific activity 20·1 Ci/mmole) during the viral DNA replication for a period of 20 h.

DNA of human KB cells was included in all hybridization tests for control purposes. Occasionally, we also examined DNA from Nil-2 cells of Syrian hamster origin[15], cytomegalovirus-infected Wi-38 cells, and the EBV-carrying D 75 line of lymphoblasts established by exposure of peripheral leucocytes to EBV[16]. Details of denaturation and DNA–DNA hybridization procedures have been given[1,13].

Hybridization with EB Virus ^{3}H-DNA

Table 1 summarizes the results of four different hybridization tests, each involving DNA from several sources, as well as data on biopsy donors. It shows that the DNA

Table 1. HYBRIDIZATION OF EBV ^{3}H-DNA WITH CELLULAR DNA AND THE SEROLOGICAL STATUS OF THE INVESTIGATED PATIENTS

Biopsies and cell lines tested	Diagnosis	Test No.	c.p.m. hybridized	Approx. No. genome equiv.	Block index	Anti-EA-titre	Anti-VCA-titre
KCC 1080 I	Burkitt's lymphoma	1	282	7	0·65	43	152
KCC 1080 III		4	166	8	0·65	43	152
KCC 1106		1	169	3	0·77	46	320
KCC 1126		2	104	2	0·79	10	806
KCC 1126		3	100	2	0·79	10	806
KCC 1021		2	204	10	0·70	349	919
KCC 1124		2	377	22	0·90	202	508
KCC 1140 I		2	445	26	0·93	160	640
KCC 1140 II		2	357	21	0·93	160	640
KCC 1046		2	192	9	0·76	306	561
KCC 1154		4	106	3	0·40	5	160
KCC 1151		4	119	4	0·68	160	640
KCC 1166		4	141	6	0·42	40	160
KCC 1095	Anaplastic carcinoma of nasopharynx	1	152	3	0·73	160	1,280
KCC 1104		1	289	7	0·77	226	640
KCC 1098		1	292	7	0·87	320	905
KCC 1101		3	121	4	0·11	28	135
KCC 1097		3	329	19	0·68	160	1,280
KCC 1138		3	154	6	0·83	320	1,280
KCC 1112		3	95	2	0·60	32	202
KCC 1165		4	201	11	0·52	10	320
KCC 1168		4	80	1	0·45	640	2,560
KCC 1169		4	148	7	0·73	160	320
SAU	Hodgkin's disease	1	74	0	n.t.	n.t.	n.t.
KCC 1164		4	66	0	0·19	< 5	57
KCC 1156		4	48	0	0·62	< 10	195
KCC 1160	Squamous cell carcinoma, myeloma-like tumour, lymphoma	4	47	0	0·33	< 5	10
KCC 1173		4	42	0	0·26	< 5	80
SCH		1	85	0	0·00	< 10	10
BRÜ I		2	63	0	n.t.	n.t.	n.t.
BRÜ III		3	60	0	n.t.	n.t.	n.t.
SÖD		2	65	0	0·00	< 10	20
KCC 1134	Follicular carcinoma thyroid, carcinoma maxilla, epiderm. carcinoma nasoph.	3	64	0	0·50	< 5	40
KCC 1139		3	63	0	0·49	< 10	80
KCC 1099		3	64	0	0·45	< 10	34
Chicken tumour	Marek's disease	1	68	0	n.t.	n.t.	n.t.
Nil-2	Syrian hamster line	1	68	0			
Nil-2		1	42	0			
KB	Human epidermoid carcinoma line	2	74	0			
KB		2	70	0			
KB		3	65	0			
KB		4	67	0			
Wi-38 infected with CMV	Human fibroblasts, CMV-infected	2	66	0			
D 75	EBV carrier line	1	3,355	110			

The filters containing cellular DNA (100 μg/membrane) were incubated with 20,000 c.p.m. of EBV ^{3}H-DNA. The specific radioactivities of the viral DNA preparations used were 22,500 c.p.m./μg (test No. 1), 11,000 c.p.m./μg (test No. 2), 10,600 c.p.m. (test No. 3), and 9,150 c.p.m./μg (test No. 4). The serological data represent mean values of multiple tests.

derived from biopsies of Burkitt tumours, as well as anaplastic carcinomas of the nasopharynx, annealed with EBV DNA to a greater extent than DNA from other tumours and cultured cells of virus-negative lines examined. The specific radioactivities of the viral DNA preparations used were different in each of the four experiments. To compare the results of the four tests, it seemed most convenient to estimate the viral genome equivalents per cell[1]. We therefore determined whether or not the hybridization tests were performed under saturating conditions. DNA from biopsies of patients KCC (Kenya Cancer Council) No. 1140, 1124, 1097 and SÖD, as well as from human KB cells, was annealed with 10,000 and 30,000 c.p.m. of EBV ^{3}H-DNA (Fig. 1). The small amount of viral DNA available precluded the use of additional inputs, as well as further saturation experiments. The result showed that the hybridization of DNA from Burkitt tumours and anaplastic carcinomas of the nasopharynx reached a plateau between 10,000 and 30,000 c.p.m. This, in addition to previous evidence obtained in similar conditions[1], supported the assumption that the DNA in the four tests presented in Table 1 annealed with saturating amounts of viral ^{3}H-DNA. This provided the basis for calculation of viral genome equivalents per cell[1].

The estimation of viral genome equivalents showed that the Burkitt tumours contained between two and twenty-six, and the nasopharyngeal carcinomas between one and nineteen per cell. From two BL patients, two different tumours were tested, both with typical histology. The ovarian tumour of patient KCC 1080 contained a similar number of genome equivalents to the left orbital tumour which was biopsied 7 months after the first. Similarly, the ovarian and maxillary tumours of patient KCC 1140 hybridized with EB viral DNA to nearly the same degree. By contrast, the DNA of all other tumours, including Marek's disease tumours of chicken, failed to anneal to a greater extent than the DNA from human KB cells, Nil-2 cells of Syrian hamster origin (negative controls), or cytomegalovirus-infected Wi-38 cells, of which nearly every one showed cytopathic changes before DNA extraction. The DNA of the D 75 line with 2 to 3 per cent EBV antigen-producing cells (positive control) hybridized with about 17 per cent of the input EBV DNA.

Hybridization with Herpes Simplex Virus ^{3}H-DNA

In order to further establish the specificity of the DNA–DNA hybridization procedure, selected samples of DNA from biopsies and tissue culture cells were annealed with HSV ^{3}H-DNA (Table 2). The high specific radioactivity of the viral DNA increased the background annealing

Table 2. HYBRIDIZATION OF HERPES SIMPLEX VIRUS ^{3}H-DNA WITH CELLULAR DNA OF VARIOUS ORIGIN

Cellular DNA from	Origin of tissue	c.p.m. hybridized	Per cent of input hybridized
Nil-2 + HSV	HSV-inf. Syrian hamster cells	3,302	16·51
Nil-2 + HSV		3,349	16·75
Nil-2	Syrian hamster cells	221	1·11
Nil-2		205	1·03
D 75	EBV carrier line	190	0·95
D 75		239	1·20
SAU	Hodgkin's disease	250	1·25
SAU		223	1·12
SCH	Human lymphoma	204	1·02
KCC 1106	Burkitt lymphoma	189	0·95
KCC 1080		270	1·35
KCC 1080		262	1·31
KCC 1098	Anapl. carc. nasophar.	268	1·34

100 μg cellular DNA per filter were hybridized with 20,000 c.p.m. of HSV–DNA. Specific activity of HSV–DNA 51,000 c.p.m./μg.

considerably. In spite of some variations, all specimens hybridized approximately within the same range. The positive control (DNA from HSV-infected Nil-2 cells) annealed with about 17 per cent of the input viral DNA. DNA from D 75 cells which showed a high degree of homology with EBV DNA (Table 1) failed to react with HSV–DNA.

Antibodies to EBV Related Antigens

Antibodies to viral capsid antigens ranged in titre from 1 : 80 to 1 : 2,560 in the BL and NPC patients, but only from 1 : 10 to 1 : 195 in the patients with other tumours (Table 1). Antibodies to early EBV-induced antigens ranged from 1 : 5 to 1 : 640 in the BL and NPC patients but were absent in the control tumour patients. Antibodies to EBV-determined cell membrane antigens, as measured by the blocking index (BI), were at high levels (BI > 0·5) in eight of the ten BL patients, as well as in eight of the ten NPC patients, but in only two out of nine of the other biopsy donors tested. Although the absence of viral genome equivalents in the control tumour biopsies correlates well with the generally low antibody levels of their donors, and the presence of such equivalents in the BL and NPC biopsies with the generally high antibody levels in these patients, there was no clear-cut relation between the number of equivalents found in biopsies and the concentration of any one of the antibodies measured by the three methods.

Interpretation

All the BL and NPC biopsies—but none of those used as controls—contained EBV DNA in varying quantities. The specificity of the observed interactions seems clear from the fact that no hybridization was detectable between EBV DNA and DNA from cytomegalovirus-infected cells or from Marek's disease tumours, nor between herpes simplex virus DNA and DNA from BL and NPC biopsies. This is consistent with the lack of significant antigenic crossing between EBV and other members of the herpes group[6].

The number of EBV genome equivalents per cell varied from one to twenty-six in tumours of different patients. These might be underestimates depending on the representation of stroma or other non-tumour cells in the biopsies. Indeed, in the case of NPC, it is still not clear whether the viral DNA was derived from the anaplastic carcinoma cells or the lymphoid elements present to varying extents, or both. It is of interest that the number of viral genome equivalents were of the same order in different tumours of given BL patients biopsied at different times. This suggests a stable virus–host relationship in individual patients which is in line with previously reported observations[4]. The clonal nature of this tumour, as shown by Fialkow *et al.*[17], also agrees with these findings.

It has been suggested that after primary infections, whether or not accompanied by signs of classical infectious mononucleosis, EBV persists, presumably in the lymphoreticular system; this would account for the persistence of antibodies to VCA for years, if not for life, and the frequent emergence of the virus in cultures of peripheral leucocytes of healthy donors or patients with various diseases[18]. The presence of EBV genome equivalents in Burkitt's lymphomas and nasopharyngeal carcinomas might merely denote an extension of the persistent infection to the tumours, leading to increased antigenic stimulation and with it to the generally high levels of antibodies to EBV-related antigens in patients with these malignancies. All tested donors of the control tumour biopsies, however, had antibodies to VCA and therefore were presumably also carriers of EBV. Further, the numbers of EB viral genome equivalents per cell at apparently constant levels seem to be incompatible with a merely casual persistent infection of the tumours but rather suggest an intimate interaction of viral DNA with most, if not all, tumour cells.

In conclusion, we have provided direct evidence for the regular presence of EBV nucleic acid in biopsies of Burkitt's lymphomas and anaplastic carcinomas of the nasopharynx. The DNA–DNA hybridization seemed to be specific for EBV DNA and up to 26 genome equivalents were estimated to be present per cell.

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Origin of Mammalian Trypanosomes which Develop in the Anterior-station of Blood-sucking Arthropods

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Two hypotheses have already been advanced on the origin of the pathogenic trypanosomes of African mammals. A third is here proposed, in which it is suggested that they originated recently from leech-transmitted trypanosomes of aquatic reptiles in the tropics.

It is generally accepted that trypanosomes are monophyletic in origin and were primary gut parasites of invertebrates[1–8]. Two hypotheses have been advanced concerning the origin of the Salivaria, which are trypanosomes that develop in the foregut of the vector and are then injected into the mammalian host (see Fig. 1). Hoare[4,5,9] suggested that these trypanosomes originated recently from the Stercoraria (trypanosomes that develop in the hind gut of the vector and infect the vertebrate host by contamination, see Fig. 2), while Baker[7,8] suggested they were originally trypanosomes of aquatic reptiles which had evolved with their hosts as they colonized the land. I propose that the anterior-station trypanosomes of mammals evolved recently in the tropics from the trypanosomes of aquatic reptiles.

Trypanosomes can be divided into three fairly homogeneous groups based on the kind of vector and the site of development: first, the trypanosomes of aquatic vertebrates (fishes, amphibians, and reptiles), which develop in the anterior-station of leeches, infection of the vertebrate host is via the proboscis of the leech during feeding; second, the trypanosomes of birds, mammals and of some reptiles, which develop in the posterior-station of arthropods, the vertebrate host is infected by faecal contamination of the wound made by the arthropod during feeding or of the mucous membranes of the oral cavity if infective stages are ingested; third, the trypanosomes of African mammals transmitted by tsetse flies, and *T. rangeli* transmitted by the reduviid bug in South America. These trypanosomes develop in the anterior-station of blood-sucking arthropods and infective stages are inoculated into the final host at the time of the blood meal. The Salivaria are generally considered to differ from other trypanosomes in being more pathogenic to their vertebrate hosts[4,7]. Trypanosomiasis in domesticated animals in Africa has been the subject of intensive study for many years. These trypanosomes are not always highly pathogenic to wild animals in Africa; however, Ashcroft *et al.*[10] pointed out that while some wild animals in Africa are usually killed by *T. rhodesiense* and *T. brucei*, others are usually tolerant or resistant to the infection. Even among the first group, individual animals survive the infection. Nevertheless, it is characteristic of the Salivaria to be more pathogenic than other trypanosomes. Morphologically, trypanosomes of the Salivaria are distinct in that their posterior ends are rounded (except in *T. suis*) and not pointed as in other mammalian trypanosomes[11].

Metabolic studies of blood stages have revealed striking differences between the Salivaria and Stercoraria[12]. Most Salivaria (for example, *T. brucei*, *T. rhodesiense*, *T. evansi*) degrade glucose to a mixture of pyruvic acid and glycerol, but *T. lewisi* and *T. cruzi* convert glucose to a mixture of lactic and acetic acids with the production of large amounts of carbon dioxide. Cyanide does not affect the respiration of *T. rhodesiense* and *T. vivax*, but does affect that of *T. lewisi* and *T. cruzi*. Also, cytochrome pigments were detected in *T. cruzi* and *T. lewisi* but not in *T. congolense*, *T. rhodesiense* or *T. equiperdum*. These metabolic differences have not been observed in the insect or culture forms of the two groups of trypanosomes, which tends to support the view that trypanosomes are monophyletic in origin and that the observed differences in the blood stages are secondary features adapted to conditions in the vertebrate hosts.

Godfrey[13] found that *T. lewisi* had considerably less sphingomyelin and more lecithin than *T. brucei*, *T. vivax*

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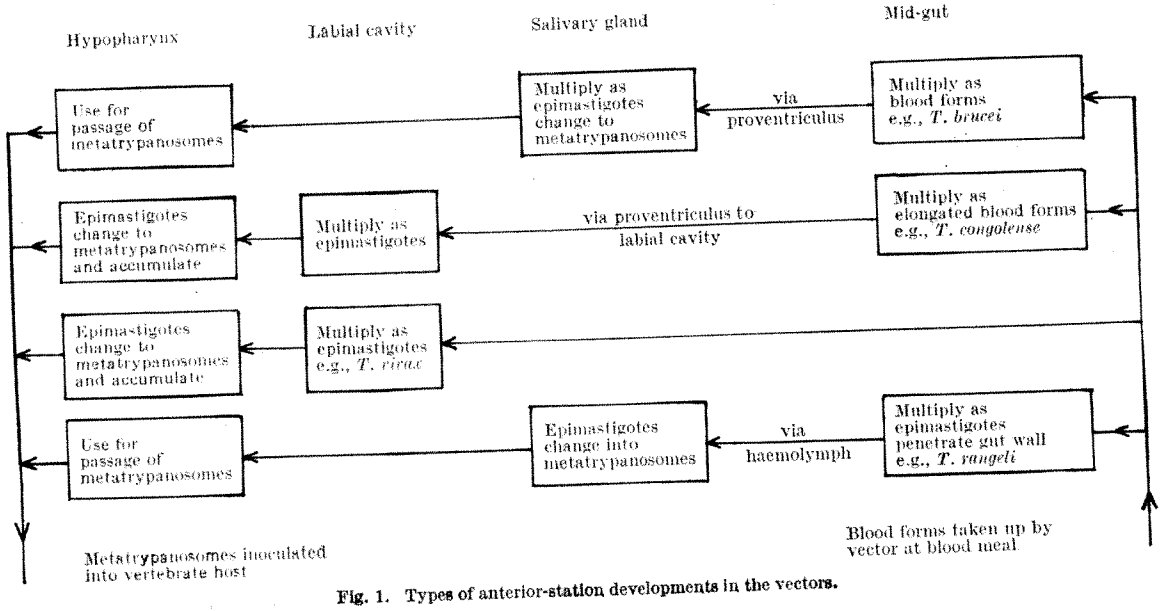


Fig. 1. Types of anterior-station developments in the vectors.

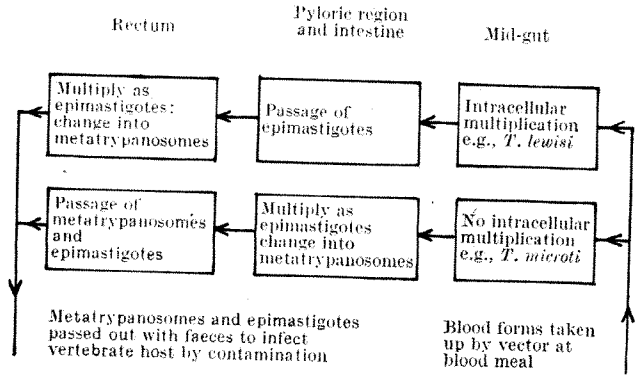


Fig. 2. Types of posterior-station developments in the vectors.

and *T. congolense*. Such differences are not confined to the phospholipids. Jaffe, McCormack and Gutteridge[14] found that the dihydrofolate reductase ". . . of *T.* (*T.*) *brucei*, *T.* (*T.*) *rhodesiense*, *T.* (*T.*) *equiperdum*, *T.* (*D.*) *vivax*, and *T.* (*N.*) *congolense* had closely similar drug sensitivity profiles, clearly distinguishable in certain respects from the reductases of *T.* (*H.*) *lewisi* and *T.* (*S.*) *cruzi*". They further pointed out that this particular protein was mutually indistinguishable among the saliverian species studied.

Although *T. lewisi* phosphohexose isomerase cross-reacts with the antisera to the *brucei* group enzyme (which suggests a close relationship between the Stercoraria and Salivaria) there are differences even between the isomerases of *T. brucei* and *T. equiperdum* and those of *T. gambiense* and *T. rhodesiense*. It is therefore doubtful if isomerase has any phylogenetic significance within a group.

The Salivaria are less host specific: for example, members of the *T. brucei* group can infect rodents, carnivores, ungulates, and primates, whereas most other trypanosomes of mammals (with the exception of *T. cruzi*) have a fairly narrow host range.

The Salivaria are unusually inefficient in infecting their vectors[9,16]. *T. vivax*, which develops in the proboscis of the tsetse fly, often infects only about 20 per cent of flies which have fed on an infected animal. *T. congolense*, which multiplies in the proboscis and mid-gut of the vector, often infects only about 10 per cent of the flies and *T. brucei*, which develops in the mid-gut and salivary glands, is even less infectious. In contrast, those trypanosomes that develop in the posterior-station of insects invariably infect almost 100 per cent of vectors that have fed on an infected animal[4,9].

Most trypanosomes transform into epimastigotes before dividing in the vector[17], but members of the *T. congolense* and *T. brucei* groups multiply as trypanosomes in the mid-gut of the tsetse fly. They are transformed into epimastigotes after migration to the proboscis or salivary glands[18]. All these common features tend to separate the pathogenic trypanosomes of African mammals from members of the rest of this genus.

The Hypotheses of Hoare and Baker

Hoare[4,5,9] suggested that the anterior-station trypanosomes of mammals might come from members of the Stercoraria. He believed that ". . . these pathogenic trypanosomes were originally parasites of wild ruminants, whose development was completed in the posterior-station of some other insect vector which transmitted them by the contaminative method"[9]. The transformation ". . . was affected either directly in the original vector (for example, *T. rangeli* in reduviid bugs) or indirectly, by secondary adaptation to cyclical development in insects that formerly served as mechanical vectors (for example *Glossina*)"[5]. So he is suggesting that the anterior-station trypanosomes of mammals came from a posterior-station trypanosome stock. Hoare[19] suggested that the Salivaria are only gradually becoming adapted to the tsetse fly for transmission, and that they first became adapted to the proboscis, then the mid-gut and finally the salivary glands. Invasion of the salivary glands was recent for Duke[20] showed that many mid-gut infections of *T. brucei* failed to become established in the salivary glands.

Baker[7] has suggested that trypanosomes which use leeches and insects as vectors had a common ancestor that parasitized the gut of the ancestor of both leeches and insects. When the annelids and arthropods diverged in the course of their evolution during the Cambrian period each took along its parasites. The trypanosomes were transferred to vertebrates when leeches and arthropods became blood-suckers. He also offered an alternative[7,8] to Hoare's hypothesis, suggesting that the anterior-station trypanosomes of African mammals represent ". . . the survivors of a line which did evolve from the leech aquatic group along with their vertebrate hosts as they gradually colonized the land"[8].

Hoare's suggestion that the trypanosomes of African mammals evolved from posterior-station forms implies that these trypanosomes develop in the anterior-station of the tsetse fly mainly because of the vector rather than their ancestral origin. *T. grayi* (which morphologically resembles members of the Stercoraria) of crocodiles in Africa is, however, transmitted by *Glossina palpalis*, one of the vectors of the *T. brucei* group. Development of *T. grayi* takes place in the hind-gut of the fly and transmission to a new host is by faecal contamination[3,21]. So it seems that there is nothing about *Glossina* which makes posterior-station development impossible. Adler and Theodor[22] demonstrated that *Trypanosoma platydactyli* of the gecko (*Taranetola mauretanica*) develops in the anterior-station of the sand fly *Phlebotomus parroti*. Recently Anderson and Ayala[23] found that a trypanosome (morphologically similar to members of the Stercoraria) of the toad (*Bufo boreus halophilus*) develops in the hind-gut of the sand fly *Phlebotomus vexator occidentis*. Although *T. rangeli*, a parasite of mammals of tropical South America, develops in the posterior and anterior station of the reduviid bug, infection of the vertebrate host is by way of the mouth parts of the vector. Infective metacyclic forms are produced only after the haemolymph and salivary glands are invaded[24]. This is in contrast to *T. cruzi*, which develops in the posterior-station of the reduviid bug, and in which infection of the vertebrate host is by faecal contamination of the wound made during feeding. Also *T. rangeli* is pathogenic to the vector[24,25], which may indicate a recent association between the reduviid bug and *T. rangeli*. These examples indicate that the site of development of a trypanosome may not depend on the vector, because anterior and posterior-station developments are possible in the tsetse fly, sand fly and reduviid bug.

Morphologically there is little evidence that the Salivaria came from the Stercoraria. If the Salivaria came recently from the posterior-station forms as suggested by Hoare, one would expect to find in African mammals some members of the Stercoraria (which develop in the posterior station of arthropods) with rounded posterior ends. So far as is known, all members of the Stercoraria have pointed posterior ends[11].

Baker[7,8] suggested that the tetrapod ancestors of modern mammals took trypanosomes along with them when they adapted to a terrestrial mode of life. Because the trypanosomes came from a leech–aquatic–vertebrate stock an anterior-station development was retained even after new vectors were secondarily acquired. If we accept Baker's view, the trypanosomes of African mammals have to be considered the oldest group of existing trypanosomes in terrestrial mammals, which makes it difficult to explain why the African trypanosomes are pathogenic to their vertebrate hosts and also have such a wide range of hosts—two factors that are usually associated

with organisms recently adapted to new hosts[4,9,26–28]. Also poor development of the trypanosomes in tsetse flies indicates a recent adaptation to the fly for transmission[4]. If we accept Baker's hypothesis that the tetrapods evolved with their trypanosomes, what were the vectors before the tsetse flies ?

An Alternative Hypothesis

I propose that trypanosomes that develop in the anterior-station of arthropods evolved quite recently from a leech–aquatic–vertebrate stock, perhaps from those of reptiles in the tropics. This hypothesis might explain why African trypanosomes tend to be unusually pathogenic to the vertebrate host and why they have such a wide range of hosts. The fact that they develop poorly in the tsetse fly may indicate a recent adaptation to the fly for transmission, as suggested by Hoare[4,9].

Experimental studies have shown that trypanosomes tend to become increasingly pathogenic while adapting to new hosts. Desowitz and Watson[29,30] have shown that rats can be infected with *T. vivax* if sheep blood or serum is inoculated as a supplement. In their earlier subpassages sheep serum was necessary and none of the rats died from the infection but "the greater percentage of rats which become heavily infected in later rat subpassages indicates that the infection becomes progressively severe"[30]. After thirty-seven subpassages in the rat the strain had lost its dependence on sheep serum[31], and this strain now produces ". . . fulminating lethal infections in that host"[32]. Moreover, *T. lewisi* can infect mice if rat serum is inoculated with it[33]. Lincicome[34] has shown that in calorically restricted mice the number of hosts that developed maximal infections and the intensity of the parasitaemia are increased by subpassaging. Although there was an initial decline in death rates of mice in earlier subpassages there was a definite increase in later subpassages, and this continued to 100 per cent fatality when C3H mice were used instead of albino mice. Also, there was a gradual reduction in the life span of infected animals and a steady diminution of the period over which the maximal infection level persisted.

Although *T. vivax* and *T. lewisi* can be adapted artificially to new hosts (by the inoculation of homologous host serum), the latter species does not lose its dependence on rat serum for survival in the mouse even after it has been subpassed 300 times, while *T. vivax* is able to do without sheep serum after 37 subpassages. This lack of plasticity of *T. lewisi* might suggest that it has been associated with the rat for a much longer time than *T. vivax* has with mammals. Other members of the Salivaria (for example, *T. rhodesiense*, *T. brucei*) are well known for their numerous antigenic changes (or variations) in response to antibodies or chemotherapeutic drugs. It should be noted that antigenic changes occur in *T. lewisi* infections[35], but are limited to two, while those of the Salivaria are believed infinite or at least very large[36,37]. This high degree of plasticity of the Salivaria also supports the idea that these are relatively more recent parasites of terrestrial mammals than the Stercoraria.

As I have indicated, anterior and posterior-station developments of trypanosomes in tsetse flies and reduviid bugs are possible; so the site of multiplication and development of a trypanosome to the infective stage may not be dependent on the vector. If, however, the Salivaria originated recently from a leech–aquatic–vertebrate stock, they might have retained an anterior-station development after becoming adapted to arthropods for transmission.

Anterior-station trypanosomes of mammals have only been found in the tropics—for example, *T. vivax*, *T. congolense*, and *T. brucei* in tropical Africa and *T. rangeli* in tropical South America. Reptiles abound in the tropics and crocodilians are confined largely to the tropics. Lindsey[38] reported 677 species of reptiles (lizards and turtles) from the tropics compared with about 150 species in each of the warm temperate and cool temperate regions. Also most trypanosomes of reptiles seem to be confined to the tropics. Of the forty-three nominal species described from reptiles, thirty-eight have been described from animals collected from the tropics whereas only five are from hosts collected in the temperate regions. Daytime temperatures in many parts of the tropics can be high for long periods: the afternoon temperatures at Bolobo in the Congo, for example, range from 30°–34° C (85°–93° F) throughout the year[39]. Trypanosomes of reptiles in the tropics would therefore have to be able to withstand relatively high temperatures. This is especially true when reptiles are basking in the sun. Conceivably, some trypanosomes of these reptiles were able to survive and multiply in the blood of warm-blooded animals.

Initial transmission could have been through the bites of infected leeches when mammals come down to the river to drink; the trypanosomes may then have lived in the blood, and later transmission to other mammals could have been taken over by tsetse flies and other blood-sucking arthropods. Alternatively, the infected reptile may have been fed on by blood-sucking arthropods while on land. It is known that tsetse flies of the *palpalis* group, vectors of the Salivaria in Africa, feed on reptiles, especially crocodiles[40–42]. Trypanosomes may at first have been transmitted mechanically but later acquired a cyclical development in the vector[4,5,9].

Adler and Theodor[22] have demonstrated that sand flies, *Phlebotomus parroti*, can transmit *Trypanosoma platydactyli* which developed in forty of forty-three sand flies that had fed on infected geckos. The flagellates adopted an anterior position in the cardia and had progressed to the oesophagus within the first four days. Flagellates were not seen in the hind gut. Possibly *T. platydactyli* was originally a leech-transmitted parasite of an aquatic ancestor of the gecko, and evolved with this ancestral reptile as it colonized land retaining the anterior-station as its site of development when sand flies became involved in the terrestrial life cycle.

As I have pointed out, the Salivaria are morphologically distinct from other mammalian trypanosomes in that their posterior ends are rounded. But they resemble in size and form some of the trypanosomes described from reptiles in Africa, for example, *T. sebae*, *T. psammophis*[43] and *T. mochli*[44].

Red-eared turtles (*Pseudemys scripta elegans*) have been infected with *T. brucei*[45]. These turtles from temperate regions had to be acclimated to 35°–37° C for a month before infection was possible. Control turtles at room temperature (20°–22° C) could not be infected. In experimentally infected turtles the trypanosomes divided in the blood during the first six days. The infection persisted for at least 12 days after which the turtles were killed. The trypanosomes were still infective and pathogenic when inoculated back into mice.

Caimans (*Caiman sclerops*), normally found in tropical South America, can be infected with *T. brucei* without prior temperature acclimation[46]. The trypanosomes isolated from these caimans after 12 weeks were still infective and pathogenic when reinoculated into mice. Apparently *T. brucei* is not pathogenic to caimans, for the experimental animals were active and seemed healthy even months after infection. This may indicate an ancestral relationship between reptiles and this species of trypanosomes.

T. cruzi can also infect reptiles[47,48] but, as I have pointed out, *T. cruzi* is somewhat different from most members of the Stercoraria in that it is unusually pathogenic and has a wide host range. I am therefore inclined, with Baker[8], to feel that its origin may also be different from that of other Stercoraria.

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Neolithic Trade Routes re-aligned by Oxygen Isotope Analyses

by

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Oxygen isotope analyses of *Spondylus* shells from neolithic sites suggest that the source for the shells used as ornaments in the Balkans and central Europe during the fourth millennium BC was the Aegean and not the Black Sea. The trade in *Spondylus* may have taken the form of an exchange of gifts.

THE distribution in the European neolithic objects, chiefly beads and bangles, made from the marine bivalve *Spondylus gaederopus* Linne gives a clear indication of a prehistoric trade in this attractive material. Clearly, either the ornaments or the unworked shells must have been transported by man from the sea where the animal lives. Recent work on other materials, supposedly traded from the Aegean region to the Balkans and central Europe, notably obsidian and faience, has suggested that prehistoric contacts between these areas were fewer than had been previously supposed. This finding seems to strengthen the possibility that the source for the *Spondylus* used in the Balkans and central Europe was not the Aegean but the Black Sea; several authors have postulated its availability there in neolithic times[1,2]. We report here preliminary investigations into the characterization of the source of *Spondylus* shell through the analysis of the oxygen isotopic composition of the calcium carbonate of which it is constituted. Our results suggest the exclusion of the Black Sea as a source, and document the earliest known trading contacts between the Aegean and central Europe, in the fourth millennium BC.

The physical principle which we are utilizing in this study is that a mollusc deposits the calcium carbonate of its shell in isotopic equilibrium with the water it inhabits. That is to say, the proportion of oxygen-18 atoms to oxygen-16 atoms is governed by thermodynamical principles rather than by biological factors. In fact, the ratio of the two isotopes differs from that in the parent water by only a small amount, the extent varying slightly with temperature. From a geological point of view this is of interest because it is sometimes possible to estimate palaeotemperatures through an analysis of the oxygen isotopic composition of a fossil shell[3]. From our point of view, it means that shells from two different sources may be differentiated clearly, either if the source regions have markedly different temperature regimes, or if they are characterized by watermasses of substantially different isotopic composition. Although we are not aware of any previous attempt to use the technique in this way, the idea was suggested some years ago by Keith *et al.*[4].

Isotopic Compositions of the Black Sea and the Mediterranean

In the initial stages of the project, we decided to restrict ourselves to differentiating between *Spondylus* from the Black Sea and from the Mediterranean. Here we are relying not on a climatic difference, but on a considerable difference in the isotopic composition of the waters.

The mechanism responsible for the fact that not all natural waters display the same $^{18}O/^{16}O$ ratio is a relatively simple one. Because water molecules containing ^{18}O are heavier than those containing ^{16}O, they have a slightly lower chance of evaporating into the atmosphere, so that water vapour in the air, and rain water, are slightly depleted in ^{18}O (ref. 5). The differences are small, and

are expressed as a deviation in parts per thousand from an arbitrary standard using the δ-notation:

$$\delta = 1{,}000\left\{\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right\}$$

where R is the ratio of ^{18}O to ^{16}O in sample and standard as indicated. All values quoted in this article are referred to the Pee Dee Belemmite standard[6]. In the oceans, the observed variations are very small except in regions where evaporation is high, leading to a high salinity and a positive δ-value in the surface waters, and in regions where the admixture of fresh water reduces salinity and gives rise to a negative δ-value[6]. Several analyses have been published for Mediterranean water, which comes into the former category, in the range +1·1 parts per thousand to +1·3 parts per thousand[6–8], these referring to water with a salinity of about 38·5 parts per thousand. (The values cited in references 7 and 8 have been converted from standard mean ocean water isotopic standard (SMOW) to PDB standard; see ref. 9.)

The Black Sea is largely isolated from the Mediterranean; the water budget given by Sverdrup *et al.*[10] is: precipitation, 7,600; runoff, 10,400; inflow from Mediterranean, 6,100; evaporation, 11,500; outflow to Mediterranean, 12,600 (all figures in m^3/s). A comparison with the Caspian, where inflow is balanced by evaporation, suggests that the Black Sea would be expected to be isotopically slightly more negative; Longinelli and Craig give a value of −2·4 parts per thousand for the Caspian[7] so that we might expect a value between −2·5 parts per thousand and −3 parts per thousand for the Black Sea. Very recently an even more negative value, −4·15 parts per thousand, has been published[8]. In any event there is clearly a substantial difference between its isotopic composition and that of the Mediterranean.

The second factor upon which the isotopic composition of shell carbonate depends is temperature, a factor whose importance was demonstrated by Urey[11]. The scale of the variation may be indicated by the fact that a seasonal change from 12° to 25° C would give rise to an isotopic variation through the shell of about 3 parts per thousand if growth continued throughout the year. Carbonate analyses are again expressed as δ-values in terms of the PDB standard. The generally used relationship between isotopic composition and temperature is[12]

$$T = 16{\cdot}5 - 4{\cdot}3(\delta_c - \delta_w) + 0{\cdot}14(\delta_c - \delta_w)^2$$

where δ_c and δ_w refer to carbonate and water respectively. The standard deviation among replicate analyses of carbonate is generally about 0·1 parts per thousand representing about ½° C.

Isotopic Analyses of *Spondylus* Shells from Neolithic Sites

The samples we have analysed are from settlement sites, datable between 4,000 BC and 2,500 BC in radiocarbon years. Tell Goljanio Delschevo[12] is in the Varna district, near the Black Sea coast of Bulgaria; samples from this site (submitted by the excavator, Dr H. Vajsová Simeonová) were selected in view of the likelihood that they would, if the Black Sea were used at all as a source for the material, be from this source rather than from the much more distant Aegean. Sitagroi is a prehistoric settlement mound near Drama in Northern Greece, lying some 40 km from the Aegean sea coast (excavations here were jointly organized by C. R. and Professor Marija Gimbutas; specimens from phase III occupation of site were selected and sampled by N. S. with permission from the Greek Archaeological Service). Gradeshnitsa in north-west Bulgaria and Vinča[15] near Belgrade in Jugoslavia are both more than 200 km from the sea (specimens from the surface of the Gradeshnitsa site were collected in 1966 with the collaboration of the excavator, Dr Bogdan Nikolov; the Vinča specimens were selected from the collection in the University of Belgrade by permission of the curator, Mme Z. Letica). The results of the analyses are given in Table 1.

Table 1. CARBONATE ANALYSES

Site and sample No.	$\delta^{18}O$ (parts per thousand)	$\delta^{13}O$ (parts per thousand)
Tell Goljanio Delschevo 3	−1·06	+0·21
Tell Goljanio Delschevo 5	+0·04	+1·14
Tell Goljanio Delschevo 6	−0·71	+0·29
Sitagroi 548	−0·27	+1·16
Sitagroi 817	−0·97	+0·50
Sitagroi 896	−0·55	+0·52
Sitagroi 1,446	−0·17	+0·87
Gradeshnitsa	−1·21	−0·04
Vinča	−0·91	+1·19

It is immediately clear that the analyses fall into a single group; the samples from the site near Varna on the Black Sea coast would be consistent with temperatures between about 24° to 19° C in water of isotopic composition +0·6 parts per thousand. A more positive water would imply a higher maximum temperature; the value chosen is a plausible summer value. If, on the other hand, our samples were by chance biased in favour of winter fragments, they would be consistent with temperatures between about 17° and 12° C in a water with isotopic composition −1 part per thousand. Thus even in the unlikely event of our having sampled fragments deposited in winter, the implied isotopic composition for the water is not nearly negative enough to be consistent with a Black Sea origin for the fragments found at Tell Goljanio Delschevo.

Analyses of Recent Shells

Because this conclusion was rather surprising, we carried out analyses of some recent shells from the Black Sea (specimens were supplied by Dr M. Demir, Marine Laboratory, Plymouth), which, in fact, confirmed our earlier findings. Three shells were analysed sequentially so as to obtain the maximum range of growth temperature (Fig. 1). The overall range is satisfactorily large; if 24° C is used as a likely value for the maximum represented, the minimum would be about 10° C and the

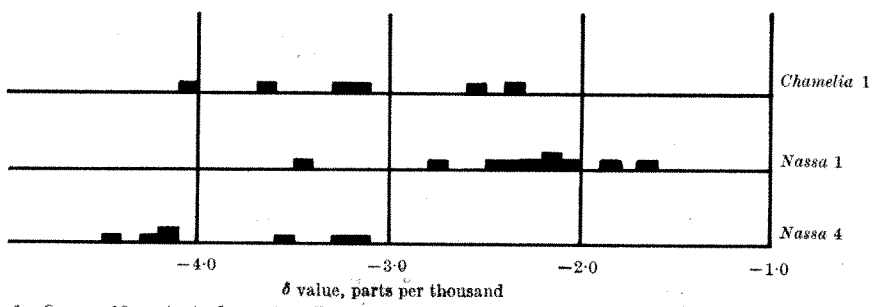


Fig. 1. Oxygen-18 content of recent shells from the Black Sea. *Nassa* 1 and 4 are *Nassa* (*Hinea*) *reticulata* L., collected at a depth of 79 m at 41° 26′ 35″ N, 29° 09′ E. *Chamelia* 1 is *Chamelia gallina* (L.) collected at a depth of 33 m at 41° 17′ 15″ N, 28° 53′ E. Each analysis is of about 0·25 mg shell sampled with a fine file; samples were taken so as to cover as much of the growth season as possible.

water isotopic composition about −2·6 per thousand, in satisfactory agreement with the value estimated. There is not any overlap at all with the values in Table 1. It is also of some significance that there is almost no overlap in carbon isotopic composition, the Black Sea samples giving δ-values for carbon of +0·28 parts per thousand to −3·77 parts per thousand (twenty-nine analyses). The factors governing carbon isotopic composition are complex[3], but from the almost total separation it seems certain that the *Spondylus* from Tell Goljanio Delschevo are, indeed, of Aegean rather than Black Sea origin.

There are two further points which must be considered before our conclusion is finalized. First, it is necessary to know that the samples analysed do, in fact, represent original carbonate, rather than the product of a later recrystallization. Carbonate undergoes isotopic exchange with groundwater if it does recrystallize; this is fortunate in our case because it means that although marine shell might give the impression of having derived from the Black Sea if some exchange with isotopically light groundwater had taken place, the reverse is not possible.

Second, it is pertinent to ask whether the Black Sea could have had an isotopic composition closer to that of the Mediterranean a few thousand years ago. The answer is that, if anything, it would have been more negative, because up to about 8,000 years ago the Black Sea was largely filled with glacial meltwater and was isolated from the Mediterranean owing to the glacio-eustatic lowering of sea level[13,14].

The extent to which our approach is capable of refinement is obviously complicated by the fact that the isotope ratio we measure depends on both the temperature and the isotopic composition of the water where the shells lived. Fortunately, however, only the first of these changes appreciably through the year (at least this is so away from river mouths). Although the objects analysed are often not large enough for many consecutive growth elements to be analysed, as the shells we chose from the modern Black Sea were, a number of small samples taken at random from several different shells is likely to represent a reasonable coverage of the growth season. Ideally, the analyses should cover a range wide enough to represent the maximum temperature range likely to have been experienced. If this were achieved, the isotopic composition of the water could be deduced within narrow limits; this in turn should narrow down the number of likely sources. In the case of the samples from Tell Goljanio Delschevo, the three analyses represent a range of about 5 degrees C. Because it is unlikely that the shell grew below about 12° C or above about 24° C, the water probably had an isotopic composition between −1·0 parts per thousand and +0·6 parts per thousand. This already suggests an Aegean source rather than, for instance, trade from a Cycladic Island. We are hopeful that, at least in some circumstances, we may be able to specify the likely sources of *Spondylus* from a critical site more precisely by analysing a greater number of samples.

Archaeological Implications

The conclusion that the *Spondylus* ornaments from Tell Goljanio Delschevo are of Aegean rather than Black Sea origin is at first a surprising one, because such ornaments are common in Bulgaria and Rumania from the time of the Hamangia culture[15] around 4,000 BC in radiocarbon years to the end of the Copper Age Gumelnitsa culture around 2,800 BC. We now plan to analyse samples from other sites near the Black Sea coast, in order to test the validity of the generalization that all the Balkan *Spondylus* is of Aegean origin. The analyses from Gradeshnitsa and Vinča, supporting those of Tell Goljanio Delschevo, strongly suggest this to be the case.

Recent finds at Sitagroi, in the north Aegean region, have yielded, in levels of phase III, pottery which is unmistakably of Balkan Gumelnitsa type. It is precisely in these levels that "bracelets" of *Spondylus* are most common, being rare in levels of the preceding phase II and absent in those of phase I. Finds of *Spondylus* are very common at this and other sites of the fourth millennium BC in the Plain of Drama, notably more so than at other sites in north Greece[16] or elsewhere in the Aegean[17], where the animal itself is abundant. The Aegean coasts near the Plain of Drama now seem the most likely source for much of the Balkan *Spondylus*.

Ornaments of the shell are particularly numerous in graves of the fourth millennium Linearbandkeramik culture of central Europe[18]. We hope to investigate further the differentiation between the Aegean and the Adriatic as possible sources for this material. Already, however, an early, long-distance trade with the Aegean is documented in the Balkans by finds of surprising number.

Definitive elucidation of the trading mechanism must await further study. Yet already the demonstration of the Aegean origin of the material sets the whole question in a different light. Finds of *Spondylus* ornaments are numerous in eastern Bulgaria and Rumania, some of the sites actually lying very close to the shores of the Black Sea. The decreasing number of finds with distance from the source might have been interpreted as conforming very approximately to a straightforward down-the-line trading mechanism, of the kind which has been postulated for the obsidian trade in the Near East[19], had the Black Sea been the source of the material. With the Aegean now recognized as the source the situation is transformed, for the quantities found do not decrease exponentially with distance from the Aegean coast as such a model would predict; finds are at least as common in Bulgaria and Rumania as in north Greece.

A different model, prestige chain exchange, is here proposed for the Balkan *Spondylus* trade. It has four defining characteristics: (1) the transfer of prestige goods takes place, often between persons of high status, on a basis of balanced reciprocity[20]; (2) the prestige goods are frequently handed on in subsequent exchanges; (3) such goods are not expended or utilized in daily life; (4) they appear in the archaeological record either as a result of deliberate burial (generally with the dead) or through loss or accidental breakage. A feature of such trade is that the traded goods are distributed over considerable distances, occurring in places remote from the source in quantities as great as at findspots nearer to it. Such now seems to be the situation in south-east Europe.

The best known exchange mechanism of this kind of recent times is the kula trade of the Trobriand islanders of the western Pacific[21], a conventionalized gift exchange whose social significance far transcended the utilitarian or "economic" value of the gifts[22]. As perhaps in prehistoric Europe, the most prized objects were bracelets of marine shell, and bead necklaces, many of them cut from shells of the genus *Spondylus*.

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Simultaneous Measurements of Clear Air Turbulence at the Tropopause by High-power Radar and Instrumented Aircraft

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Large amplitude Kelvin–Helmholtz billows observed by radar at a height of almost 11 km are shown to have been associated with clear air turbulence which produced a vertical aircraft acceleration of 0·65*g*.

A RECENT article[1] has reviewed the principles of the detection of clear air turbulence (CAT) by radar. Briefly, radar detects the incoherent backscatter from inhomogeneities of refractive index in the atmosphere occurring on a scale of half the radar wavelength (the most commonly used wavelength for the detection of CAT is about 100 mm). In the upper troposphere the inhomogeneities are associated mainly with fluctuations of temperature, these fluctuations being associated with small scale turbulence generated by Kelvin–Helmholtz instability (KHI). The vertical extent of KHI may be anything from metres to hundreds of metres, but only that occurring on a large scale gives rise to turbulence hazardous to aircraft. For this reason, attention was concentrated in the earlier communication[1] on the use of radar to detect deep KHI. A case study was presented showing the evolution of Kelvin–Helmholtz billows with crest-trough amplitude 400 m, across which the vector wind change was as large as 16 m s^{-1}. It was suggested that the breaking of KH billows such as these was likely to give rise to intense CAT. It is necessary to obtain direct measurements by aircraft, however, to establish this conclusively. The purpose of this article is to present other radar observations of large amplitude KH billows in which simultaneous measurements have been obtained by an instrumented aircraft.

Combined radar-aircraft measurements of CAT have already been reported by Glover *et al.*[2], but these were concerned mainly with situations of light turbulence in which the amplitude of the KH billows was too small to be resolved by radar. Moreover their turbulence measurements were made subjectively using an uninstrumented aircraft. The data presented in this paper are believed to be the first observations of moderate CAT in which

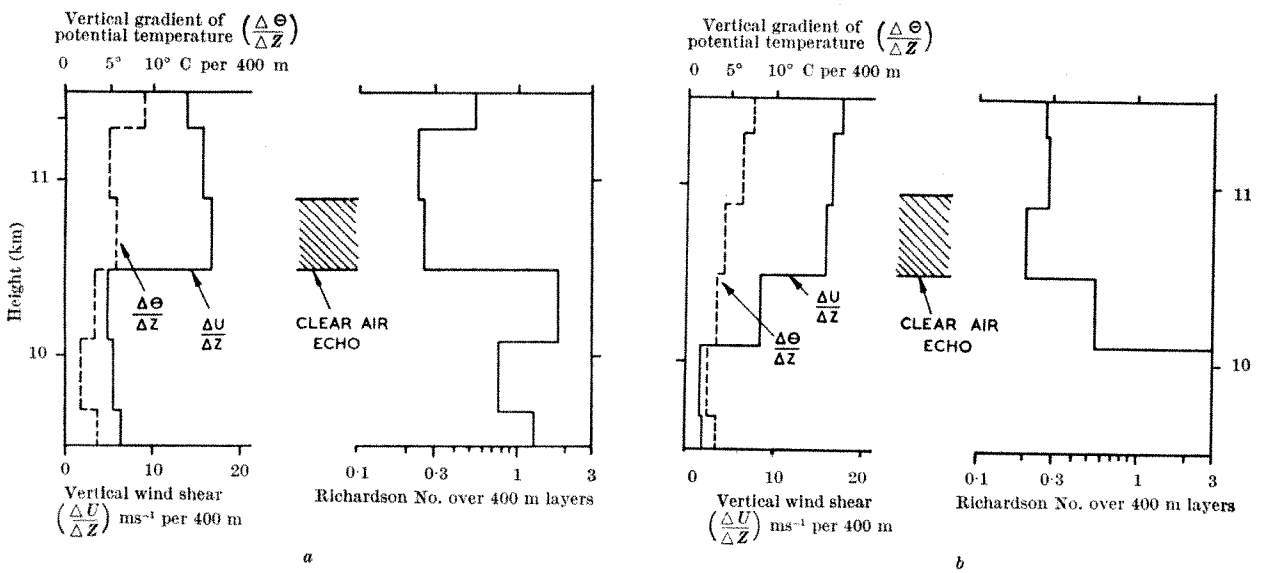


Fig. 1. Structure of the atmosphere in the vicinity of the tropopause on February 3, 1970, as derived from radiosondes released at Pershore at (*a*) 1243 GMT and (*b*) 1453 GMT. Each of the plotted parameters has been evaluated over layers 400 m deep.

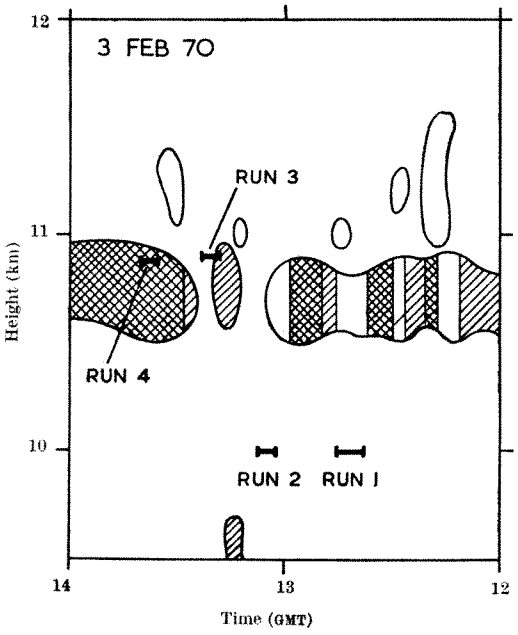


Fig. 2. Vertical extent of detectable clear air echoes associated with the KH billows on February 3, 1970, as a function of time. The horizontal extent of echo is represented by shading as follows: > 15 km (cross-hatched); 5–15 km (hatched); < 5 km (unshaded). Times and heights of aircraft runs are also indicated; registration between the radar and aircraft data is thought to have been closer than 1 min and 200 m respectively.

measurements by a fully instrumented aircraft are related to simultaneous radar observations of a specific patch of KH billows of known amplitude.

Observations of Clear Air Turbulence

The observations were made on February 3, 1970, while a strong upper tropospheric jet stream was overhead. The radar used in this study was the same one, at Defford, Worcestershire, that was described by Browning and Watkins[1]. It was operated to make 0°–90° elevation scans into the direction (280°) of the high level winds at a rate of roughly one scan every 3 min. KH billows were observed at tropopause level throughout almost the entire period of radar observations, from 1115 to 1530 GMT. The maximum range at which they were detectable was 50 km, and the amplitude of the billows (crest-trough) varied from less than 200 m to more than 500 m. (The amplitude may have been underestimated at times when the echo intensity was close to the threshold of detectability.) Vertical profiles of wind, temperature and humidity were obtained by means of radiosondes released at 2 h intervals from Pershore, Worcestershire. These showed that wind speeds reached 70 m s^{-1} from 280° beneath a tropopause which was situated between 10·5 and 10·9 km. Data from two of the radiosonde ascents are reproduced in Fig. 1 to show the structure of the atmosphere in the vicinity of the tropopause. The vertical extent of radar echo from KH billows is also indicated in Fig. 1, and it can be seen that the 400 m layer Richardson number Ri at the height of the billows was close to or less than 0·25 which, according to theoretical studies[3], is the critical value for the onset of KHI.

The aircraft was a Canberra from the Royal Aircraft Establishment, Bedford, fully instrumented for gust research studies. The instrumentation includes a wind vane mounted on a nose pole, a sensitive thermometer and an inertial platform[4]. The aircraft made ten almost straight and level runs at five different levels between 1240 and 1524 GMT along a line 170 km long from 52° 30′ N 04° 00′ W to 52° 00′ N 01° 45′ W. Each run was parallel (within ±10°) to the section scanned by the Defford radar, always remaining within ±7 km of this section when within radar range (5–50 km) and being within ±3 km during one of the more interesting runs (No. 4). The three highest flight levels, 11·8, 13·6 and 15·0 km, were entirely free of turbulence; no clear air radar echoes were detected from these levels. During parts of the flights at 10·0 and 10·9 km (runs 1–4), the aircraft encountered clear air turbulence within range of the Defford radar, and the remaining analysis consists of an interpretation of the radar observations in relation to the aircraft measurements at these two flight levels.

Fig. 2 depicts the height of runs 1–4 in relation to the vertical extent of radar echo associated with the KH billows. It shows that, whereas runs 1 and 2 were 500 m below the base of the principal layer of detectable echo, runs 3 and 4 were within this layer. The aircraft detected turbulence during only 14 per cent of runs 1 and 2 but continuously during runs 3 and 4. Fig. 3 shows that detectable billows occurred in a patchy manner, amplitudes greater than 300 m rarely being detected continuously for longer than 10 min or over distances greater than 20 km. Aircraft runs 3 and 4, plotted in Fig. 3, appear to have missed the billows of largest amplitude, although part of run 4 went close to a patch of billows with a maximum amplitude of 500 m. The aircraft accelero-

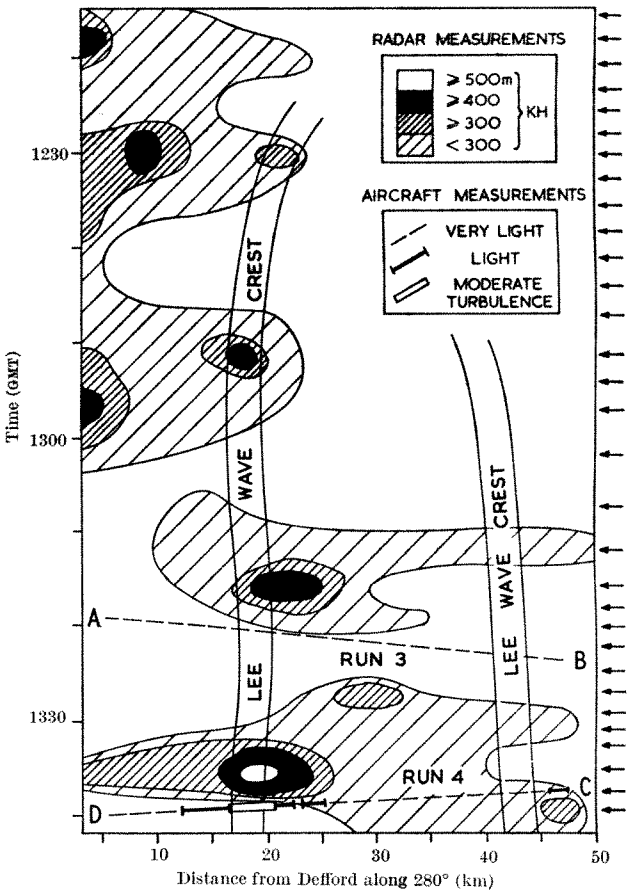


Fig. 3. Locations of detectable radar echoes associated with the KH billows at tropopause level, plotted in an x–t diagram, where t is time and x is distance from the Defford radar towards 280°. The crest–trough amplitude of the billows is indicated by shading (see key in diagram). Also shown are the positions of crests in the lee wave pattern as observed by radar. Arrows on right side of diagram denote times of individual RHI scans. The aircraft turbulence reports during runs 3 and 4 are plotted along AB and CD according to the code indicated in the key: very light, light, and moderate turbulence correspond to vertical accelerations of 0·1–0·2, 0·2–0·4, and 0·4–0·7g, respectively. Time and space registration between the radar and aircraft data is thought to have been closer than 1 min and 3 km.

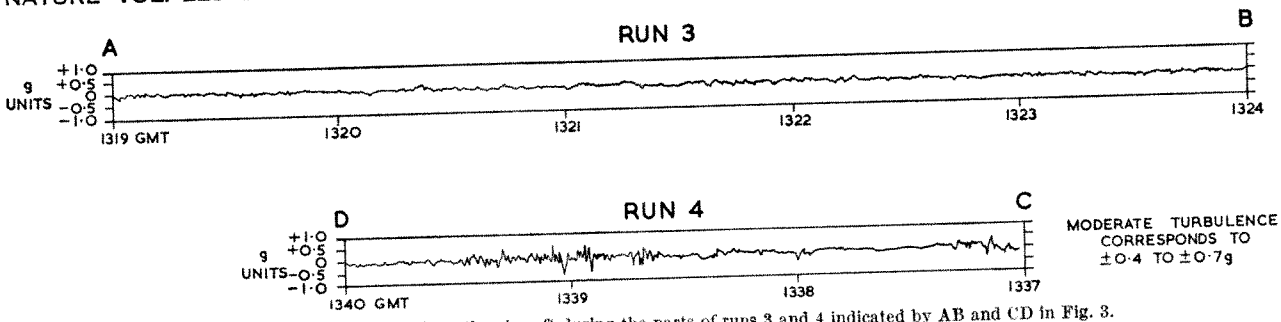


Fig. 4. Accelerometer traces from the aircraft during the parts of runs 3 and 4 indicated by AB and CD in Fig. 3.

meter traces for the parts of runs 3 and 4 labelled AB and CD in Fig. 3 are reproduced in Fig. 4. Perhaps the most striking feature of these records is that the maximum vertical acceleration of 0·65*g* (corresponding to moderate, almost severe, turbulence) occurred in close proximity to the patch of large amplitude billows observed by radar. This is not to imply, however, that the presence of large amplitude billows identifiable by radar is a sufficient condition for moderate CAT; it is also necessary that the billows should occur in association with very strong vertical wind shear. In this study, for example, there was a vector wind change of 17 m s^{-1} over a 400 m height interval.

Table 1. COMPARISON OF RADAR AND AIRCRAFT MEASUREMENTS OF BILLOW TURBULENCE AT TROPOPAUSE LEVEL MADE BETWEEN 5 AND 50 KM WEST OF DEFFORD DURING THE PERIOD 1320 TO 1340 GMT

	Radar measurements	Aircraft measurements (runs 3 and 4)
Height	Radar echo from billows at heights between 10·5 and 11·0 km	Aircraft flying at 10·9 km
Max. amplitude of billows (crest–trough)	500 m	220 m
Wavelength of billows	1 to 2 km	1·5 km
Percentage of range interval for which turbulence was detected	Radar echo from billows detected on average over about 50 per cent of 5–50 km range interval	Turbulence of at least very light intensity detected over 100 per cent of range interval. Turbulence of at least light intensity detected over 14 per cent of range interval
Principal turbulence event	One occurrence of billows with 500 m amplitude detected over a 3 km range interval	One occurrence of moderate turbulence detected over a 3 km range interval

A comparison of several aspects of the radar and aircraft measurements during runs 3 and 4 is presented in Table 1.

Although there is good agreement in the wavelength of the billows as measured by the two techniques, the agreement in amplitude is not as good. The amplitude and wavelength of the billows, as estimated from the radar data, have been obtained in a straightforward manner from the shape and dimensions of the echo. On the other hand, the amplitude and wavelength, as estimated from the aircraft data, have had to be obtained indirectly from the magnitude and periodicity of the fluctuations in potential temperature θ. The vertical excursions of air parcels have been estimated from the magnitude of fluctuations at a constant flight level, knowing the vertical gradient of potential temperature from the combined aircraft and radiosonde data. Although such a technique is reasonably valid for billows before they break, it will under-estimate their amplitude after they have broken owing to turbulent mixing. Probably the main reason for the aircraft giving a smaller amplitude than the radar, however, is that the aircraft narrowly missed the billows of largest amplitude.

The evidence in Table 1 and in Fig. 3 suggests that the radar detected all turbulence of at least light intensity encountered by the aircraft at 10·9 km. As noted earlier, however, the radar detected nothing at 10·0 km, corresponding to the aircraft flight level during runs 1 and 2. Although 86 per cent of runs 1 and 2 were indeed free of turbulence, the aircraft did encounter a small patch of light turbulence during run 2 which the radar failed to detect. The capability of the Defford radar for detecting CAT thus appears to be broadly consistent with the results of Glover *et al.*[2], who used a radar with a minimum detectable reflectivity of $1{\cdot}3 \times 10^{-15}$ m^{-1}, almost identical to that of the radar used in this work. They found that, for flights made at altitudes between 9 and 12 km, very light turbulence was detected by radar in 21 per cent of cases and light or moderate turbulence was detected in 41 per cent of cases. At lower altitudes the detection capability improves significantly.

Effect of Lee Waves

As is often the case when strong jet streams cross a barrier such as the Welsh mountains, lee waves of large amplitude occurred in the vicinity of Defford on this occasion. The lee waves were detected by the Defford radar as a result of the distortion of otherwise horizontally stratified echoes from the clear air (and also occasionally from cirrus). Because these layer echoes sometimes occurred simultaneously at several altitudes from 1·5 km to the tropopause, it was possible to determine the amplitude and wavelength of the lee waves as a function of height with a single radar scan taking only 3 min to complete. The wavelength was found to vary from 15 to 30 km. Crests and troughs were aligned vertically to within 1 or 2 km. The crest–trough amplitude of the lee waves was as large as 400 to 600 m at 2 to 4 km and, although the amplitude decreased above this, it was still as large as 100–300 m at tropopause level. According to Scorer[5], the development of KH billows is encouraged locally in parts of lee waves where isentropic surfaces are tilted appropriately, and similarly discouraged in other parts. This would partly account for the patchy nature of the distribution of KH billows shown in Fig. 3. Indeed, one might expect to find some overall relationship in Fig. 3 between the location of the billows with large amplitudes and the lee wave pattern. Pinpointing the regions of largest amplitude billows is made difficult by the range dependence of the echo intensity, and by the fact that the amplitude of the billows was underestimated at 10–15 km range because of the use of a moving target indicator radar system.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Separation of Matter and Antimatter

DOES the universe contain equal amounts of matter and antimatter? Unless one invokes an asymmetric initial condition, charge symmetry follows from the conservation of baryons. An asymmetric initial condition cannot be ruled out, however, and in fact is postulated in the standard version of the big-bang cosmology.

With the possible exception of cosmic ray statistics[1], observations yield essentially no information on the relative amounts of matter and antimatter beyond the solar system[2]. What the observations do tell us is that matter and antimatter are rarely, if ever, found together. Stringent upper limits on the amount of mixed matter and antimatter can be derived from dynamical arguments[3] and from gamma ray observations[4]. Even more conclusive is the existence of condensed objects, such as stars, each of which must be composed exclusively of either matter or antimatter. This high degree of separation cannot possibly be explained by statistical fluctuations[4,5]. It is therefore important from the standpoint of charge-symmetric cosmologies to explore possible physical mechanisms by which matter and antimatter can be separated. Several such mechanisms have been described by Alfvén (unpublished). One of these occurred to me independently and I think merits further attention.

Consider a gas of protons, antiprotons, electrons, and positrons which is sufficiently dilute that annihilation can be neglected. In general such a gas will be situated in a magnetic field $\mathbf{B}$, a gravitational field $\mathbf{g}$, and an electromagnetic-radiation field of flux $\mathbf{F}$. Each of these fields will be assumed static and homogeneous. In particular, the length scale for variations in $\mathbf{B}$ must be large enough that particle drifts arising from magnetic inhomogeneities are negligible.

The protons and antiprotons will usually be much more strongly influenced by the gravitational field than by the radiation field. As well as spiralling around the magnetic lines of force, the heavy particles will therefore have a drift velocity[6]

$$\mathbf{V}_h = \frac{m_p \mathbf{g} \times \mathbf{B}}{qB^2} \tag{1}$$

where m_p is the proton mass and q is the particle charge. Because of their smaller mass and larger scattering cross-section, the electrons and positrons will feel a much weaker gravitational force and a much stronger force due to radiation pressure. If the radiation force much exceeds the gravitational force, the drift velocity of the light particles will be

$$\mathbf{V}_l = \frac{\sigma \mathbf{F} \times \mathbf{B}}{qcB^2} \tag{2}$$

where σ is the Thomson cross-section of an electron (or an appropriate value of the Compton cross-section if the energy per photon is sufficiently high) and c is the speed of light.

As will be shown later there is a natural tendency in the universe for $\mathbf{g}$ and $\mathbf{F}$ to be antiparallel. When the radiation flux is sufficiently large for equation (2) to hold and when $\mathbf{g}$ and $\mathbf{F}$ are, in fact, antiparallel, it follows from equations (1) and (2) that protons and electrons will drift in one direction while antiprotons and positrons drift in the opposite direction; that is, matter and antimatter will tend to separate.

When B is measured in Gauss and the other quantities are measured in c.g.s. units, the magnitudes of the drift velocities (1) and (2) are

$$V_h \approx 10^{-4} \frac{g_\perp}{B} \tag{3}$$

and

$$V_l \approx 10^{-15} \frac{F_\perp}{B} \tag{4}$$

where the subscript $\perp$ specifies the vector component perpendicular to $\mathbf{B}$. It is clear from equations (3) and (4) that B must be very small if the separation mechanism just described is to have any cosmological significance. In this respect the mechanism contrasts sharply with Alfvén's electrolytic mechanism[2,7], which implies the existence of strong magnetic fields if significant amounts of matter and antimatter are separated.

It should be noted that, just as an electric current does not necessarily result in a separation of charge, the opposed drifts of matter and antimatter need not produce an actual matter–antimatter separation. The simplest example of a failure to separate would be an infinite matter–antimatter gas with $\mathbf{B}$, $\mathbf{g}$, and $\mathbf{F}$ uniform throughout. On the other hand, matter and antimatter in an isolated cloud or in an extended medium with an appropriate field configuration should achieve some degree of separation. Because the proton (antiproton) and electron (positron) fluxes will not be equal, in general, there will also be some separation of charge, leading to an electric field $\mathbf{E}$ and an $\mathbf{E} \times \mathbf{B}$ drift (which is the same for all particles). As the $\mathbf{E} \times \mathbf{B}$ drift increases, the heavy particles acquire an inertia drift which tends to remove the original difference between the proton (antiproton) and electron (positron) fluxes (unpublished work of Alfvén). Alfvén has pointed out that this fact makes separation possible even in the absence of an appreciable gravitational field.

There are two reasons why $\mathbf{g}$ and $\mathbf{F}$ may, in general, be expected to be antiparallel. The more obvious reason is that major sources of radiation are usually massive objects. Let us suppose that the gravitational and radiation fields originate at an object equivalent to a point source of mass M and luminosity L. The requirement that $\mathbf{g}$ and $\mathbf{F}$ be antiparallel is obviously satisfied. Matter and antimatter will tend to separate provided

$$10^{-11} < M/L < 0{\cdot}057 \tag{5}$$

where M and L are measured in solar units. The upper limit in expression (5) results from the requirement that the radiation force on each electron or positron exceeds the gravitational force; the lower limit follows from the condition that the gravitational force on the protons and antiprotons be greater than the radiation force.

Expression (5) is satisfied by supergiant stars and by main-sequence stars of types O and B. But stars obviously cannot be used to explain the initial stages, at least, of matter–antimatter separation in a charge-symmetric universe. Instead, we might appeal to objects deriving their luminosity from annihilation. Alfvén and Elvius, among others, have suggested that quasi-stellar objects are such entities (ref. 8 and a paper at the IAU Symposium No. 44, Uppsala, 1970). Although very little information on the masses of these objects is available (see ref. 9), it is likely that they obey condition (5). For example, if a quasi-stellar object with $L \approx 10^{47}$ erg s^{-1} has a respectable galactic mass of 10^{11} solar masses, then $M/L \approx 4 \times 10^{-3}$ solar units.

Another possibility is provided by the cosmology of Alfvén and Klein[7]. According to this scheme, an initial contraction of the metagalaxy (containing equal amounts of matter and antimatter) resulting from its self-gravitation was turned into an expansion by the pressure of radiation produced by annihilation reactions. If we suppose that the proton–antiproton and electron–positron gases are effectively coupled by a magnetic field which does not otherwise affect the dynamics, we obtain the equation of motion adopted by Alfvén and Klein

$$N_p m_p \frac{\mathbf{du}}{dt} = N_p m_p \mathbf{g} + N_e \frac{\sigma \mathbf{F}}{c} \qquad (6)$$

in which $\mathbf{u}$ is the gas velocity, N_p is the number density of protons (or antiprotons), and N_e is the number density of electrons (or positrons). Equation (6) may be rewritten in the form

$$N_p m_p \mathbf{g}_{eff} = -N_e \frac{\sigma \mathbf{F}}{c} \qquad (7)$$

where

$$\mathbf{g}_{eff} = \mathbf{g} - \frac{\mathbf{du}}{dt}$$

is the effective value of the gravitational acceleration, which should replace $\mathbf{g}$ in equation (1). With this substitution, we see from equations (1), (2) and (7) that matter and antimatter drift in opposite directions and, moreover, that the flux of proton gyration centres perpendicular to the magnetic field is equal to the flux of electron gyration centres (and similarly for antiprotons and positrons). Although the gravitational and radiation fields in this case are metagalactic, the scale of the matter–antimatter separation is determined by the magnetic field and is likely to be much more local.

The second general case in which $\mathbf{g}$ and $\mathbf{F}$ are antiparallel does not involve radiation generation by the source of the gravitational field but relies on the (presumably) black-body radiation of the universe. This radiation is usually discussed in terms of the big-bang theory and may[10] or may not[4] be consistent with a charge-symmetric cosmology. Let us consider a gravitationally bound system which is not participating in the universal expansion. As pointed out by Peebles[11], the black-body radiation tends to flow out of such a system because the radiation density decreases as the universe expands. Thus there is a net radiation flux which is antiparallel to the gravitational field of the system as required for the operation of the separation mechanism.

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Observations of High Velocities in H II Regions

SEVERAL investigators using a variety of optical instruments have indicated that small components of the ionized matter in H II regions are in a highly turbulent state[1–5]. In particular, Shcheglov[3], using a photographic, single etalon Fabry–Perot at Hα in a non-classical combination with a telescope, found small components with turbulence of up to 100 km s^{-1} in M17 (NGC 6618), M8 (NGC 6523), the Orion nebula (NGC 1976) and M16 (NGC 6611). With this instrument he effectively took photographs at three adjacent positions in wavelength through a tunable 0·7 Å filter of the whole of these nebulae. They changed shape at different wavelengths though this was only apparent with widely varying exposure times.

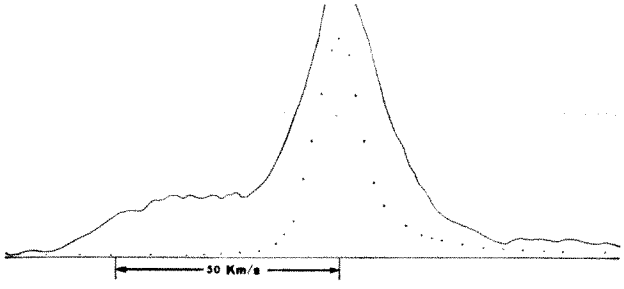


Fig. 1. The [O III] (5007 Å) line from a position in the centre of M16 is shown compared with the instrumental profile (dotted curve).

An exact measurement of the line shapes from the dense cores of several bright H II regions was desirable to reveal this turbulence and, if confirmed, to indicate its nature. For this it was necessary to reduce the ambiguities introduced by the single etalon Fabry–Perot but to retain its high potential luminosity–resolution product (L × R pot)[6]. I used a two etalon, propane scanned photoelectric Fabry–Perot, which has a high L × R pot, and a resolution of 0·2 Å but a very large free spectral range. A thermo-electrically cooled (−20° C) EMI 6256A photomultiplier was used as the detector. This spectrometer was combined classically with the 1 m reflecting telescope at the Pic-du-Midi observatory to convert its entrance cone into a smaller solid angle on the sky without significantly diminishing its L × R pot. The [O III] (5007 Å) line was chosen in preference to Hα because it is as bright, its thermal broadening is approximately four times less than Hα whereas the turbulent broadening, which is of interest here, remains unchanged, it is predominantly emitted by the dense cores of H II regions in the hardest radiation field of the exciting stars where any high velocity gas is expected to originate, and this line can be detected at three times the quantum efficiency of Hα. A 30 Å passband Fabry–Perot type filter was used to isolate the line.

Twenty-eight positions in the bright cores of three nebulae (M8, M16 and M17) in the Sagittarius arm were

observed with a 1′ beamwidth. The spectrometer and telescope combination was then being used well below L×R pot. Consequently, even extreme variations in brightness across the field of view could not significantly affect the shape of the profile.

The most remarkable scan of the 5007 Å line obtained from one position in the centre of M16 is shown in Fig. 1, together with the primary maximum of the instrumental profile. Clearly the line is split into two components; the faintest is being emitted by ionized matter approaching at 35 km s^{-1} with respect to the bulk of the nebular material. From other regions of M16, single though very broad lines (0·55 Å wide) are being emitted. The thermal width of the 5007 Å line at 10,000 K is only 0·1 Å.

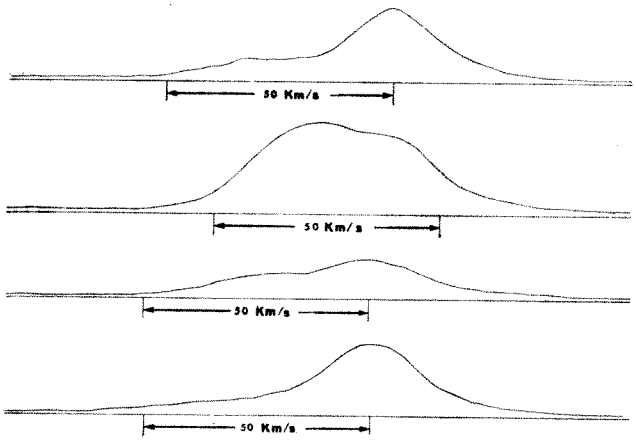


Fig. 2. The [O III] (5007 Å) line from four positions in the centre of M17.

In Fig. 2 the four most unusual scans that were obtained from four positions on M17 are shown. It is apparent that the nebular lines, that are convolved with the instrumental profile to give the scans that are presented here, must in some cases be strongly split and in other cases have turbulently broadened wings. Scans from many other positions in the core of this nebula indicate broad (around 0·3 Å) though more symmetrical lines. A very high signal to noise ratio (500 to 1) was achieved in the scan of the 5007 Å line from the region of M8 containing the 'Hourglass'. It is evident that the bulk of the matter is emitting an asymmetric 5007 Å line approximately 0·35 Å wide. The only other feature to produce a signal greater than 0·2% of this principal line within ±1·75 Å from it was a feature with 0·6% of the signal strength of the primary but 0·93 Å to its short wavelength side. This could be a 0·6% split component of the 5007 Å line being emitted by a small percentage of the matter approaching at 55 km s^{-1} with respect to the bulk of the nebular matter. But this feature is exactly on the level of uncertainty introduced by the partially transmitted [O III] line at 4959 Å. This will lie somewhere between 0·9 Å and 2·7 Å to the short side of the primary profile of the [O III] line at 5007 Å. Other possible sources of confusion for all these scans are negligible and need not be considered. These are the contributions from the He I (5016 Å) nebular line, airglow lines and Fabry–Perot ghosts. Only further observations will resolve the nature of this interesting feature on the scan of M8.

Here I communicate only the unambiguous confirmation and initial explanation of Gershberg and Shcheglov's[2,3] observations of M16 and M17. Some doubt still remains about M8 and no study of the Orion nebula has yet been carried out with this two etalon Fabry–Perot spectrometer. The physical interpretation of the exact deconvolution of all the irregular profiles will be reported elsewhere.

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Photometric History of BL Lacertae

IN the discovery paper of BL Lac forty years ago, Hoffmeister[1] classified the object as a short-period variable with a range of m_{pg} of 13 to 15. Subsequent unpublished observations were made by van Schewick[2], and Semakin[3] observed a range of m_{pg} of 13·0 to 16·1 between 1950 and 1954. Schmitt[4] identified BL Lac with the radio source VRO 42.22.01, whose radio spectrum has been investigated by MacLoed and Andrew[5]. Optical and near infrared spectrophotometry by Visvanathan[6] and by Oke *et al.*[7] showed a steepening decline in flux density from 10^{14} to 10^{15} Hz. The nonthermal nature of the radiation and the optical variability resemble the characteristics of compact galaxies and quasistellar sources. Recently Tschäpe[8] re-examined the Sonneberg plate material and found a range of $m_{pg} = 12{\cdot}4$ to 16·1 during 1928 and early 1929. Modern photographic and photo-electric monitoring[9–12] showed that the variability of BL Lac is extremely irregular over periods of a few hundred days of continual observation. In view of the interest in the past variability of BL Lac expressed at the recent meeting of the International Astronomical Union, we present here photometric data derived from the archive plate collection of Harvard College Observatory. Our conclusions, based on the available BL Lac light history, are (1) the brightness of the object is fainter than 14 magnitudes most of the time; (2) the object has reached or exceeded magnitude 13 during two periods in its history; (3) during the second outburst, the brightness increased secularly throughout practically its entire range, and subsequently decreased again, the whole episode lasting about 10 years; and (4) there is a similarity in the optical behaviour of BL Lac and the N-galaxy 3C371.

We divided the Harvard plate material into two groups: (i) 114 plates taken with *f*/7 cameras of small aperture (3 inch) such as the RH and related patrol series; (ii) 51 plates taken with cameras of similar focal ratio but larger aperture (≥8 inch) such as the IR, MA, MC series. These 165 plates comprise all those available, excluding series with camera apertures < 3 inches and series in which BL Lac is confused with the image of the nearby star b in the comparison sequence of Bertaud *et al.*[9].

Fig. 1 summarizes the Harvard results as well as published data of other authors, and contains 60 Harvard data points of which 27 are determined by the Harvard Askania iris astrophotometer and 33 are based on visual estimates. The probable error of the iris-photometric points, as estimated from the scatter in plate calibration curves, is almost always ± ≤0·2 magnitudes. The visual estimates based on plates of group (ii) agree well with iris-photometric values in those cases where both exist. The visual estimates based on plates of group (i) are usually of poorer accuracy; the probable error can be as high as 0·5 magnitudes in the worst cases. Consequently,

although visual estimates have been made for all available Harvard plates, we have included in Fig. 1 only 12 group (i) visual estimates of special interest. The remaining available visual estimates, most of which merely conform to the trends shown in Fig. 1, have not been included pending iris-photometric evaluation. The photo-electric comparison sequences of Bertaud *et al.*[9] and of Kinman were used in our photometry. In view of the large brightness changes shown in Fig. 1, we consider it unnecessary to correct for the small differences in the photometric systems used by the various authors whose data are represented in Fig. 1 or for a small putative hour-angle effect[13] for nonthermal sources.

Fig. 1 reveals an interesting property of the light curve, that is, the brightness lies between magnitudes 14·0 and 16·6 most of the time but undergoes rare excursions to magnitude 13·0 or brighter. Only two such giant outbursts have been recorded. The first, observed by Tschäpe on Sonneberg plates, occurred over at least 400 days in 1928 and early 1929 during which the brightness increased to about 12·4 magnitudes with several rises and declines through almost the entire known range of variability[8]. The second was a much more prolonged and steady rise, again through almost the entire known range of variability, between 1950 and the late 1950s. This rise is documented by eight Semakin data points and fifteen visual and iris-photometric points from Harvard RH plates (group i) as well as by a visual estimate based on Palomar Sky Survey prints. The rise is shown in Fig. 2 on an expanded time scale. The Sonneberg data (open rectangles) and Sonneberg upper limits shown in Fig. 1, together with Harvard visual estimates not included in Fig. 1, also describe a subsequent decline in the later 1950s to fainter than magnitude 15·5 by the 1960s. Despite the comparatively large maximum probable error of the group (i) visual estimates ($\pm \leq 0{\cdot}5$ magnitudes), it can be reasonably inferred that BL Lac has undergone a long-term brightness increase and decline spanning 10 to 15 years. Inspection of our visual estimates of RH plates (group i) shows no other such giant outburst after 1930. Available Harvard material before 1928 is too scanty to decide whether outbursts occurred then.

The optical behaviour of BL Lac bears some resemblance to that of the N-galaxy 3C371 (ref. 14). This object

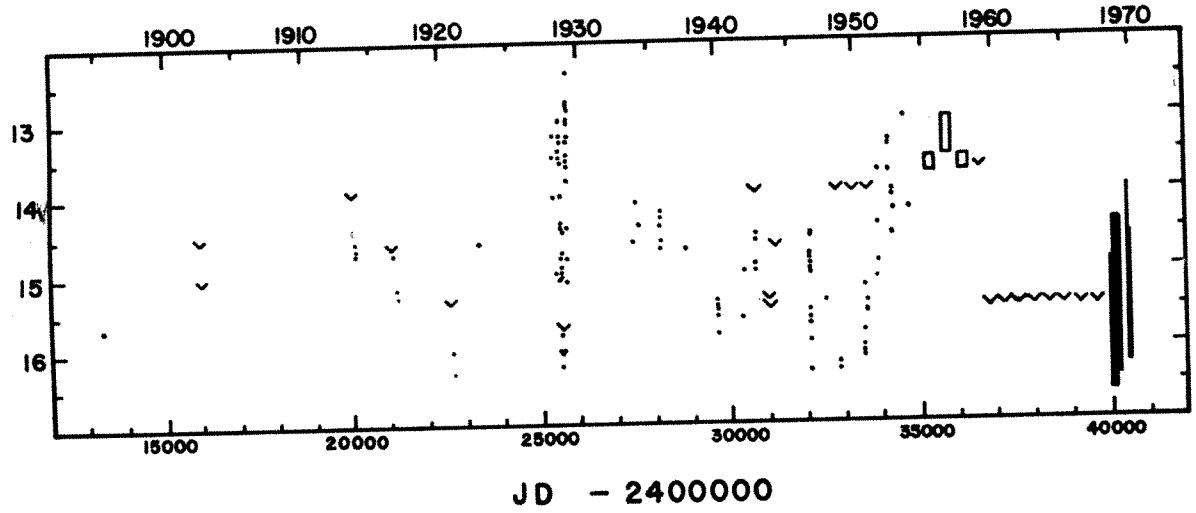


Fig. 1. Composite photometric history of BL Lac from 1895 to 1970. Axes are magnitudes (B or m_{pg}) and Julian days. The tip of a "V" denotes an upper limit in brightness. Filled rectangles enclose recent observations by various authors[9–12], open rectangles the data of Tschäpe[8]. All data in 1928 and all upper limits at 15·5 after 1958 are from Tschäpe. Eight points in the early 1950s are from Semakin[3] (see Fig. 2). The remaining points are from the Harvard plate collection. All upper limits brighter than magnitude 14 have been omitted.

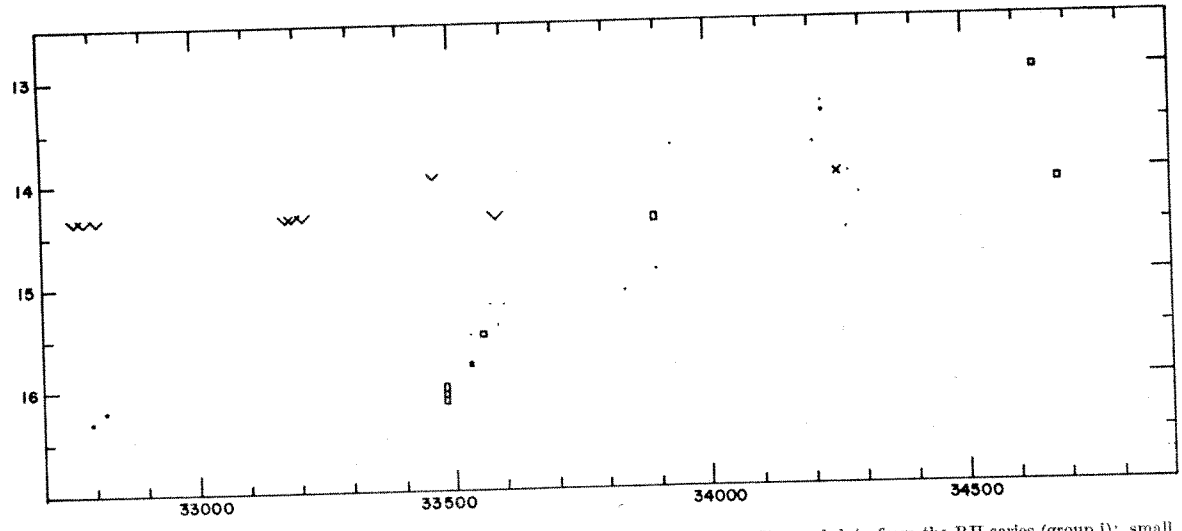


Fig. 2. A portion of the light curve of BL Lac on an expanded time scale. Dots are Harvard data from the RH series (group i); small dots are visual estimates, large dots iris-photometric values. Squares are Semakin's data. The cross is a visual estimate from the Palomar Sky Survey prints. Upper limits are indicated by "V". All upper limits brighter than magnitude 14 have been omitted. Axes are as in Fig. 1.

too has undergone a sustained outburst which lasted about fifteen years or more from 1895 to 1911. There is also scant evidence that a second outburst of 3C371 may have occurred fifty years later which lasted no more than ten years and which, like the 1928 outburst of BL Lac, was slightly brighter than the previous outburst. Kinman, Harlan and Marosan have pointed out still other similarities between BL Lac and 3C371. Terrell and Olsen[15] have shown that the light curve of 3C273 could be reproduced by a shot-noise simulation. It would be interesting to ascertain whether such a shot-noise model could also reproduce with reasonable probability the variations exhibited by BL Lac and 3C371.

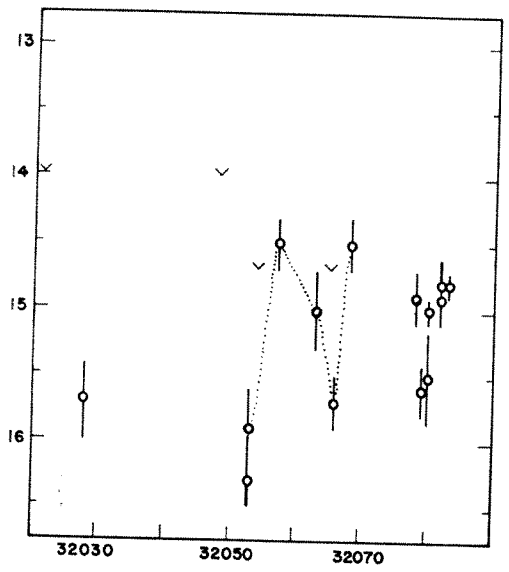


Fig. 3. A portion of the light curve BL Lac on an expanded scale. Upper limits are indicated by "V". All points and upper limits are derived from plates of the Harvard IR series (group ii). Axes are photographic *B* magnitudes and Julian days.

Fig. 3 shows another portion of Fig. 1 on an expanded scale. The dotted line indicates a brightening of some 1·5 magnitudes over four days (at a rate of 0·4 magnitudes per day) followed by a decline of 1·2 magnitudes over nine days (0·13 magnitudes per day) which is followed again by an abrupt return to the previous maximum value in two days (0·6 magnitudes per day). All these rates rely on single plates, but are consistent with the high rates found in modern observations[9–12]. One other well documented pulse, observed by Bertaud *et al.*[9] near JD 2440065, exhibits very similar properties; a brightening by almost 1 magnitude in three days followed by a decay over seven days to the original brightness. In both the case of Bertaud *et al.* and our case, the decay rate is about 0·13 magnitudes per day. It is interesting to recall that Type 1 supernovae show a comparable (0·1 magnitudes per day) decay rate near maximum. Multichannel-scanner observations of BL Lac have so far shown no obvious spectral features[6,7]. Because type I supernovae characteristically show line spectra a few days after maximum, it would be interesting to see whether spectral features could be detected in BL Lac near the end of an outburst of the kind shown in Fig. 3.

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Pulsar Ejection in Supernova Events

Although pulsar formation is generally attributed to supernova events, only two pulsars are associated convincingly with known supernova remnants (the Crab Nebula and Vela). Other pulsars are close enough to remnants to suggest association[1], but only if they are moving away from the remnant at velocities of the order of 10^3 km/s. Another problem seems to be that a supernova core may well be too massive to form a gravitationally stable object[2] (for example a "neutron star"), and gravitationally collapsing objects ("black holes") do not presently seem promising candidates to be pulsars.

Fowler and Hoyle[3] suggested some time ago that the symmetric ejection of radio-luminous material from galaxies could be caused by asymmetrical processes occurring in the collapse of very massive objects. Indeed, it is known that such an imploding system is Rayleigh–Taylor unstable. Thus the more massive core could fission into several less massive objects. The same idea can be applied to a supernova event, wherein the core fragments into some distribution of neutron stars, black holes and general debris (planetesimals, dust and so on). The number of fragments should be few, for the disparity between core mass and neutron star masses is not large. Such a multiple system would be readily detectable either directly as superimposed pulsars or indirectly from the orbital perturbations to the observed pulsar's period. At present, there is only a suggestion[4] that some pulsars have companions and then only of planetary mass.

Our point here is to show that the resultant system can be expected to become unbound, with the component objects (not all of which need be pulsars) ejected with velocities of the order of 10^3 km/s. Consider the binary fission of a supernova core into equal mass neutron stars. The collapse time to form two stars is expected to be of the same order as the time for the two stars to fall together. Furthermore, the conservation of the angular momentum of the original core would instead place them in highly excentric orbits as shown in Fig. 1. The semi-major axis would be comparable with the initial core radius, while the closest approach distance would be determined by the initial angular momentum of the core. Because final condensation occurs at the highest system

density, this will also be when the fragments are closest together.

At closest approach, the kinetic energy relative to the barycentre can be as high as a tenth of the rest mass (for "contact" trajectories), yet the system is only slightly bound. If now the rest mass of the system is rapidly reduced by a fraction f, the system would become unbound[4-7] by f times the gravitational binding energy less the total binding energy. The velocity at infinity would then be given from

$$V^2/c^2 = GM(2f-1+e)/2a \qquad (1)$$

where a is the closest approach distance to the barycentre, f is the mass loss fraction and e is the orbital eccentricity (nearly unity for the model described above). The eccentricity can be estimated roughly from

$$(1-e)/(1+e) \approx a/R_{\rm core} \geq R_{\rm pulsar}/R_{\rm core} \qquad (2)$$

which gives values for $1-e$ of about 5×10^{-3} for present supernova models. Escape velocities are thus obtained if $f \geq (1-e)/2$ or about $2{\cdot}5\times10^{-3}$. If nongravitational forces between the two fragments could be neglected, we would have

$$1-e = 2w_0^2 r_0^3/GM \qquad (3)$$

where w_0 is the initial rotation frequency of the core (radians/s) and r_0 is the initial distance between the centre of mass of each fragment-to-be and the total centre of mass ($r_0 \approx \frac{1}{2}R_{\rm core}$). Thus for one solar-mass object

$$1-e = 1{\cdot}8\times10^{-12}\, w_0^2\, R({\rm km})^3 \qquad (4)$$

and for nominal values ($w_0 = 0{\cdot}1$, $R = 10^3$), $1-e$ is even smaller than given in equation (2). Because nongravitational forces must act if the closest approach is less than the diameter of the neutron star, "contact" trajectories should be favoured. Note that relatively little energy is required to adjust the closest approach distance. The rest-mass energy fraction released in the neutron star formation event is perhaps of the order[8,9] of $f = 3\times10^{-2}$, which would give a recession velocity of 2×10^4 km/s for $a = 10$ km. The mechanism is less efficient if the energy is lost slowly, and a rough correction may be estimated from the replacement

$$f \rightarrow f^* \approx f(t_a/t_{\rm decay})^{2/3} (t_{\rm decay} > t_a) \qquad (5)$$

where

$$t_a^2 = a^3/GM(1+e) \qquad (6)$$

is the time for one radian of orbital motion at periapsis, and t decay is the characteristic time to release energy in the formation event.

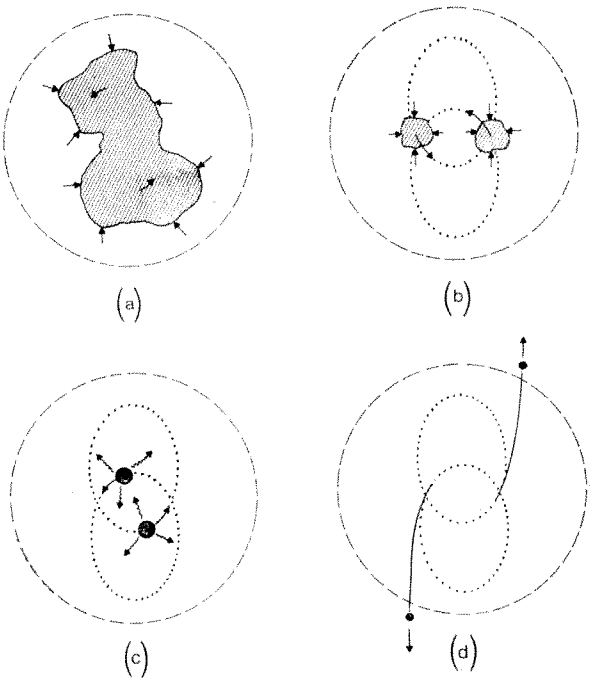


Fig. 1. Schematic development of run-away pulsars at the phases (*a*) collapse and fragmentation, (*b*) condensation, (*c*) radiation and (*d*) disruption. Dashed line shows boundary between ejected shell and collapsing core. Dotted lines show bound orbits after separation but before radiation. A complete orbit is not executed.

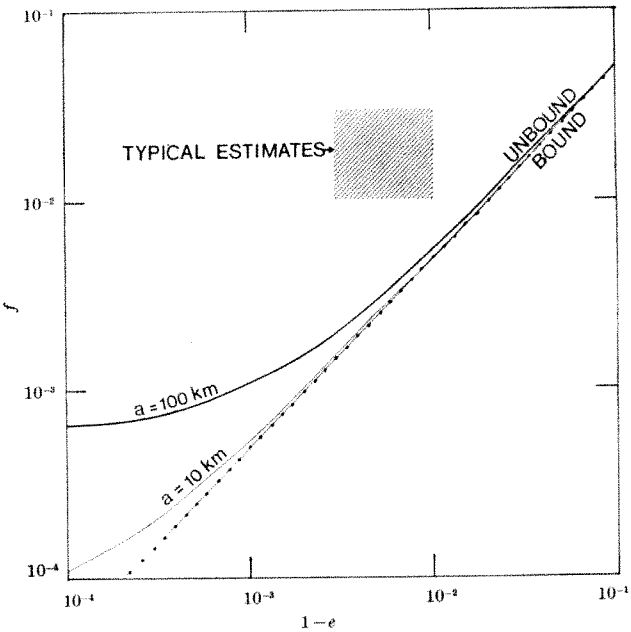


Fig. 2. Regions of f and e that permit disruption for various values of a assuming one solar mass pulsars. The mass-loss fraction f should be replaced by f^* if the emission is slow (see equation (5)). Above the solid lines for the appropriate value of a, the velocity exceeds 10^3 km/s, while below the dotted line, the system remains bound.

Fig. 2 shows the values of f, e and a required to produce runaway pulsars at a velocity in excess of 10^3 km/s. We see that nominal energy release values and stellar dimensions permit recession velocities of the order of or greater than 10^3 km/s. Similar ideas could be applied also to massive objects[3,7-9].

It is not clear from the available data whether such runaway motions are required for the pulsars. The absence of observed pulsars associated with the other supernova remnants could be attributed to the formation of non-pulsar objects (M. Rees, private communication) or of pulsars that are not beamed towards us. On the other hand, the mechanism proposed here seems capable of producing runaway pulsars whether they are yet required by the data or not. The detection of such objects would thereby be suggestive of fragmentation in the supernova event.

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Absence of Average Polarization at 111·5 MHz in Pulsar NP 0532

THE average polarization properties of NP 0532 have been measured at about 400 MHz by us[1], by Manchester (private communication) and by Schonhardt[2]. Strong linear polarization is present in the precursor while the other pulse components are only weakly linearly polarized and the position angle is independent of pulse phase. The polarization properties of strong single pulses have been measured at high frequency by ourselves[3,4] and at lower frequencies by Staelin and Sutton[5], who have shown that large linear polarization does not occur in strong pulses. We have now measured the four Stokes parameters of the average pulse at 111·5 MHz.

The only difference in technique from our previous work was the use of a feed which yielded two orthogonal linear polarizations. The details of obtaining the Stokes parameters then differ slightly from our previous experiment in which two circular polarizations were provided by the feed. The four channels produced at baseband were passed through identical filters having a double-sideband width (to the 3 db points) of 5 kHz and were then digitized and recorded synchronously with the pulsar at 100 μs intervals throughout the entire pulsar period. We assumed the average intensity from the pulsar and the Crab Nebula itself to be unpolarized and used this as a calibration to eliminate instrumental polarization; thus the experiment was insensitive to time-independent linear polarization in the pulsar.

We carried out the observations for 3 h on both January 7 and January 11, 1970. The critical frequency of the ionosphere was measured at approximately 15 min intervals during the observations, using the ionosonde at Arecibo Observatory, and ionospheric Faraday rotation was calculated approximately using ionospheric density profiles supplied by G. Nelson.

Having accounted for rotation of the feed during tracking, we found that ionospheric Faraday rotation had rotated the position angle of linear polarization through about 140 degrees during each run. Each day's data were reduced in a series of short blocks accounting properly for Faraday rotation, and additionally in a series of short blocks in which the effects of Faraday rotation were ignored. In no case did we find appreciable linear or circular polarization. The present observations thus imply

$$P = \frac{\text{Peak-to-peak variation in } Q, U, \text{ or } V}{\text{Peak-to-peak variation in I}} \leq 0{\cdot}2 \quad (1)$$

The precursor pulse component at 430 MHz is essentially 100 per cent linearly polarized and is responsible for about 0·4 of the integrated (continuum equivalent) flux density. If the relative energies and polarization properties of the emitted pulse are similar at 111·5 MHz then the broadened observed pulse should exhibit more than 50 per cent linear polarization in the vicinity of the main pulse. Several explanations for the observed lack of polarization are plausible: the precursor may have become weak relative to the other pulse components at 111·5 MHz; it may not be strongly polarized on emission at the pulsar; or a strongly polarized precursor may have become depolarized in the medium in transit to Earth.

None of the above possibilities are subject to direct confirmation; however, Rankin *et al.*[6] have measured the integrated flux density of the precursor relative to the main pulse and found that the ratio is roughly independent of frequency in the interval 430 to 196·5 MHz. They argue that although the main pulse and precursor cannot be resolved at 111·5 MHz it is unlikely that their relative flux changes greatly in the octave between 196·5 and 111·5 MHz. In this case we may ask whether the depolarization is intrinsic to the source or occurs in the medium between Earth and the pulsar. Clearly, a strongly polarized precursor is severely broadened by scattering in the interstellar medium[6]; the scattering will not in itself change the fraction of linear polarization or its sense. But scattering introduces the possibility that differential Faraday rotation may occur on the various paths of propagation. We expect the scattering angle to be about 6×10^{-7} radians on the basis of the current knowledge of interstellar scintillation[6] and we believe it is unlikely that depolarization due to differential Faraday rotation occurs over such a small angular extent. (An observational indication of the importance of differential Faraday rotation at these low frequencies is provided by polarization measurements on quasars, which are observed to be linearly polarized at high frequency.)

Thus it seems most probable that either the pulsar's 111·5 MHz radiation is depolarized in the Crab Nebula, or in the immediate vicinity of the pulsar, or that the pulsar's radiation at low frequency is not strongly polarized on emission. A need for further polarization measurements of the Crab Nebula pulsar at intermediate frequencies is clearly implied.

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Battery Effect in Neutron Stars

RECENTLY there has been interest in the problem of intense magnetic fields in gravitationally collapsed bodies[1,2]; in particular the radio emission from neutron stars (pulsars) suggests strong magnetic fields of the order of 10^{12} G (refs. 1, 3 and 4). The battery effect for nonuniformly rotating early main sequence stars has been considered recently[5,6], but we will show in this communication that there is a possible battery effect in neutron stars which has a different physical cause.

Let us consider a rotating star which is composed only of neutrons, protons and electrons, and assume that they are completely degenerate. I have considered such a model of the neutron star and shown that the magnitude of the stationary magnetic field cannot be more than a few gauss. Here I shall show that there is a possibility that a non-stationary magnetic field of the order of 10^{12}G can be generated.

The effect of gravity will be considered using the post-Newtonian approximation. We assume that $\varphi/c^2 \ll 1$, so that only first order corrections in φ/c^2 to the Newtonian theory will be included in the basic equations of this problem. Then the space–time metric will be[3]

$$ds^2 = g_{ik}\, dx^i\, dx^k \quad (1)$$

where

$$g_{00} = 1 + \frac{2\varphi}{c^2}, \quad g_{\alpha\beta} = \left(1 - \frac{2\varphi}{c^2}\right)\delta_{\alpha\beta}$$

$$g_{0\alpha} = 0 \quad \alpha,\beta = 1,2,3$$

and where φ is the gravitational potential. If we denote **E** and **D** as the vectors of the electric field, and **H** and **B** as the vectors of the magnetic field, then Maxwell's equations have the form[8]

$$\text{div}\,\mathbf{D} = 4\pi e n_1 \quad (2a) \qquad \text{curl}\,\mathbf{E} = -\frac{1}{c}\frac{\partial \mathbf{B}}{\partial t} \quad (2d)$$

$$\text{div}\,\mathbf{B} = 0 \quad (2b) \qquad \text{curl}\,\mathbf{H} = \frac{4\pi}{c}\mathbf{S} + \frac{1}{c}\frac{\partial \mathbf{D}}{\partial t} \quad (2e)$$

$$\mathbf{D} = \frac{\mathbf{E}}{\sqrt{g_{00}}} \quad (2c) \qquad \mathbf{B} = \frac{\mathbf{H}}{\sqrt{g_{00}}} \quad (2f)$$

where en_1 and **S** are the densities of the electric charge and the electric current respectively. The macroscopic equation of motion for plasma in the space we are considering has the form[11]

$$\frac{\mathbf{S}\wedge\mathbf{B}}{cn_e} - \sqrt{g_{00}}\,\nabla P + mn(\nabla\varphi - \Omega^2\tau)\sqrt{g_{00}} = 0 \quad (3a)$$

$$\frac{\mathbf{S}}{\sigma} = \mathbf{E} + \frac{\mathbf{S}_p\wedge\mathbf{B}}{cn_e} + \frac{\sqrt{g_{00}}}{en_e}\nabla P_e - \frac{\mathbf{S}\wedge\mathbf{B}}{ecn_e} \quad (3b)$$

$$n_e = \alpha^{3/2} n \quad (3c)$$

$$\mathbf{S} = \mathbf{S}_p - \mathbf{S}_e = en_e(v_p - v_e), \quad v^\beta = \frac{dx^\beta}{dt} \quad (3d)$$

where τ is the distance from the axis of rotation, n is the density of neutrons, P is the pressure of neutrons, σ is the conductivity, $-e$ is the charge of the electrons, c is the velocity of light and v_p and v_e are the velocities of protons and electrons respectively. We assume that the density of electrons n_e is equal to the density of protons and the mass of the proton m equals the mass of the neutron. α is the ratio of the mass of the electron to the mass of the proton, and $1+\alpha$ is replaced by 1 since $\alpha \sim 5\times10^{-4}$. Equation (3) was obtained from the equation of hydrodynamic equilibrium written in its correct Einsteinian form[11].

If we take the curl from equation (3*b*), taking into account the fact that P is a function of n only and P_e of n_e only, and using equation (3*a*), then we have

$$\frac{\partial \mathbf{B}}{\partial t} = \frac{m}{e}c\Omega^2\frac{1}{c^2}\frac{\partial\varphi}{\partial R}\sin\theta\cdot\cos\theta\,\tau - c\,\text{curl}\frac{\mathbf{S}}{\sigma} - c\,\text{curl}\frac{\mathbf{S}\wedge\mathbf{B}}{cen_e} \quad (4)$$

where τ is a unit vector in the direction of the azimuthal angle φ. R, θ, φ are spherical coordinates. To obtain this equation we have used Maxwell's equation (2*d*) and the relations

$$\text{curl}\,\mathbf{E} = (\nabla\varphi - \Omega^2\tau)\wedge\nabla\sqrt{g_{00}}, \quad P_e = \alpha^{3/2}P$$

We assume that at the moment $t=0$ the star rotates like a solid body with angular velocity Ω. Then $\mathbf{v}_e^0 = \mathbf{v}_p^0$ and according to (3*d*) $\mathbf{S}=0$. The last two terms in equation (4) will vanish but the first term will be finite and thus a toroidal magnetic field is generated in the star. Provided that we include in the investigation the first order corrections of general relativity to the Newtonian theory, then we have the result that the effective angular velocity $\Omega(g_{00})^{1/4}$—where Ω is constant—depends on R. The electrons are driven relative to the protons, and the electric currents produced grow in time[5,6] and generate a toroidal magnetic field. The first term in equation (4), which describes the battery effect, is a constant in time, if Ω is a constant. But the next two terms (which have a tendency to stop the increase of the magnetic field) will grow because, according to Maxwell's equation

$$\mathbf{S} = \frac{c}{4\pi}\,\text{curl}\,\mathbf{B}$$

the electric currents **S** increase with the toroidal magnetic field. The length of time in which the Ohm term balances the battery term is $D^2\sigma/c^2$, where D is a magnitude commensurate with the radius of the star. The conductivity of a neutron star has been calculated[9] and was found to be of the order of 10^{29} s^{-1}. The length of time in which the Hall term becomes equal to the battery term is

$$\left(\frac{D^2\,en_e}{cA}\right)^{1/2}$$

where A is the speed at which the magnetic field increases and is given by

$$A \sim \left|\frac{m\Omega^2}{ec}R\cdot\frac{\partial\varphi}{\partial R}\right|$$

The speed at which the magnetic field increases depends on the central density of the matter of the neutron star. For a lower central density $\rho_c = 8\times10^{13}$ g cm^{-3} for which the mass is $M = 0{\cdot}12\ M_\odot$, the radius $R = 1{\cdot}2\times10^7$ cm, the maximal angular velocity $\Omega = 37$ s^{-1} and the average density of the electrons of the order of 10^{34} cm^{-3} (ref. 10). The speed of growth of the magnetic field is of the order of 10^{-4} G s^{-1}. For this model of the neutron star, the length of time in which the Ohm term balances the battery term is 10^{15} years, and the length of time in which the Hall term becomes equal to the battery term is of the order of 10^9 years. In order to find the magnitude of the maximum magnetic field, it can be assumed that on the time scale of the order of 10^9 years, the growth of the magnetic field depends linearly on time; this gives a field $\sim 10^{12}$ G.

For a higher central density $\rho_c = 5{\cdot}5\times10^{14}$ g cm^{-3}, for which the mass is $M = 0{\cdot}64\ M_\odot$, the radius $R = 10^6$ cm, the maximal angular velocity $\Omega = 6\times10^3$ s^{-1} and the average density of the electrons of the order of 10^{35} cm^{-3}, the speed of growth of the magnetic field is of the order of 3×10^2 G s^{-1}. The length of time in which the Ohm term balances the battery term is 10^{13} years, and the length of time in which the Hall term becomes equal to the battery term is only 10^5 years. This means that the maximum magnetic field is of the order of 10^{14} G.

The pulsar, which is known to exist in the Crab Nebula, has an age of the order of 10^3 years. If we identify this pulsar with the higher density model, and suppose that its angular velocity decreases linearly from 6×10^3 s^{-1} to 30 s^{-1}, then in 10^3 years the magnetic field can grow to $\sim 10^{12}$ G.

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Comparison of Precise Radio Source Positions with Independent Optical Positions

In a recent letter[1], Wade *et al.* pointed out a systematic difference in right ascension between two independent surveys carried out at 2,695 MHz, one by the National Radio Astronomy Observatory, Green Bank, and the other by the Royal Radar Establishment, Great Malvern. The weighted mean difference in the sense NRAO minus RRE for 27 sources was $-0{\cdot}044$ s with a standard error in this mean of $\pm 0{\cdot}006$ s.

Each survey used essentially the same method to fix the scale in right ascension. The interferometer gave differential measurements to a high degree of accuracy, and the zero point was then fixed by comparison with astrometric positions for the optical counterparts of certain sources of small angular diameter. These optical positions were taken from various sources in the literature, and Wade *et al.* concluded that most of the discrepancy between the NRAO and RRE surveys must have arisen because of systematic differences between the various sets of astrometric measurements used by the two groups.

We have measured optical positions for thirteen sources in the RRE catalogue. We used the 43 cm classical Schmidt telescope at Cambridge. The reference frame was AGK 3 exclusively. It is legitimate to compare with the radio surveys because none of our measurements has been used in any of the calibrations. A comparison with RRE is set out in Table 1. 3C 9 is badly discordant and will be omitted from further discussion.

The mean difference Cambridge optical minus RRE in right ascension is $-0{\cdot}029$ s with a standard error in this mean of $\pm 0{\cdot}006$ s (Table 2). This difference is therefore highly significant, being five times its standard error. Comparing this with the difference NRAO minus RRE = $-0{\cdot}044$ s already quoted, we conclude that our optical measurements are somewhat closer to NRAO than to RRE as regards right ascension. In declination, on the other hand, there is no significant difference shown in Table 2 between us and RRE.

The RRE zero point in right ascension was fixed using 36 calibrators. The optical data were derived[1] from Griffin, from Veron, from Bolton and from Murray *et al.* Griffin's reference frame was the Yale zone catalogue except for declinations between $+30°$ and $+50°$ for which he used AGK 2. Veron used AGK 2 for declinations north of $-2°$, otherwise the Yale catalogue. Bolton used the Yale catalogue only. Murray *et al.*[2] used BD stars linked directly to FK 4 by transit observations, giving a system essentially equivalent to AGK 3. But since Murray *et al.* cannot have contributed more than 16 out of the total of 36 calibrators, it is clear that the RRE catalogue is tied mainly to a system based partly on the Yale catalogue and partly on AGK 2.

The NRAO survey used optical positions provided by Kristian and Sandage (unpublished). These authors measured with reference to an AGK framework for declinations north of $-2°$, and against the Smithsonian star catalogue southwards. At the time of writing, AGK 3 is available only north of declination $+10°$. Not having exact details it is impossible to make precise statements, but it seems that most sources in the Kristian and Sandage catalogue are tied to AGK 3, with a zone of intermediate declinations extending down to $-2°$ where measurements are tied to AGK 2 without proper motion updating, and finally a more southerly zone tied to the Smithsonian catalogue. Generally good agreement with our AGK 3 based measurements is therefore to be expected and has indeed been found.

Finally, we may note that the standard errors for one position difference in Table 2, namely $\pm 0{\cdot}022$ s in right ascension and $\pm 0{\cdot}28$ arc s in declination, are significantly lower than the corresponding values given by Wade *et al.*[1] for the differences between the two radio surveys, $\pm 0{\cdot}034$ s and $\pm 0{\cdot}50$ arc s respectively. These figures indicate that our optical measurements are of higher internal consistency than at least one of the radio catalogues. It would be inappropriate to discuss this in detail on the basis of the present limited series of optical observations, except to remark that it indicates that radio and optical astrometry are of comparable accuracy. If the structure of extra-galactic objects is to be investigated by intercomparing radio and optical configurations, then it is clear that a severe limitation may be imposed by inconsistencies between the radio and optical reference frames. Radio astronomers who rely on optical calibrations would have to exercise great care in the selection of optical data. Its reference frame would need to be self consistent over the sky to an accuracy of one or two-tenths of an arc s if the radio accuracy is to be fully

Table 1. RADIO AND OPTICAL SOURCE POSITIONS

Source	Right ascension 1950.0 RRE	Right ascension 1950.0 Cambridge optical	Right ascension 1950.0 Cambridge optical—RRE	Declination 1950.0 RRE	Declination 1950.0 Cambridge optical	Declination 1950.0 Cambridge optical—RRE
3C 9	00 h 17 m 50·10 s	(49·90 s)	−0·20 s	+15° 24′ 13·8″	(16·5″)	+2·7″
3C 48	01 h 34 m 49·86 s	49·82 s	−0·04 s	+32° 54′ 20·8″	20·4″	−0·4″
3C 84	03 h 16 m 29·58 s	29·55 s	−0·03 s	+41° 19′ 52·1″	52·2″	+0·1″
3C 138	05 h 18 m 16·56 s	(16·53 s)	−0·03 s	+16° 35′ 27·0″	(27·2″)	+0·2″
3C 147	05 h 38 m 43·49 s	(43·50 s)	+0·01 s	+49° 49′ 43·1″	(43·1″)	0·0″
3C 153	06 h 05 m 44·46 s	(44·44 s)	−0·02 s	+48° 04′ 49·0″	(48·8″)	−0·2″
3C 186	07 h 40 m 56·75 s	(56·75 s)	0·00 s	+38° 00′ 31·3″	(31·1″)	−0·2″
3C 196	08 h 09 m 59·42 s	(59·39 s)	−0·03 s	+48° 22′ 07·2″	(07·6″)	+0·4″
3C 277·1	12 h 50 m 15·19 s	(15·13 s)	−0·06 s	+56° 50′ 37·0″	(36·4″)	−0·6″
3C 286	13 h 28 m 49·72 s	49·65 s	−0·07 s	+30° 45′ 58·4″	58·4″	0·0″
0Q 208	14 h 04 m 45·65 s	45·63 s	−0·02 s	+28° 41′ 29·2″	29·1″	−0·1″
3C 345	16 h 41 m 17·64 s	17·61 s	−0·03 s	+39° 54′ 10·6″	10·8″	+0·2″
3C 380	18 h 28 m 13·54 s	13·51 s	−0·03 s	+48° 42′ 40·5″	40·5″	0·0″

Most of our results are based on three or four photographs. 3C 286 was so faint as to be satisfactory on only one. For six other sources the images were also either too faint or invisible, and we measured the "secondary reference stars" used by Murray *et al.*[2] from which we derived corrections to apply to the source positions given by these authors. These so-called "corrected" positions are given in brackets in Table 1.

Table 2. MEAN POSITION DIFFERENCES AND STANDARD ERRORS

	Right ascension	Declination
Mean difference (Cambridge optical—RRE)	$-0{\cdot}029$ s $\pm 0{\cdot}006$ s	$-0{\cdot}05″ \pm 0{\cdot}08″$
Standard error of one position difference	0·022 s	0·28″

utilized. It is not reasonable to expect regional variations in the reference frame to cancel out. This would require a sufficient number of local calibrators, and even if sufficient numbers could be measured accurately in the radio survey their optical measurement would in general need large telescopes and would only proceed slowly and laboriously.

We do not wish to propose here the adoption of any particular "system", but to emphasize that the time has now come for agreement to be reached between radio and optical workers.

We thank Dr C. A. Murray for furnishing finding charts and full details of the Herstmonceux measurements before publication, and Dr H. Gent for a pre-publication copy of the RRE catalogue.

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Two Infrared Sources in Nebulae

In a systematic survey of the Caltech infrared catalogue (IRC)[1], 210 of the 5612 listed sources have been examined optically using the prints of the National Geographic Society–Palomar Observatory sky survey. Several criteria were adopted in the selection of sources for optical examination. Objects were first selected for which no previous identification was listed on the basis of comparison with star catalogues, and which further were very red as indicated by their I (0·84 μm) minus K (2·2 μm) colour index. The reddest sources among these have $(I-K) \geq 6^{m}\cdot00$ and all such cases were examined, whereas for the moderately red objects ($(I-K) \gtrsim 4^{m}\cdot00$) only sources at high galactic latitude ($|b^{II}| \geq 40°$), or lying in the $+40°$ declination zone of the two micron survey, or located near stellar rings[2], were inspected. During the work, a number of infrared stars of $(I-K) \gtrsim 4^{m}\cdot00$ but catalogued as belonging to spectral class B were also located. Examination of the sky survey prints revealed that the infrared object, in sixteen cases out of twenty investigated, was a fainter, very red star lying near the class B star in question. Some further named stars in the catalogue whose right ascension and declination offsets relative to the infrared source exceeded ~ 6 s and/or ~ 1′·2 were also examined, and again in nine cases out of fourteen a more satisfactory identification was made. On the basis of this admittedly small sample of stars with large offsets, it is suggested that such errors of identification may number about 100 stars throughout the catalogue, or about 2 per cent of the total.

Two particularly interesting sources coincided with small nebulae on the sky survey prints, and the main purpose of this letter is to give details of these. The first source is IRC+40091, with $(I-K) = 5^{m}\cdot72$, which has the position[1] (1950) 4 h 26 m 59 s ± 2 s, +35° 10′·2 ± 0′·3. This is identified with NGC 1579 which is a small, irregular, diffuse nebulosity, some 10 arc minutes across, with a prominent dark lane[3], and lies at[4] (1950) 4 h 26·9 m, +35° 11′. Pease[3] describes the brightest part of the nebula as a broad arrowhead; its appearance is very similar to that of the nebula about R Monocerotis. Very close to this cometary nebulosity, in the dark lane, is the faint star Lk Hα-101. Herbig[5] has discussed the source of illumination of the nebula, based on the unusual spectral features of the nebula and this star. He concludes[5] that Lk Hα-101 may be the exciting star if it is "very heavily obscured by dark material in the dark channel", and notes several spectral similarities between NGC 1579 and R Mon. The increasing brightness of the star through the photographic infrared to 2·2 μm seems strong evidence for this obscuration, and the brightness of R Mon at 5 μm, as reported by Low and Smith[6], makes NGC 1579 an object of great interest.

The second source closely associated with nebulosity is IRC+30407 with an $(I-K)$ index of $4^{m}\cdot48$, at[1] (1950) 19 h 59 m 55 s ± 2 s, +33° 22′·4 ± 0′·3. The position of NGC 6857, listed as a planetary nebula by Perek and Kohoutek[7], is (1950) 19 h 59 m 52·0 s, +33° 23′ 12″ and its extent is about 40 arc s. Four sources of radio emission have been detected in close proximity to NGC 685 by Wynn-Williams[8]. One is coincident with this nebula and a second has been shown by Higgs[9] to coincide with K3−50, a neighbouring nebula also listed as planetary. Both authors have interpreted the radio data as implying that these supposed planetary nebulae are in reality compact H II condensations excited by a cluster of OB stars. Elldér, Rönnang and Winnberg[10] have discovered an intense OH emission source at (1950) 19 h 59 m 51 s ± 15 s, +33° 25′ ± 3′, which is evidently associated with this region of nebulosity. It is unusual in that it is strongest in the 1720 MHz line whereas most infrared stars associated with OH emission are strongest in the 1612 MHz line, with little or no emission at 1720 MHz[11].

The recognition of an infrared source in this nebulosity strengthens the existing evidence from the radio observations that NGC 6857 is a region of active star formation.

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Strong New X-ray Object in the Cetus Region

In a recent flight of a Black Brant rocket, AKF-IIIB-51, we have observed a new intense source of X-radiation with a photon flux of 16·9 cm^{-2} s^{-1} in the 1·5–5·0 keV range. The rocket was launched on October 26, 1969, from Resolute Bay, Canada, at 0640 h GMT and reached an apogee of 196 km. It carried two proportional counters nested in a plastic scintillator mould, with their fields of view (~20° × 20°) in opposite radial directions. The detailed experimental configuration was similar to that used in earlier University of Calgary flights[1]. The detec-

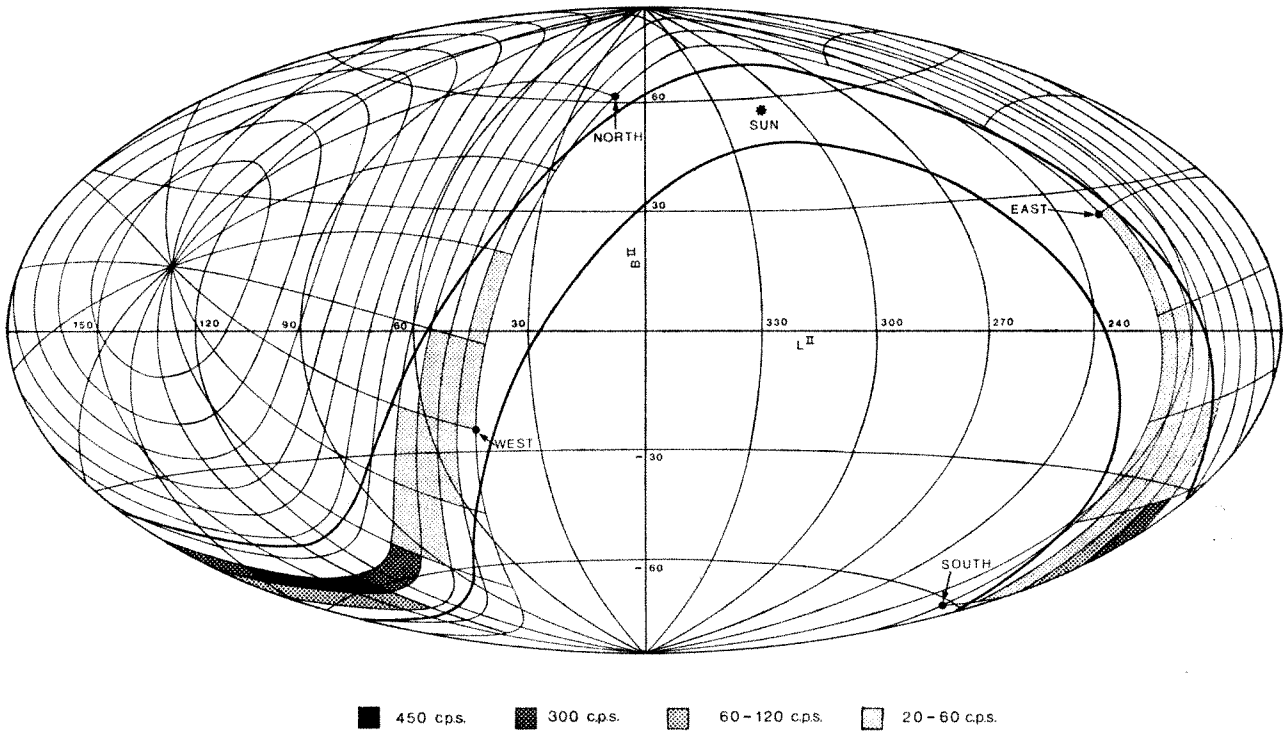


Fig. 1. Distribution of count rate in galactic coordinates (energy range 0·2–12·5 keV). Solid dark curves are the boundaries of the total area scanned. Unshaded region within the scanned area has either negligible scan time or the count rate attributed to detector background. Experimental horizon is defined by the curve with the north, west, south and east directions indicated on it by solid points.

tors had windows of $3{\cdot}0\times10^{-3}$ inch beryllium and $2{\cdot}5\times10^{-4}$ inch mylar so that together they covered the range of energies 0·2–12·5 keV. Above the atmosphere the rocket spun with a period of 3·34 s and precessed very slowly (period $\sim$52 min) about a cone of half angle 18°, consequently scanning almost the same region of sky on each sweep. The aspect was determined, using the data collected by three on-board magnetometers and a system of lunar sensors, to less than 2° uncertainty. At the time of the flight the Sun was 28° below the horizon with a shadow height of 750 km.

The general features of the flight are illustrated in Fig. 1. The count rate in the region of highest intensity was about six times the normal value. The data of the intense region were analysed separately after subtracting the contribution of both the diffuse X-radiation and the instrumental background.

Attempts were made to fit the data to the predictions of theoretical models of synchrotron emission, black body radiation and thermal Bremsstrahlung from a hot, thin plasma. The fit was poor with the first two models but was consistent with the third model giving the spectral distribution as

$$I=(20{\cdot}9\pm1{\cdot}0)\exp(-E/(7{\cdot}17\pm0{\cdot}57))\ \text{keV (keV cm}^2\ \text{s)}^{-1}$$

with the plasma temperature being $(8{\cdot}3\pm0{\cdot}7)\times10^7$ K. The fitted energy spectrum is presented in Fig. 2. Our estimate for the position of the object is $l^{II}=159°$ and $b^{II}=-52°$ (R.A. 2 h 14 min, dec. 4° 33′), the error circle being $\sim10°$.

Table 1 gives comparative figures for the energy and photon flux for a typical Sco-XR1 intensity[2] and the present object which we designate Cet-XR2. It is interesting to note that Cet-XR2 has an intensity comparable with that of Sco-XR1.

Table 1. CET-XR2 AND SCO-XR1 FLUX COMPARISON

Energy range (keV)	Photon flux (cm^{-2} s^{-1}) Cet-XR2	Sco-XR1	Energy flux (ergs cm^{-2} s^{-1}) Cet-XR2	Sco-XR1
1·5–5·0	16·9	25·9	$0{\cdot}7\times10^{-7}$	$1{\cdot}13\times10^{-7}$
1·5–10·0	22·3	32·9	$1{\cdot}35\times10^{-7}$	$1{\cdot}91\times10^{-7}$

A localized source in the Cetus region has been reported by Barnden and Francey (UAT group)[4] which has been designated as Cet-1 in the LRL catalogue[3] of X-ray sources. The position and intensities of Cet-XR1 and Cet-XR2 are summarized in Table 2.

The error circle estimated in the UAT experiment was approximately 15°. Within errors the two measurements could be referring to one and the same object. If this is so, the disparity in fluxes suggests that either the UAT measurement did not have a good geometry head-on view of the source or there has been a time variation in

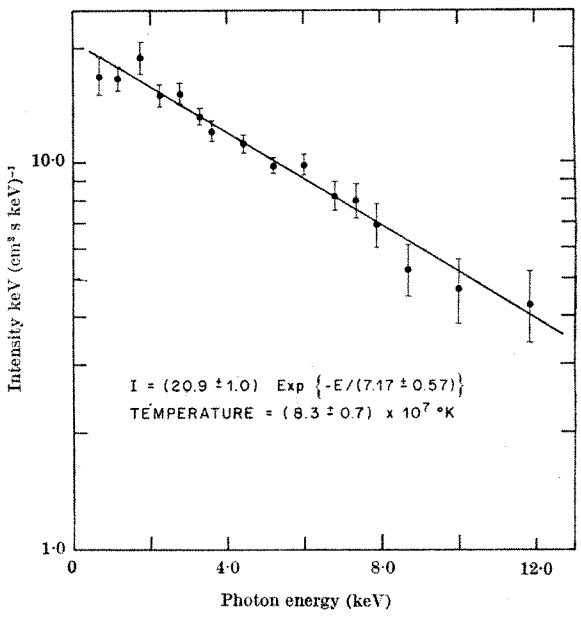


Fig. 2. Intensity of Cet-XR2 as a function of energy.

Table 2. COORDINATE AND FLUX DATA FOR CET-1 AND CET-2

	Cet-XR1	Cet-XR2
Galactic longitude (lII)	145°	159°
Galactic latitude (bII)	−60°	−52°
Intensity (photons cm^{-2} s^{-1} in the range 1·5–5·0 keV)	1·5	16·9
Date of observation	Dec. 1, 1967	Oct. 26, 1969

the intensity of the source, as has been observed, for example, for Centaurus-XR2, a nova-type object which has a similar exponential spectrum. The provisional location of Cetus-XR1 appears to have been very close to the experimental horizon so that the intensity of the source may have been underestimated.

On the other hand there may be two distinct sources in this region of the sky. The provisional location of Cetus-XR2 appears to be somewhat below the horizon of the UAT observation and consequently it would have been unobservable at the time of the earlier flight. Unfortunately neither of the two experiments yields sufficiently precise positional information to clarify the situation at the present time. Tentatively we consider that these are two different sources. Future experiments with more precise determination of the position should clarify the situation.

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Nocturnal Variation of the Intensity and Rotational Temperature of the OH (8,3) Band in the Airglow

THE OH airglow is a sensitive indicator of conditions in the mesosphere. We report the results of measurements of the intensity and excitation temperature in the OH (8,3) vibrational rotational band, carried out with a spectrophotometer of simple design. Light entering a 3 mm diameter iris is collimated with a 170 mm focal length lens and impinges on a narrow band interference filter of 5 cm diameter; the filter has a bandwidth of approximately 3 Å and can be tilted to scan the band between 7300 Å and 7403 Å. The filtered light is detected with a cooled photomultiplier 'EMI 9558A (S20)'; photon counting is accomplished with a multichannel analyser 'Laben 100'. An entire scan, including dark current measurements, is carried out automatically in about 20 min. After removal of sky background and dark current the experimental spectra are compared with synthetic spectra obtained by considering the theoretical contribution of all lines of the OH (8,3) band under the assumption of thermal equilibrium. The χ^2 criterion is utilized to optimize the fitting; best values for the relative intensity and the rotational temperature are obtained at intervals of one hour every night. Observations reported here were carried out for a total of 22 nights and 276 scans during July to December 1969 and for a total of 7 nights and 72 scans during July to December 1968. Data were taken near l'Aquila (42° N, 13° E) at two locations: at Campo Imperatore (2,200 m above sea level) until August 1969 and at Preturo (700 m above sea level) after that date.

The graphs in Fig. 1 indicate the average variations of rotational temperature and intensity throughout the night, referred to the hour of sunset and to the local midnight. The graphs were obtained by considering, for each night, the difference in rotational temperature and relative intensity at successive hourly intervals. The intensity was referred to the minimum value obtained during each night. A decreasing trend during the night is evident for both intensity and temperature. The intensity variation is in reasonable agreement with estimates for the rate of emission of OH* obtained by Hesstvedt[1] with his photochemical model. The variation is probably because of the decrease in the OH* production rate associated with the decrease in the ozone and hydrogen concentrations at night near the mesopause. A decreasing trend with a slightly larger variation is found in the results of Shimazaki and Laird[2]. Hunt[3] also calculated the rate of formation of OH* and found a much larger nightly variation. His results, however, cannot be directly compared because they do not include quenching. In previous studies of the diurnal variation of the OH emission intensity[4] an overall fall of intensity from evening to morning was often present, but contamination by O_2 bands may have affected those earlier broad band measurements. Present measurements are thought to be relatively immune from contamination of other airglow emissions in view of their selectivity and the procedure used in reducing the data.

Measurements of intensity in general can be affected also by variations in atmospheric transparency. Measurements of rotational temperature are, in contrast, less sensitive to such possible cause of error. They are, however, difficult to interpret, because OH emission takes place at heights where radiative and collisional deactivation processes are competitive, and because rotational temperature may not reflect the ambient temperature. We point out that no systematic measurements of OH rotational temperature or of mesospheric kinetic temperature exist.

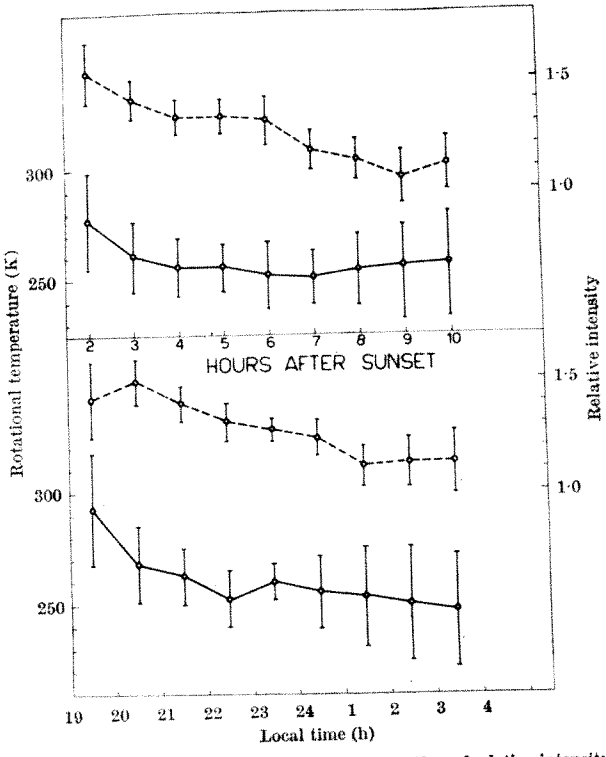


Fig. 1. The graphs indicate the nocturnal variation of relative intensity (- - - -) and rotational temperature (———) for the OH (8,3) band. Time variations are referred to local midnight and to the sunset.

A change in rotational temperature may thus reflect changes in the excitation process and in the height where the process takes place, as well as changes in ambient kinetic temperature. According to Hesstvedt's model, the change in intensity from sunset to midnight is accompanied by a change in the bottom height of the layer of only 1 km approximately. If the rotational temperature was constant this change could account for a measured variation of only a few degrees. The difference that we notice appears to be larger; if Hesstvedt's model is valid, it could point either to large variations in ambient temperature or to substantial differences between rotational temperature and ambient temperature.

Experiments are in preparation to observe simultaneously the kinetic temperature of OH lines and the rotational temperature of two OH bands.

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Night-time Anomalies in Very Low Frequency Propagation produced by a Galactic X-ray Source at Centaurus

Night-time propagation of very low frequency (VLF) waves in the terrestrial ionosphere shows considerable variability, both in phase and in amplitude, in comparison with the relatively stable daytime conditions. The night-time anomalies are not yet well understood, a number of them being associated with the presence of energetic particles originating from solar proton events[1]. The explanation of others remains obscure.

It has been demonstrated, however, that the night-time ionization at heights around the upper boundary of the Earth–ionosphere waveguide, for VLF waves, may contain an appreciable contribution from the stronger celestial X-ray sources[2,3], and, indeed, long-term observations confirm the influence of the transits of Scorpius XR-1 and Taurus XR-1 on the amplitude of 164 kHz signals[4] and on the phase at frequencies below 30 kHz[3]. Calculations have shown, however, that the phase effects are indeed very small and that they can often be masked by large seasonal variations of other origin[3,5].

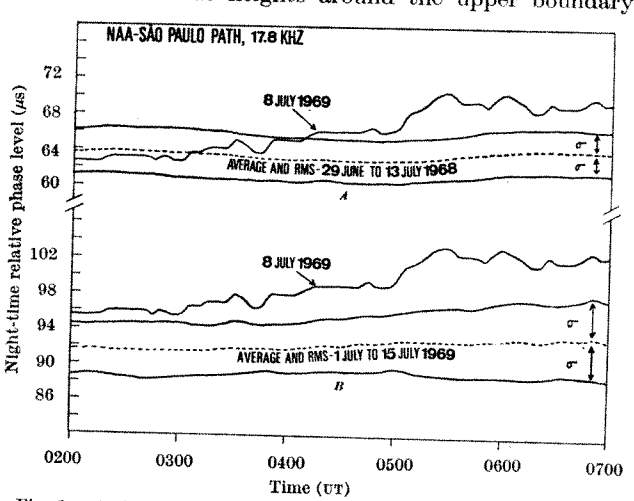


Fig. 1. *A*, The night-time phase anomaly recorded on July 8, 1969, compared with the mean value and standard deviation range for the fifteen days containing July 8, one year before, when the reported source was absent. *B*, The July 8, 1969, night phase compared with average values and σ range in the period July 1–15 of the same year.

The appearance of a very strong X-ray source in the southern sky, reported by Conner *et al.*[6], and observable between July 6 and 9, 1969, produced on July 8 a very clear night-time ionospheric disturbance on VLF transmissions from NAA (17·8 kHz, 7·4 mm path) received at São Paulo by a receiver controlled by a rubidium standard oscillator. One preliminary method to witness such an event is to compare night-time levels with the standard deviation range (σ) in a period of at least 15 days, containing the anomalous day. Fig. 1*A* shows the night phase record of July 8 together with the mean value and σ range in the period of June 29 to July 13, 1968. Night-time phase daily values were plotted around the mean value for the period of one year before fitting the averages in the interval 0200 to 0400 UT. In the period July 1–15, 1969, only the night-time phase from July 8 presented a significant departure from the σ, but some small deviations were also noticed, after 0400 UT, for July 5, 6, 7, 9 and 11. The reported Centaurus location[6] ($\alpha = 14$ h 56 m and $\delta = -32° 15'$) indicated that at that epoch the effect should be noticeable for nearly 8 h after sunset throughout the night.

A similar treatment was given to the period July 1–15, 1969, taking averages of 5 min intervals from 0200 to 0700 UT and establishing σ (corrected for the daily oscillator drift). Again, the night-time phase on July 8 presented a significant departure from σ throughout the night (Fig. 1*B*). A small deviation could also be seen on July 7.

Conner *et al.*[6] also reported that since the source discovery its spectra showed a daily softening. The rate of softening was irregular with no change in the period from July 16–18. The new source became stable after July 24, with flux and spectrum comparable with the neighbouring Scorpius SR-1, and was again observed on August 7 by Kitamura *et al.*[7]. On July 8 the X-ray hardness ratio between the 6–12 keV and 3–6 keV ranges was greater than one[6], and was enough to ionize the night-time upper boundary of the Earth–ionosphere waveguide for VLF waves—(85–90 km). There are at least two modes of propagation dominant[8] so that the observed phase retardation, $4 \lesssim \Delta\varphi \lesssim 6$ s, is the result of the relative changes of the first and second order modes and not a direct measurement of the reflexion height lowering, as is usually inferred from daytime SIDs. A more intense and persistent ionizing source would certainly produce a subsequent phase advance similar to that which occurs at sunrise.

In the following periods of July 17–31 and August 1–15, 1969, averages and r.m.s. ranges were taken. The phases from July 23 and 25, and August 7, were below the average through the whole night although the decrease was less than 2σ, an effect that could be caused by inaccuracies in the experimental determination of the drift. The night phase of July 30 and August 14 were above the average all the time, below or equal to 2σ. The whole period, from July 1 to August 15, 1969, was geomagnetically rather quiet, excepting July 26, when an SC was reported by ESSA, Solar Geophysical Data, apparently unrelated to the discussed anomalies. These results would suggest a possible recurrent activity, at least on the hardening of the celestial X-ray source spectrum, but the quality of the data, the lack of knowledge on the other possible causes for night-time anomalies, and the too small ionizing intensities, make it diffi-

cult to determine the sidereal dependence of the anomalies.

The effect on amplitude was much less evident, because of the even bigger variation of NAA signal level at night. On July 8, 1969, the attenuation effect after 0400 UT was less than 0·6 dB/mn.

In VLF data handling we usually assume a normal distribution of deviations. We tried different representations, deciding in favour of the one described. If we simply plot all the night-time phase records together, or peak-to-peak range, corrected for daily experimental drift, the July 8, 1969, record shows a clear departure from the others. In the periods of June 29–July 13, 1968, and July 1–15, 1969, only a few night-time phase records from the 1969 period exceed σ, but they are always below 2σ—as pointed out in the text. Taking 15 days phase data, departures as large as 2 to 3σ are not common.

In conclusion the anomaly produced by hard and intense galactic X-rays is more pronounced and definite than long-term effects, and the present result encourages a detailed search of other night-time anomalies which could be associated with the appearance of celestial X-ray sources and to the variability of others already known[3]. VLF data on long propagation paths, controlled by atomic frequency standards, are very suitable for that purpose.

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Aleutian Deep-sea Channel on the Aleutian Abyssal Plain

THE Aleutian abyssal plain is a vast turbidite deposit south of the Aleutian trench (Fig. 1). Hurley noted[1] that the plain had been cut off from turbidity currents as a result of the downbowing of the trench which had interrupted the turbidity current process along the bottom. In support of this thesis, seismic reflexion evidence[2] indicated an unusually thick layer of pelagic sediments overlying the turbidite layers on the Aleutian plain, and, using pelagic sedimentation rates, he placed the cessation of turbidity current activity in the early to middle Tertiary. The discovery of the Seamap channel[3], which is beheaded by the Aleutian trench, provided striking further evidence of the fossil nature of the Aleutian plain.

Another prominent channel trends south-westerly for more than 1,000 km across the abyssal plain (Fig. 2). This feature consists of a series of deltaic structures

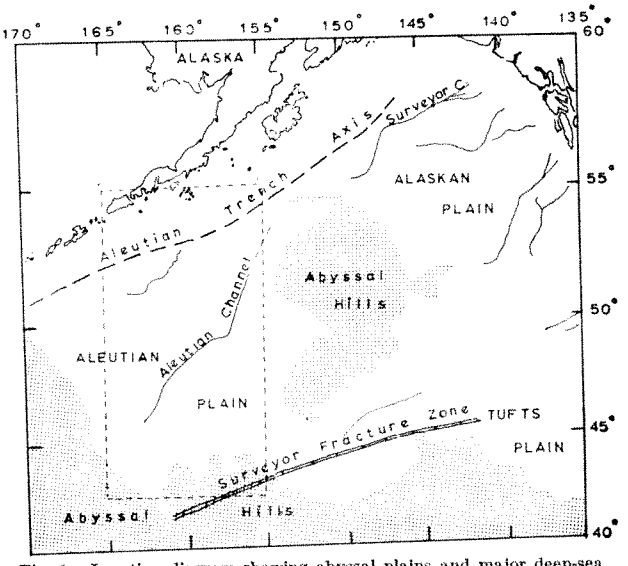


Fig. 1. Location diagram showing abyssal plains and major deep-sea channels in the north-east Pacific. Dashed line indicates area shown in detail as Fig. 2.

extending along its length. It is the largest single sedimentary feature on the Aleutian plain and is here named Aleutian channel. This unique deposit seems to occupy the axis of what was once a very broad south-west trending valley or elongate basin. This valley (basin) seems to have been a shadow zone to turbidity currents during an early stage of sedimentation in this region. This shadow zone coincides with the region where linear magnetic anomalies 25 to 32 (ref. 4) bend abruptly from a northerly to a westerly trend forming the Great Magnetic Bight[5], suggesting that during the initial stages of sedimentation turbidity currents were diverted away from the axis of the Bight by an associated topographic grain. A prominent topographic grain parallels these magnetic anomalies in the region immediately west of the Aleutian plain[6]. Remnants of the old shadow zone are now present in the form of two "secondary" shadow zones flanking the Aleutian channel. Although these flanking zones have undergone a certain amount of lateral filling they still show some of their original characteristics. Lateral filling has resulted in several enclosed basins (shown by depression contours in Fig. 2) that occur along the axes of both shadow zones. The largest basin lies within the eastern portion of the plain (Fig. 2). It apparently became an enclosed feature when a secondary channel, branching off Aleutian channel in a southerly direction, bridged the south-west trending shadow zone.

The Aleutian channel thus seems to represent one of the last stages in Aleutian plain turbidite sedimentation; the following is offered as a possible sequence of events leading to its formation. (In Fig. 2 deltaic units are assigned numbers 1 through 6.) Early turbidite sedimentation was controlled by the initial volcanic topography and a broad shadow zone trending north-easterly across the Aleutian plain remained. A point was reached when further inflow of sediment bridged obstructions to the north-east and found its way down the axis of the shadow zone, which now had become a receptacle for turbidity currents. Several local basin features—perhaps the result of differential sedimentation during the earlier stage—were located along the axis of the shadow zone. The initial inflow of sediment was primarily channelized, although various local depressions had to be filled in turn as the channel extended itself down the regional slope. Because the deltaic segments of Aleutian channel are best developed downstream from delta 2, this portion has been chosen for the discussion of their origin. A channel coming off of delta 2 found its way south of a pronounced east–west ridge (appearing now as two ridges, Fig. 2). Turbidity currents remained chan-

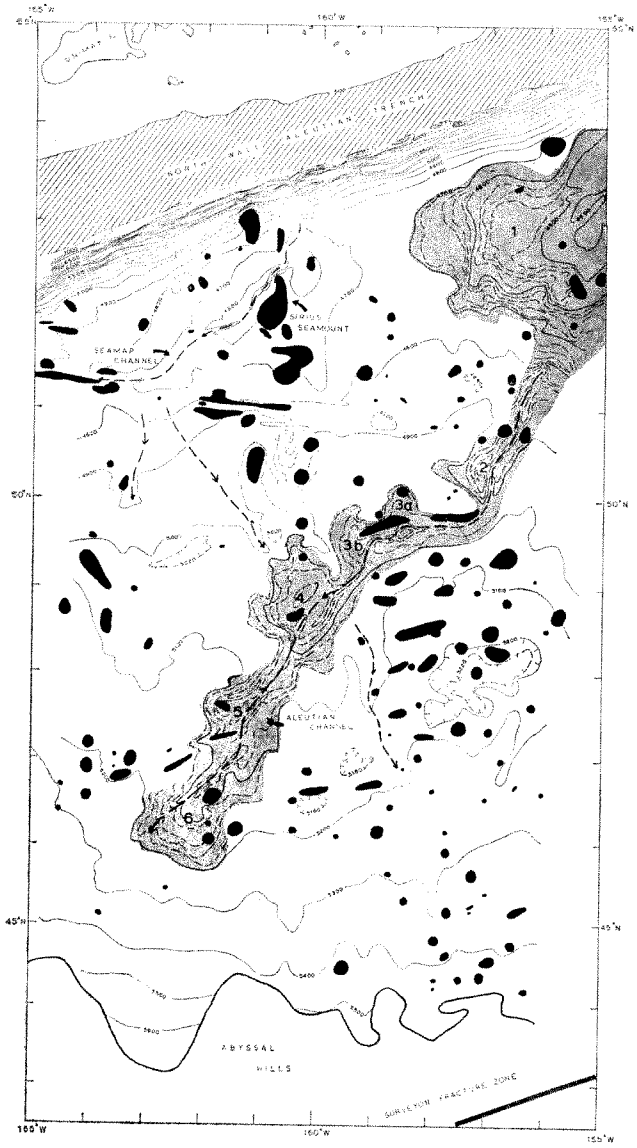


Fig. 2. Aleutian abyssal plain based on bathymetric data (north–south tracklines spaced at 10 nm (18·5 km) collected during ESSA's Seamap survey)[11]. The 100 m contour interval is shown by solid lines and selected 20 m contours are shown by light dashed lines. Aleutian channel deposit is shaded. Heavy dashed lines indicate channels. Black areas indicate volcanic topography as judged from an examination of fathograms.

nelized until they reached the western limit of the ridge, although a small amount of sediment apparently spilled over a low spot in the ridge at a later date and formed delta 3*a*. At this point the turbidity currents encountered a local depression and proceeded to fill it, primarily by means of sheet flow (or unchannelized turbidity currents). Sheet flow, radiating away from the point where the turbidity currents had lost their channel, continued until a small deltaic structure (delta 3*b*) occupied what was once a depression. When slope relationships made it possible for the channelized portion to extend itself farther down the regional slope, channelized flow broke away from the delta at its most downslope location. This process seems to have repeated to produce deltas 4, 5 and 6, with channelized flow always extending the feature from the downslope portion of the deltaic unit. Turbidity currents ceased before the principal channel had become aggraded to its slope, as is shown by the relatively steep slope in the channel axis at each location where the channel leaves a delta. Delta 1, although similar in morphology to the other deltas, is considerably larger and apparently represents a late-stage surge of sediment on to the Aleutian plain. This is based on the observation that the channelized portion of delta 2 ends abruptly on encountering delta 1 (Fig. 2), suggesting that its upstream portion has been overlapped.

It seems significant that the deltaic units of Aleutian channel are positioned on the right side of the channel (looking downstream). This bias may be purely the result of the initial topography coupled with a tendency of the principal channel to break away in a downstream direction; alternatively, it may reflect an influence of Coriolis force. The well documented asymmetrical levee heights of deep-sea channels[2,7] are clearly the result of Coriolis tilting of channelized turbidity currents; and the left hook of channels on mature abyssal plains (in the northern hemisphere), though somewhat more difficult to explain, also seems related to the Coriolis phenomenon[7]. The effect, if any, of Coriolis force on an unchannelized turbidity current is straightforward. Without confining levees turbidity currents would tend to turn toward the right in the northern hemisphere.

The source of the turbidity currents that deposited the Aleutian plain is difficult to determine because the upstream portion of the plain has been obliterated by the trench. The region of abyssal hills east of the Aleutian plain and immediately south of the trench (Fig. 1) precludes a present-day access to turbidity currents from the Alaskan plain which is a region of relatively recent turbidite deposition. It is possible that the Aleutian plain source lay north of the trench and that an ancient continuously sloping pathway from the continental margin of the Alaskan Peninsula was interrupted by downbowing of the trench, but recent views on sea-floor spreading favour an alternative explanation. By introducing a mobile sea-floor, a turbidity current pathway could have existed between the abyssal-hill region and the trench and subsequently migrated into the trench. In the New Global Tectonic model[8,9], the oceanic crust in this region is now moving north-westerly. Accordingly, the Aleutian plain may have been some 350 to 500 km south-east of its present location when its turbidity current supply was cut off[10]. The orientation of Aleutian channel and the turbidite morphology of the adjacent region strongly suggest that the source of sediment was immediately north of the abyssal-hill region. It is thus likely that the Aleutian plain is a distal portion of the Alaskan abyssal plain. This suggestion becomes increasingly more possible the farther south the crust is placed at the time turbidity current activity on the Aleutian plain ceased.

The relation between Aleutian channel and Surveyor channel (Fig. 1) suggests they were connected before the time the turbidity current pathway north of the abyssal-hill region migrated into the trench. If so, turbidity currents probably flowed through Surveyor channel and into the Aleutian trench, along a slightly diverted path, for a while after its distal segment (Aleutian channel) was severed by the trench.

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Elemental Variations of Transport Coefficients Across Density Interfaces in Multiple-diffusive Systems

We report here laboratory measurements of the fluxes of several solutes across a density interface, between two fluid layers which are in a state of turbulent convection. Transport coefficients defined for the different components are shown to vary systematically with the molecular diffusivities, and it is suggested that this separation must be kept in mind when using the distribution of trace elements as an indicator of the mixing (of water with different salinities) in the ocean.

The instabilities which can arise in a fluid the density of which is affected by two components with different diffusivities are now well understood[1]. When the vertical density gradient of one component is stable, but the other is in the opposite sense and partly counteracts this, convective motions can be driven by the potential energy associated with the latter. Typically an initially smooth density distribution overturns in a series of steps, with well mixed convecting layers separated by sharper interfaces across which molecular processes are important[2]. The simplest example of the system of interest here is a warm salty layer of water underneath a cold fresh layer. Convection occurs in both layers because of the transfer of heat across the interface, while the more slowly diffusing salt preserves the density step across it. More generally, at such "diffusive" interfaces the substance with the higher molecular diffusivity is unstably distributed, and this drives a flux of the stable component between the convecting layers.

Quantitative laboratory experiments have been carried out[3] using heat and salt, and it has been found that over a wide range of conditions the ratio of the two fluxes is substantially constant. Preliminary experiments[2] using sugar and salt have shown that the fluxes must depend on the ratio of the diffusivities, but the process is still incompletely understood. In the work described here, we have attempted to answer a related but different question: what are the relative rates of transport across a "diffusive" interface between convecting layers when there are three or more components present, all with different diffusivities?

Three experiments have been conducted using different solutes and techniques; the initial conditions are summarized in Table 1. In the first, two 8 cm deep layers of tap water above a solution containing approximately 2 per cent sodium chloride and 1 per cent sucrose by weight were set up in a tank 30 cm in diameter and heated at a constant rate from below. Small samples were withdrawn from both layers at regular intervals, and the concentrations of salt and sugar were later deduced as functions of time. The basic measurements were of density (by weighing a 27 cm³ sinker in the sample), and sugar concentration (using a polarimeter with a 20 cm light path through the sample). Salt in the concentration present

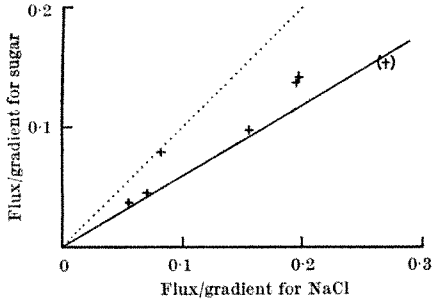


Fig. 1. Heating from below: the "transport coefficients" for sugar (the flux divided by the difference in concentration across the interface: see text) plotted against the corresponding values for NaCl. The scales are arbitrary, but the same for the two components; the relative values are directly as measured, except for the bracketed point whose coordinates have been halved for convenience of plotting.

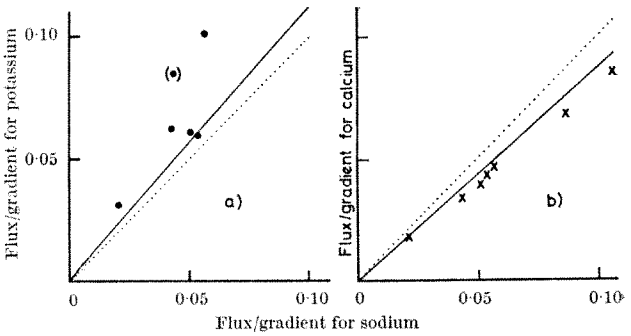


Fig. 2. As in Fig. 1, except that the transports of (*a*) potassium and (*b*) calcium are compared separately with sodium. The dotted line in these and other figures has unit slope, and the full line is drawn with a slope equal to the square root of the relevant diffusivity ratio (see Table 2).

made a negligible contribution to the optical rotation; the salt concentration was calculated as a difference, using a prior calibration.

The results are conveniently presented (Fig. 1) by defining "effective eddy transport coefficients" for sugar and salt, that is, the flux divided by a mean gradient. In terms of the concentrations of each property measured in the upper and lower layers at times t_0 and t_1 (say C_{u0}, C_{u2}; C_{l0}, C_{l1}), these may be written in difference form as

$$K \propto \frac{C_{u1} - C_{u0}}{\frac{1}{2}(C_{l0} + C_{l1}) - \frac{1}{2}(C_{u0} + C_{u1})}$$

The scales are arbitrary (for they depend on arbitrarily chosen time intervals and depths), but of course are the same for the two components so that a meaningful comparison is possible. The estimated errors are of the order of 15 per cent, but there is still clearly a systematic difference between the transports of sugar and salt, defined in this way.

The second experiment was carried out in just the same way, except that the heated lower layer now contained about 3 per cent by weight of NaCl, and a tenth of this concentration of KCl and $CaCl_2$. Analyses were carried out for the three metal ions by atomic absorption spectrometry, diluting the samples gravimetrically with distilled–deionized water to the appropriate concentration range, and aspirating them into a lean air–acetylene flame. A short path burner, mounted sideways, was used so as to minimize the amount of dilution required and to enable a wide range of concentrations to be accommodated on a single scale; calibrations were made directly using standard solutions. The results are shown in Fig. 2, where transport coefficients for both potassium and calcium are plotted relative to the sodium values. The potassium concentrations appear more variable than expected, possibly due to ionization in the flame; previous experience has shown the analytical error to be approximately ±4 per cent.

Finally, we give results from an experiment in which a layer containing 8 per cent NaCl and 0·4 per cent KCl was placed above a layer of more slowly diffusing sucrose (a 9 per cent solution). Again the interface is "diffusive", but there are now two components which can drive the convection, one much less concentrated than the other. The concentrations of sodium and potassium (but not sugar) were measured as functions of time using the atomic absorption technique. The timescale of this experiment was much longer than the previous two (a day instead of a few hours), but transport coefficients can be defined as before (Fig. 3).

Table 1. THE INITIAL PROPERTIES OF THE TWO LAYERS

	Exp. 1	Exp. 2	Exp. 3
Upper layer	Cold fresh water	Cold fresh water	8% NaCl, 0·4% KCl, 9% sucrose
Lower layer	2% NaCl, 1% sucrose. Heated	3% NaCl, 0·3% KCl, 0·3% $CaCl_2$. Heated	

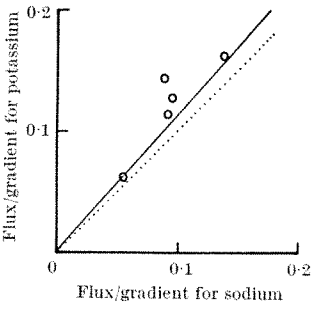


Fig. 3. Relative transports across a diffusive interface with NaCl and KCl above a sugar solution. See legend to Fig. 1.

In each of the three experiments a systematic molecular effect is evident when the transport of the various other components is compared with the rate for NaCl; the substance with higher diffusivity (KCl) goes faster and those with lower diffusivities (calcium and sugar) more slowly. The diffusivities used in making the comparisons are set out in Table 2 (taken from the *Handbook of Chemistry and Physics*). The values quoted are those appropriate for low concentrations, for it is difficult to evaluate absolute diffusivities in concentrated mixtures, but the ratios should remain roughly the same.

Table 2. DIFFUSIVITIES OF THE VARIOUS SOLUTES IN WATER AT 25° C AND LOW CONCENTRATIONS

Substance	KCl	NaCl	$CaCl_2$	Sucrose
Diffusivity $\varkappa$ (unit: 10^{-5} cm^2 s^{-1})	1·92	1·55	1·19	0·52
$\sqrt{\varkappa_x}/\sqrt{\varkappa_{Sa}}$	1·11	1·00	0·88	0·58

The only theoretical suggestion about the relative magnitude of the transports has come from the model of the mixing process described by Veronis[4] (for a two component system). He supposed that diffusive boundary layers form intermittently at the edge of the interfacial region before being swept away by the convection; this leads to a ratio of fluxes proportional to the square root of the diffusivity ratio. More work needs to be done to extend this argument to the present case, but nevertheless the full line on each figure has been drawn with this slope. In each case it is in fair agreement with the measurements, especially for the sugar–salt experiment (Fig. 1) which should be given most weight because of the greater diffusivity ratio.

Perhaps a transport rate increasing with the molecular diffusivity was only to be expected in this situation. We must remember, however, that the process described is largely a convective one, which operates because the large scale turbulent motions in the layers are continually sharpening the interface, and that the flux rates are much larger than can be accounted for by molecular diffusion through the mean gradient between the centres of the layers. If one sampled the motion and the concentrations without knowing that the interface existed, then one might say that the whole body of fluid was turbulent, with "eddy transport coefficients" which are different for the different components. Herein, we believe, lies the importance of the results when applied to oceanic processes. It does not seem permissible to assume, in the absence of a detailed knowledge of the transport mechanisms involved, that the "eddy diffusivity" deduced from the distribution of one component applies to another. Dissolved oxygen, for example, has a diffusivity comparable with that of KCl, and large molecular complexes will diffuse more slowly than NaCl. Because there is an increasing body of evidence that the typical distributions of temperature and salinity in the ocean are not smooth, but step-like[5,6], molecular diffusion near interfaces could certainly often be relevant.

Many other variants of this experiment remain to be explored, and it would clearly be desirable to measure all the transports simultaneously, not just selected pairs. We have not covered the whole range of relative concentrations of the components, though the behaviour of very small quantities of (radioactive) tracers, so small as to have a negligible effect on density, is of special interest. It would also be valuable to study systems of multiple layers, in which the observed differences could be enhanced by successive separations at several interfaces. Interfaces containing "salt fingers", which occur when the substance of lower diffusivity is unstably stratified[2], should be examined too; the experiments with two components suggest that in that case a higher molecular diffusivity will imply a relative reduction in the transport rate. There is the additional intriguing possibility of "mixed" interfaces, across which one pair of components will act so as to produce fingers, while another tends to make the interface sharp and "diffusive"; what actually happens then must depend strongly on the relative concentrations as well as the diffusivities. Our results using the simplest kind of interface do, however, indicate a need for caution when interpreting measurements of mixing rates in all multiple-diffusive systems.

Oceanographers have long been concerned with the simultaneous use of chemical tracers in oceanic studies. Broecker[7] has described a model to predict the observed distributions of ^{14}C, ^{226}Ra, ^{137}Cs, ^{90}Sr in the sea using one set of mixing conditions. Keeling and Bolin[8,9] have carried out a detailed examination of the use of box models, and pointed out that we lack direct evidence that the transfer coefficients behave as physical constants independent of the tracers under investigation. It may be that simple laboratory experiments such as we have described here can throw some light on the matter.

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Adsorption of Hydroxy-aluminium Polymers by Muscovite and Biotite

THE wide occurrence of hydroxy-aluminium coatings on expansible phyllosilicate clays in soils and sediments has been extensively reported[1,2]. In view of the marked effects of hydroxy-aluminium coatings on many properties of clays, considerable efforts have been devoted to studying the process by which they are deposited on the interlayer surfaces of phyllosilicates, and to characterizing the hydroxy-aluminium interlayers formed both in nature and in the laboratory[1–11]. Little is known, however, about the extent of adsorption of hydroxy-aluminium polymers by the external surfaces of phyllosilicates. This article reports the adsorption of hydroxy-aluminium

Table 1. ADSORPTION OF HYDROXY-ALUMINIUM POLYMERS BY VARIOUS SIZE FRACTIONS OF MUSCOVITE AND BIOTITE

Size fraction (μ)	mg of Al adsorbed/g of mineral: Muscovite, Ratio of hydroxy-aluminium solution to mineral			Biotite, Ratio of hydroxy-aluminium solution to mineral		
	25 ml./g	50 ml./g	100 ml./g	25 ml./g	50 ml./g	100 ml./g
5–2	1·4	1·6	3·7	1·7	1·8	3·0
2–0·2	4·0	4·6	5·7	4·5	4·5	5·0
0·2–0·08	13·8	16·5	19·1	13·1	14·8	18·1
<0·08	13·6	28·4	37·9	13·8	28·3	38·9

Muscovite (Effingham township, Ontario, Canada) and biotite (Bancroft, Ontario, Canada) samples were broken into thin flakes and then wet ground in a Waring blender. They were then fractionated[13] and saturated with K by five washings with 1 M KCl. The excess chloride was removed by one washing with H_2O followed by four washings with 100 per cent methanol. The hydroxy-aluminium solution of OH/Al ratio 2·0 and of Al concentration 0·02 M was prepared and added to the minerals at a rate of 5 ml./min with continuous stirring. The freshly prepared suspensions, stored in flasks, were shaken in an end-over-end shaker for 30 min and then aged for two weeks at room temperature. After ageing, the suspensions were centrifuged and the supernatant saved for Al determination[13].

polymers by various size fractions of muscovite and biotite which have external surfaces only.

The amount of hydroxy-aluminium polymers adsorbed by the minerals increased as the ratio of hydroxy-aluminium solution to mineral was increased (Table 1). This can be explained on the basis of the principle of mass action. The adsorption of hydroxy-aluminium polymers by muscovite and biotite increased consistently with the decrease of particle size. Moreover, the amounts of aluminium adsorbed by the same size fractions of the two micas are comparable. At the ratio of 100 ml. of hydroxy-aluminium solution to 1 g of mineral, the amounts of aluminium adsorbed by the 5–2, 2–0·2, 0·2–0·08 and <0·08 μ fractions were around 3, 5, 19 and 38 mg/g of mineral, respectively. The <0·08 μ fractions of micas therefore adsorbed more than ten times as much hydroxy-aluminium as the 5–2 μ fractions. The increase in the adsorption with the decrease of particle size is attributed to the increase of external surfaces, for there are no interlayer spaces in the K-saturated mica particles. The X-ray diffraction data of the minerals before and after adsorption of hydroxy-aluminium polymers show the characteristic d spacings for micas. Raman and Mortland[14] reported that the external surface area of muscovite and biotite increased considerably with the increase of fineness of particles. They further stated that there was a reasonably close agreement between the calculated and the measured values for the <5 μ fractions of muscovite and biotite. Moreover, their data show that muscovite and biotite had comparable external surface area for the size range studied. This further strengthens the contention that the increase in the adsorption of hydroxy-aluminium polymers with the decrease of particle size is essentially because of the increase of external surfaces of micas (Table 1).

The data obtained in this study demonstrate the importance of the adsorption of hydroxy-aluminium polymers on the external surfaces of micaceous minerals. They also indicate that the differentiation of the hydroxy-aluminium polymers adsorbed on external surfaces of 2 : 1 expansible phyllosilicates from interlayer hydroxy-aluminium, particularly in the <0·2 μm fractions, deserves close attention in studies on, for example, ion fixation and selectivity, acidity and swelling of clays.

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Nonisothermal Chemical Kinetics

IN a recent article[1], MacCallum and Tanner suggest that the traditional homogeneous reaction rate expression

$$\frac{dC}{dt} = A \exp(-E/RT)\, f(C) \qquad (1)$$

is applicable only to isothermal processes, and that an additional term

$$\left(\frac{\partial C}{\partial T}\right)_t \frac{dT}{dt} \qquad (2)$$

should be added when the temperature varies over the course of the reaction. This suggestion has several disturbing features. It implies, contrary to logic, that the instantaneous reaction rate depends not only on the present state of the system (the frequency of collisions and relative energies and orientations of all of the system molecules) but also—through dT/dt—on previous and future states. The proposed correction is impossible to calculate, because in a real system temperature cannot be varied holding time constant, and therefore the factor $(\partial C/\partial T)_t$ is devoid of physical meaning; moreover, even for the hypothetical case of a step-function change in temperature, the instantaneous change in concentration must be 0, which eliminates the correction. A particularly far-reaching implication of the suggestion is that essentially all of the nonisothermal reactor modelling and analysis that has taken place over the past 80 years has been fundamentally unsound, by virtue of having been based on equation (1).

We hold that the suggestion of MacCallum and Tanner is incorrect: equation (1) certainly has shortcomings, but they do not include the one proposed. Mathematically, the authors err in proceeding from the statement $C = f(t,T)$ to the equation

$$dC = \left(\frac{\partial C}{\partial t}\right)_T dt + \left(\frac{\partial C}{\partial T}\right)_t dT \qquad (3)$$

Equation (3) is valid if and only if C is a state function of the variables t and T. That it is not can be seen by considering two paths from an initial state (t_1, T_1) and concentration C_1 to a final state (t_2, T_2) (Fig. 1): the first path proceeds along an isotherm at T_1 to $t = t_2$, and then jumps instantaneously to $T_2 = T_1$, and the second path jumps immediately from T_1 to T_2 and then proceeds isothermally to $t = t_2$. The concentration C_2 in the first case lies on an isotherm at T_1, and in the second on an isotherm at T_2. Because these isotherms intersect at t_1, they cannot also intersect at t_2. The value of C_2 thus depends on the path taken from state 1 to state 2, and C is accordingly a path function rather than a state function. This violates the condition for the validity of equation (3), and negates the conclusions reached by MacCallum and Tanner, which are based solely on this equation.

We may then ask why the kinetic parameters of equation (1) are likely to be in error when evaluated using nonisothermal techniques, such as differential thermal

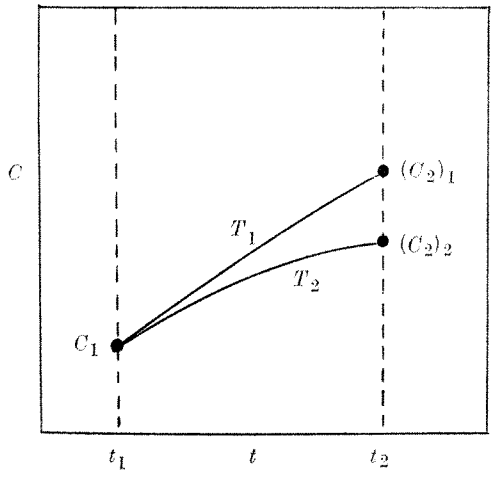


Fig. 1. Concentration as a function of time for two paths between (t_1, T_1) and (t_2, T_2).

analysis (DTA), or the thermogravimetric (TG) techniques referred to by MacCallum and Tanner. There are several possible explanations, apart from the inherent difficulty of obtaining precise measurements for nonisothermal systems. Everything that has been said about the temperature dependence of reaction rates is based on an implicit assumption of instantaneous thermal equilibrium, or something approximating it; if this assumption is invalid, then the concept of temperature loses its meaning. This equilibrium condition is certainly less likely to hold in nonisothermal than in isothermal systems, and consequently, as departures from isothermality increase, rate equations of the type of equation (1) become increasingly suspect. In extreme cases, it becomes necessary to invoke the formalism of nonequilibrium statistical mechanics to arrive at a meaningful description of the occurring rate processes.

On a less fundamental level, there is nothing at all sacred about the form of equation (1). The Arrhenius expression is a frequently erroneous semiempirical formula, with the temperature independence of the activation energy E and the pre-exponential factor A becoming increasingly questionable as the reaction temperature range broadens. Moreover, a fixed expression for $f(C)$ implies an invariant mechanism; even for isothermal conditions, the mechanism is sometimes a function of the extent of reaction, and it is common to find a varying mechanism (and therefore a varying $f(C)$) as the temperature changes.

Any of these circumstances might lead to errors of the type noted by MacCallum and Tanner, and their suggestion that isothermal methods be used whenever possible is reasonable. If the Arrhenius function is valid and the mechanism is fixed, however, equation (1) as written is applicable to nonisothermal systems, and if the driving function $T(t)$ in a DTA or TG experiment is accurately determined, then the conversion history is explicitly given by

$$C(t) - C(0) = \int_0^t \left(\frac{dC}{dt}\right) dt = \int_0^t A \exp\left[-E/RT(t)\right] f[C(t)]\, dt \quad (4)$$

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BIOLOGICAL SCIENCES

IgG Synthesis in Hybrid Cells from an Antibody-producing Mouse Myeloma and an L Cell Substrain

Proliferating hybrid cell strains have been produced using Sendai virus to mediate fusion of a cloned, culture-adapted mouse plasmacytoma, MOPC 315 (my unpublished work with M. Potter), with the thymidine kinase deficient cell strain LM(TK$^-$) C1 1D (ref. 1), referred to as C1 1D. Because the hybrid cell strains contained soluble enzymes with the electrophoretic mobility characteristic of the inbred strains of mice from which MOPC 315 and C1 1D were derived, at least part of both parental genomes was expressed (my unpublished work with H. G. Coon). Here I report an investigation of the synthesis of immunoglobulin by clones of these hybrid cell strains.

In cell culture, MOPC 315 secretes easily detectable amounts of immunoglobulin with an A type heavy chain and lambda type light chain. This immunoglobulin has antibody activity against dinitrophenyl (DNP) groups[2] as does immunoglobulin found in the serum of mice with the MOPC 315 tumour. When tissue culture supernatant from five independently produced hybrid cell strains was concentrated and assayed by agar gel diffusion (Ouchterlony) for immunoglobulin, no reactions were observed. But what seems to have been a small amount of immunoglobulin with anti-DNP activity was detected by three more sensitive techniques.

The MOPC 315 immunoglobulin binds specifically to DNP attached to 'Sepharose'; the protein can then be eluted by a hapten, DNP-glycine[3]. Two different hybrid cell strains resulting from the fusion of MOPC 315 and C1 1D (referred to as D1 and D2), C1 1D, or MOPC 315 were incubated for 16 h in Eagle's medium with 1 per cent foetal calf serum, without lysine and threonine, to which 2 or 10 μCi/ml. of ^{14}C-lysine and ^{14}C-threonine (Schwarz Bioresearch) had been added. The supernatants were aspirated, dialysed exhaustively against a phosphate buffered saline (PBS), *p*H 7·4, and passed through DNP-'Sepharose' columns (1 × 5 cm). Each column was prepared from fresh DNP-'Sepharose' and used once only. After the radioactive culture supernatant had been applied, the columns were washed extensively with PBS, and then eluted with 1–2 ml. of a 20 mg/ml. solution of DNP-glycine (Sigma). The total radioactivity in trichloroacetic acid (TCA) precipitable protein was determined by the paper disk method[4]. The results are shown in Table 1. No radioactive protein was eluted by DNP-glycine from the DNP-'Sepharose' columns which received supernatant from C1 1D cultures. Some labelled protein was always eluted from columns receiving supernatant from the hybrid cell cultures.

Table 1. Elution by DNP-glycine (20 mg/ml.) of TCA precipitable labelled protein from DNP-'Sepharose'

	Cell strain	Total radioactivity (c.p.m.) eluted as TCA precipitable protein
Experiment 1	C1 1D	0
	Hybrid D1	130,012
	Hybrid D2	60,314
Experiment 2	C1 1D	0
	Hybrid D1	1,754
	MOPC 315	28,382

A different preparation of DNP-'Sepharose' and a different amount of ^{14}C-lysine and ^{14}C-threonine were used in each experiment. Supernate was obtained from incubations of approximately 5×10^6 cells of each strain in both experiments.

Radioimmunoelectrophoresis[5] was also performed on dialysed supernatant from cell cultures incubated with either amino-acids labelled with ^{14}C or with reconstituted protein hydrolysate similarly labelled (Schwarz Bioresearch). The hybrid cells secreted protein labelling the

precipitation arcs that identify A heavy chains, λ light chains, and anti-DNP activity. If F1 (C3H × Balb/c) mice were inoculated with C1 1D, MOPC 315 or the hybrid cells, tumours developed. The hybrid tumours appeared much later than either of the parent tumours, and the one tumour tested was not transplantable (Table 2). One of the C1 1D tumours and a hybrid D1 tumour were minced and incubated with labelled amino-acids as before. The hybrid tumour secreted material which labelled the A, λ, and anti-DNP activity precipitation arcs: supernatant from the C1 1D tumour labelled no precipitation arcs. Occasionally, supernatant from cultures of C1 1D labelled the A, λ, M, κ and anti-DNP activity precipitation arcs, when large numbers of C1 1D cells (usually $0{\cdot}5$–$1{\cdot}0 \times 10^8$) were incubated and either serum or urine from mice bearing the appropriate plasma cell tumour was used as a carrier. In two experiments when MOPC 315 protein purified from a column of DNP-'Sepharose' was used as a carrier, no labelling by C1 1D supernate was observed.

The Farr technique has been used to measure the equilibrium constant for MOPC 315 binding to DNP-L-lysine[6]. I examined concentrated supernatants from C1 1D, MOPC 315, hybrids D1 and D2 as well as hybrids produced from another plasma cell tumour in culture, MOPC 265, and C1 1D or the L cell strain, A9 (ref. 7). The MOPC 265 cell strain was adapted to cell culture by the technique of alternate cell culture and animal passage[8]. MOPC 265 secretes only a κ chain which does not bind DNP. The unbound DNP-L-lysine hapten is not precipitated by 50 per cent $(NH_4)_2SO_4$. Anti-DNP antibodies present in the culture supernatants bind DNP-L-lysine labelled with ^{14}C (given to me by R. Asofsky). Precipitation with 40 per cent $(NH_4)_2SO_4$ will then remove bound radioactive hapten from the supernatant (Table 3). Supernatants from the MOPC 315 × C1 1D hybrid cultures removed 20–30 per cent of the radioactivity. Supernates from the MOPC 265 × C1 1D MOPC 265 × A9 and from C1 1D cell strains did not contain proteins which bound DNP-L-lysine.

Table 2. TUMOUR PRODUCTION IN F1 (C3H × Balb/c) MICE

Cell strain	Injected	Takes		Transplantable
C1 1D	4	4	Appeared within 44 days	Yes
MOPC 315	6	6	Appeared within 30 days	Yes
Hybrid D1	6	5	Appeared after 60 days	No
Hybrid D2	3	2	Appeared after 90 days	Not tested

Cells (usually 5×10^6–1×10^7) were inoculated subcutaneously into each host. The hybrid D1 was not transplantable; both C1 1D and MOPC 315 were. The C1 and 1D strains were derived from a C3H mouse; MOPC 315 from a Balb/C2 mouse.

Table 3. AMMONIUM SULPHATE PRECIPITATION OF PROTEINS BINDING ^{14}C LABELLED DNP-L-LYSINE

	Source of supernatants	Radioactivity (c.p.m.) left in supernatant fluid (50 μl.)	Per cent removed
Experiment 1	Culture media only	332	0
	C1 1D	322	3
	Hybrid D1	236	29
Experiment 2	Culture media only	186	0
	C1 1D	183	2
	Hybrid 265 × C1 1D	191	0
	Hybrid 265 × A9	184	1
	Hybrid D1	149	20
	Hybrid D1	144	23
	Hybrid D2	130	30

Supernatants were aspirated from cultures between 45 and 90 days after cell fusion and were concentrated twenty times by vacuum dialysis. Supernatants (0·1 ml.) and radioactive hapten (0·1 ml.) were incubated for 1 h at 37° C before precipitation in 50 per cent $(NH_4)_2SO_4$.

These data suggest that these MOPC 315 × C1 1D hybrid cells secrete a small amount of DNP-binding protein which may be identical to the immunoglobulin secreted by the MOPC 315 cell strain. Less immunoglobulin is synthesized than in MOPC 315. The hybrid cells may lack the intracellular organization (membrane systems) needed to secrete large amounts of protein. It may also be that the mixing of two different cell strains with variant translation mechanisms[9,10] results in a hybrid cell strain that secretes an altered immunoglobulin undetectable by the analytical methods used.

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Induction *in vitro* of Hapten Specific Plaque Forming Cells

THE method developed by Mishell and Dutton[1] for the *in vitro* stimulation of an apparently primary response to red cell antigens offers advantages over *in vivo* experiments in analysing the cellular basis of the immune response. The possible uses of the *in vitro* system would be much increased were it possible to get reproducible responses to a well defined hapten.

Success in achieving this with the dinitrophenyl group as hapten has been reported by Segal *et al.*[2] using explants of mouse spleen and lymph nodes, and also by Kettman and Dutton[3] using single cell suspensions and the trinitrophenyl group as hapten. We have shown that cells producing antibodies to the hydroxy-nitro-iodophenyl group (NIP) can be induced in mouse spleen cell suspensions. The response is erratic unless the donors of the spleen cells have been primed by injection of the red cell to which the hapten is attached to form the *in vitro* inducing antigen.

Both Balb/c and F1 hybrid C57Bl/6 × DBA/2 mice were used. The haptens and related chemicals used[4] were 4-hydroxy-3-iodo-5-nitrophenacetyl azide (NIP-Az), 4-hydroxy-3-nitrophenacetyl azide (NP-Az), 4-hydroxy-3-nitrophenacetylamino-N-caproic acid (NP-CAP) and 4-hydroxy-3-iodo-5-nitrophenacetylamino-N-caproic (NIP-CAP). The culture media and conditions of culture of spleen cell suspensions, the collection of the cells and the haemolytic plaque assay, were all similar to those described by Mishell and Dutton[1]. Exact adherence to the culture conditions and the use of media screened for effectiveness was necessary to obtain satisfactory results. The method of coupling haptens to red cells was modified from that of Pasanen and Mäkelä[5]. To prepare inducing antigens a 10 per cent red blood cell suspension was coupled with $2{\cdot}5 \times 10^{-3}$ M NIP-Az in 0·125 M carbonate–bicarbonate buffer, *p*H 9·25 at 4°, for 30 min. Both NIP-coupled sheep red cells (NIP-SRBC) and NIP-coupled rabbit red cells (NIP-RRBC) were used. No cross reactivity between sheep and rabbit red cells was observed. To assay cells forming antibody to the NIP determinant, NIP-coupled donkey red cells (NIP-DRBC) were used ($7{\cdot}5 \times 10^{-4}$ M NIP-Az in 0·125 M carbonate–bicarbonate buffer, *p*H 9·25 at 4°, for 30 min). Using this ratio of reagents gives a

maximum of plaques and the reacted cells are stable and can be used for several days after coupling. NIP-coupled donkey red cells were chosen to assay the cells which form NIP antibody because they gave no detectable reaction with anti sheep red cell antibody and yielded very few background plaques in the control cultures using SRBC as antigen. The specificity of the assay for cells synthesizing antibodies to the NIP hapten was demonstrated by the 90 per cent inhibition of plaque-formation by NIP-CAP at 5×10^{-5} M. At the same concentration NP-CAP had little or no inhibitory power. Moreover, neither NIP-CAP nor NP-CAP at this concentration inhibits plaques on SRBC.

Table 1. RESPONSE OF SPLEEN CELL CULTURES FROM CARRIER RED CELL IMMUNIZED AND UNIMMUNIZED MICE TO INDUCTION WITH NIP-COUPLED RED CELLS

Experiment	Cells from mice immunized with carrier RBC Antigen added		Cells from unimmunized mice Antigen added	
	NIP-SRBC	SRBC	NIP-SRBC	SRBC
1	1,531	33	342	43
2	1,492	66	17	25
3	2,185	68	433	22
4	1,022	40	—	—
5	857	12	86	12
	NIP-RRBC	RRBC	NIP-RRBC	RRBC
	1,732	12	581	17
	445	25	41	13

Plaques per 10^6 recovered cells were assayed on NIP-DRBC, day 5.

Table 2. INHIBITION OF INDUCTION OF PLAQUE FORMING CELLS

Experiment	Inhibitor	Assayed on NIP-DRBC	Percentage of control	Assayed on SRBC	Percentage of control
A	None	295	—	1,966	—
	10^{-4} M NP-CAP	304	103	1,960	100
	10^{-4} M NIP-CAP	77	26	2,000	102
B	None	1,531	—	7,285	—
	10^{-4} M NP-CAP	1,150	75	6,415	88
	10^{-4} M NIP-CAP	350	23	10,500	144
C	None	1,022	—	1,502	—
	10^{-4} M NP-CAP	778	76	2,320	154
	10^{-4} M NIP-CAP	333	32	1,810	120

Inhibitors added at same time as the inducing antigen, NIP-SRBC. Number of plaque formers is given per 10^6 recovered cells, Day 5.

When spleen cells from unimmunized mice were cultured with NIP-coupled sheep or rabbit red cells and the culture tested after 3, 4, 5 and 6 days by the plaque assay using NIP coupled donkey red cells, the maximum number of plaque forming cells were found after 5 days. The same kinetics of response were found when cells that form plaques on SRBC were assayed in cultures that had either coupled or uncoupled sheep cells as antigen. In the hapten system, using spleen suspensions from unimmunized mice, about half the cultures contained a significant number of cells synthesizing antibodies to the hapten (Table 1). The number of plaque forming cells induced with hapten was between one and twenty times greater than the number in control cultures, to which either unsubstituted red cells or no red cells had been added. We thought that a better hapten response was obtained with older mice and this, together with other reports of spontaneous immunization of mice against sheep red cell antigens[6], suggested that the origin of the very variable hapten response might lie in differences in the state of immunity to sheep or rabbit red cells of the donor mice.

To attempt to augment the hapten response we then used, in culture, spleens of mice primed by a single intraperitoneal injection of 0·5 ml. of 1·25 per cent suspension of either sheep or rabbit red cells. When the spleen cells of these immunized animals were cultured with NIP coupled to the kind of red cell used for immunization a consistently high response of anti-hapten plaque forming

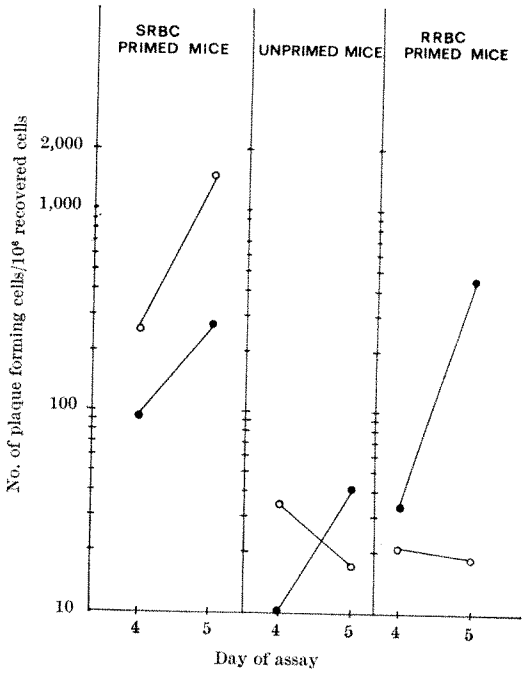


Fig. 1. Specificity of effect of immunization by carrier cell; plaque formers are assayed on NIP-DRBC. Immunized Balb/c mice used as donors of spleen cells were primed by injection of SRBC or RRBC, 33 days before setting up cultures. Antigens used in culture: ○, NIP-SRBC; ●, NIP–RRBC.

cells was obtained (Table 1). The enhanced response was specific for the cell used for immunization, being an order of magnitude higher when the hapten was coupled to the immunizing red cell compared with the response when the hapten was coupled to a non-cross reacting red cell or if unimmunized animals were used in the same experiment (Fig. 1). The effect on hapten response of prior immunization with the carrier red cell is only seen when the *in vitro* inducing antigen is the hapten coupled to the red cell used for immunization of the donor animal. Moreover, a poor response occurred when NIP-RRBC and SRBC were added to a culture of spleen cells from a mouse which had been immunized against SRBC. The effect of prior immunization was particularly large when 3–4 month old Balb/c mice were used (Fig. 1). It was smaller with 6–7 month old hybrid mice (Fig. 2). The cells from unimmunized mice sometimes gave a good response (Table 1), as we have already pointed out.

In a preliminary investigation enhancement of the antihapten response was estimated at varying times after injection of red cells. No effect was apparent after 4 days and little after 13 days, but a consistently positive response was obtained from spleens 30–60 days after injection. In contrast there was a strong anti-SRBC response in cultures of cells taken from animals immunized 4 days previously.

The hapten specificity of the induction with NIP-coupled SRBC using spleen cells from immunized mice was tested by addition of NIP-CAP or the non-cross reacting hapten NP-CAP to the cultures together with the NIP-coupled red cells. At a concentration of 10^{-4} M of NIP-CAP there was about 75 per cent inhibition of formation of cells giving plaques on NIP-DRBC but no inhibition of cells giving plaques on SRBC (Table 2). At the same concentration the inhibition by NP-CAP was much lower and is probably not significant.

We tried to evaluate the contribution of the spleen cells from mice immunized with red cells to the anti-hapten response. We mixed various proportions of cells from spleens of primed and unprimed mice and tested their capacity in culture to produce plaque forming cells on NIP-DRBC. At a ratio of 1/4 of primed/unprimed cells

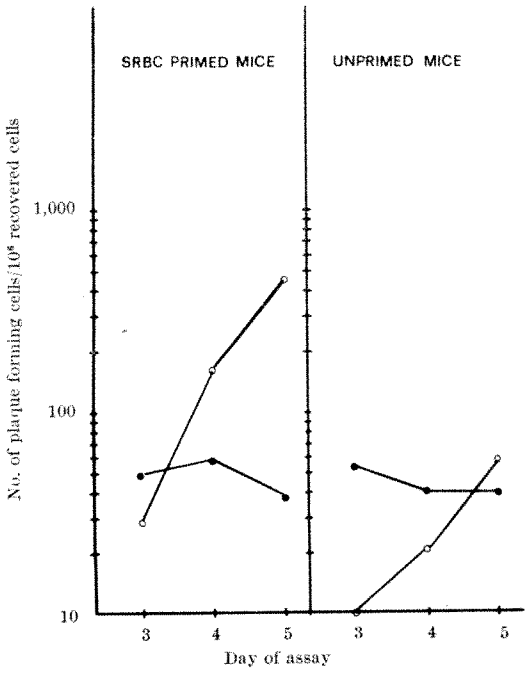


Fig. 2. Effect of prior immunization by carrier red cell; plaque formers are assayed on NIP-DRBC. Immunized F1 hybrid C57Bl/6×DBA2 mice were primed by an injection of SRBC 44 days before setting up cultures. Antigens used in culture: ○, NIP-SRBC; ●, none.

there was no diminution in the plaque forming cells, which suggested that at least two cells were involved in the anti-hapten response, and that the carrier specific cell in the spleen from a primed animal was in excess over the hapten specific cell.

Cells synthesizing antibodies to a hapten can be induced consistently *in vitro* by hapten coupled to red cells and by using spleen cells from mice immunized previously with the red cell carrier. When spleen cells from unimmunized mice are used, the response is variable. Because prior immunization with the carrier erythrocyte seems to be essential for hapten response some correlation between the *in vitro* response to the hapten and to the carrier cell might be expected. This was found insofar as the highest responders to one specificity were the highest to the other and the lowest also the lowest, but intermediate values did not correspond. This may be related to the fact that dilution of the primed cells with cells from unimmunized animals showed that the primed cells were in excess. The specificity of induction by the hapten seems clear, as does the specificity of the carrier cell.

The results with the hapten carrier complex could be interpreted in terms of current hypotheses[7] as being a consequence of cooperation of cells primed against the carrier with cells capable of synthesizing antibodies to the hapten.

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Mechanism for Joining *V* and *C* Genes

I would like to suggest an alternative to the mechanism suggested by Gally and Edelman[1] for the joining of the *V* gene to the *C* gene. This mechanism has three principal features: (1) no "cross-over" event (for excision and integration) is required; (2) the joined product, the coupled *VC* into one gene, could either be an RNA or a DNA molecule; and (3) the *C* gene in its joining end must have a few redundant bases which are almost identical to the joining end of the *V* gene.

Consider the genes *V* and *C* in Fig. 7 of Gally and Edelman's article and assume that the attachment site is the overlap region in the joining ends of *V* and *C* genes (Fig. 1*a*). Let the transcription enzyme transcribe opposite strands of *V* and *C* genes to generate a partially double stranded RNA molecule. Alternatively, these could be as in Fig. 1*b*, single stranded pieces of DNA. At this point a repair enzyme together with a ligase converts it into a double stranded molecule which would now replicate with the help of a replicase (Fig. 1*c*). A mechanism to generate diversity could be introduced by errors in the repair process; in this case the heterozygous molecule must segregate very early.

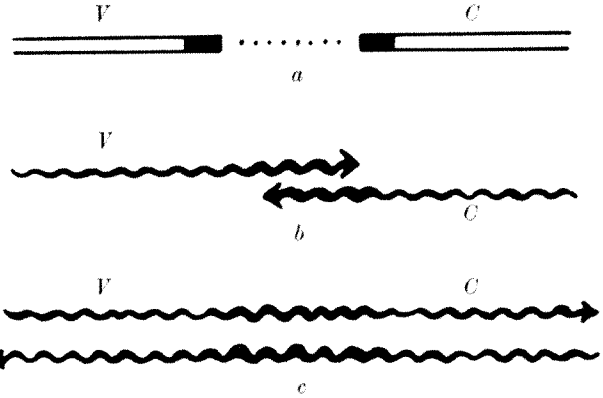


Fig. 1. The joining of *V* and *C* genes. See text for explanation.

This self replicating, intermediate template might be a special inheritable material of somatic cells producing immunoglobulin in very large amounts. If such templates are present they would perhaps be unique to immunoglobulin producing cells or cells similar to it—which require to produce many adventitious specificities built into molecules which also share common specificities.

Another mechanism of joining *V* and *C* genes is suggested in Fig. 5 of ref. 1; excision of the genetic region between the right end of any *V* gene and the left end of the *C* gene will achieve the joining by only one "cross-over" event.

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Cyclophosphamide Tolerance as a Test for Antigenicity of Nucleic Acids

It has been generally thought that nucleic acids are unable to elicit an immune response without carrier protein and adjuvant[1]. We have recently succeeded in accelerating the spontaneous formation of antibodies to RNA and DNA in New Zealand mice with polyinosinic · polycytidylic acid (poly I·C) without carrier protein or additional adjuvant[2]. We have also made New Zealand mice immunologically tolerant to subsequent challenge with poly I·C in complete Freund's adjuvant by pretreatment with poly I·C followed by cyclophosphamide[3]. These studies in mice that spontaneously develop antibodies to nucleic acids suggested that poly I·C was immunogenic but did not prove that nucleic acids could be recognized immunologically by normal mice. We now report the induction of immunological tolerance to poly I·C in normal strains of mice as a method of demonstrating immunological recognition of this nucleic acid.

Multiple injections of poly I·C without adjuvant failed to immunize three normal strains of mice[3]. We have further attempted to detect very low levels of antibody to poly I·C in C57Bl/6, Balb/c, and C3H/He mice treated with twelve injections of poly I·C (75–100 μg/injection). Antigen binding capacities by the ammonium sulphate precipitation method[3] have been less than 0·04 μg/ml., the limit of sensitivity of our assay.

To study further whether normal strains of mice immunologically recognize poly I·C without carrier or adjuvant, we tried to induce immunological tolerance with cyclophosphamide. One month old Balb/c and DBA/2, and 1–3 month old C57Bl/6 mice of both sexes were injected intraperitoneally with either poly I·C (100 μg) or bovine γ-globulin, BGG (500 μg, not ultracentrifuged), and 24 h later given cyclophosphamide intraperitoneally (75 μg/g body weight). Eight days later all mice were challenged intraperitoneally with 150 μg of poly I·C in complete Freund's adjuvant. Antibody levels measured 13 days later by the ammonium sulphate precipitation assay[3] are listed in Table 1. Much lower levels of antibody to poly I·C were found in mice pretreated with poly I·C plus cyclophosphamide than in control mice pretreated with BGG plus cyclophosphamide. Thus these mice were made immunologically hyporesponsive to challenge with poly I·C in adjuvant by pretreatment with poly I·C plus cyclophosphamide, indicating that immune cells were induced to proliferate by antigenic recognition of poly I·C and were destroyed by the cyclophosphamide.

Table 1. TOLERANCE TO POLY I·C IN NORMAL MICE

Strain*	Mean poly I·C binding capacity (μg/ml.) BGG plus cyclophosphamide	Poly I·C plus cyclophosphamide†
C57Bl/6	1·5	< 0·04
Balb/c	6·1	0·2
DBA/2	8·7	0·6

* Twenty animals in each group.

† $P < 0.01$ compared with BGG controls for each strain (t test).

The specificity of the tolerance to poly I·C was investigated in DBA/2 and C57Bl/6 mice. These mice were treated with poly I·C plus cyclophosphamide and then immunized with sheep erythrocytes. Antibody forming cells to sheep erythrocytes were enumerated by the Jerne plaque assay. Mice made tolerant to poly I·C made normal immune responses to sheep erythrocytes, indicating that the tolerance to poly I·C showed immunologic specificity.

Using this technique of cyclophosphamide induced tolerance, we have also found that a double stranded DNA, dAT, is antigenic in New Zealand mice.

The exact mechanism of this phenomenon is not clear. We may be eliminating the small number of cells capable of responding to the nucleic acid, a number perhaps too small to produce a detectable antibody response witho adjuvant. On the other hand, if cooperation of two c types is necessary for the response to the antigen adjuvant, we may be selectively eliminating one of t populations of cells necessary for the response. Whatev the exact mechanism, cyclophosphamide tolerance m be a useful test for the antigenicity of a variety of su stances which, like nucleic acids, have not previous been thought to be recognized immunologically.

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Antigenic Determinants in Amyloid Deposits

Precise analyses of human amyloid laden tissues ha shown that they contain at least two specific components- a fibrillar protein of uniform cross-sectional diameter and a rod-shaped protein (P-component). Units of t latter look pentagonal under the electron microscope a show antigenic identity with an α-globulin present human plasma[3–5]. Preliminary studies have also suggest that the fibrillar component of amyloid is non-immun genic whereas the P-component is capable of eliciting strong antibody response[6–8]. Recently, we have succeed in preparing antisera not only to the P-component b also to alkali degraded amyloid fibrils (DAM), according the method of Pras *et al.*[9]. Here we show that the antisera react not only with DAM but also with amylo

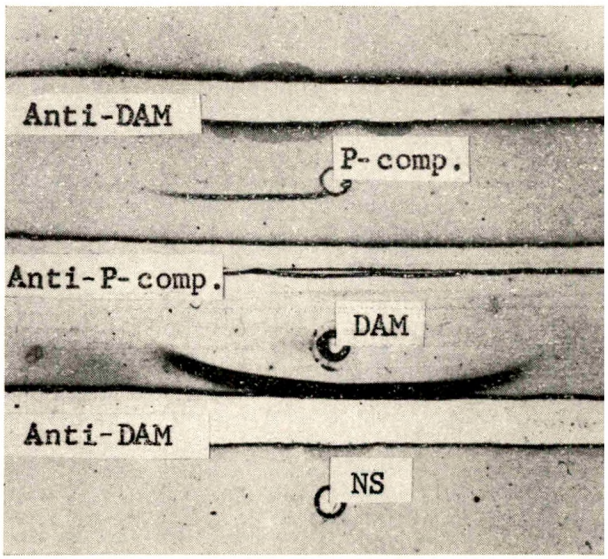


Fig. 1. Immunoelectrophoresis of amyloid components. P-component (P-comp.) forms a single precipitin line against anti-P-component antiserum (anti-P-comp.) but fails to react with anti-DAM antiserum: DAM forms a single precipitin line against anti-DAM antiserum but fails to react with anti-P-comp. Normal serum (NS) fails to react with anti-DAM.

deposits in various tissues, particularly liver, spleen and kidney. Data are also presented which compare the tissue reactivity of anti-DAM and anti-P-component antisera measured by the fluorescent antibody technique.

No identity between P-component and DAM was detectable by the Ouchterlony technique. By immunoelectrophoresis, P-component did not react with antiserum to DAM and DAM did not react with antiserum to P-component (Fig. 1). As has been seen before, normal serum formed a single precipitin line when tested against antiserum to P-component; antiserum to DAM, on the other hand, failed to react with normal serum (Fig. 1). The fluorescent antibody studies were performed on 8 μm thick sections of liver, spleen and kidney, snap frozen in a mixture of dry ice and acetone immediately after the deaths of two patients with primary amyloidosis, AM-Sn and AM-Gi, and two patients without amyloidosis, NL1 and NL2. Appropriate blocking controls included pretreatment of non-amyloid and amyloid laden tissues with non-immune rabbit serum and rabbit antiserum to human immunoglobulin G (IgG). For these studies all antisera were adsorbed several times with a lyophilized powder containing non-amyloid liver, kidney and spleen. Antisera to P-component and to DAM were further adsorbed with human neonatal cord blood which was previously shown to contain neither P-component[10] nor DAM. The indirect or "sandwich" technique was adopted using fluorescein labelled anti-rabbit globulin antiserum.

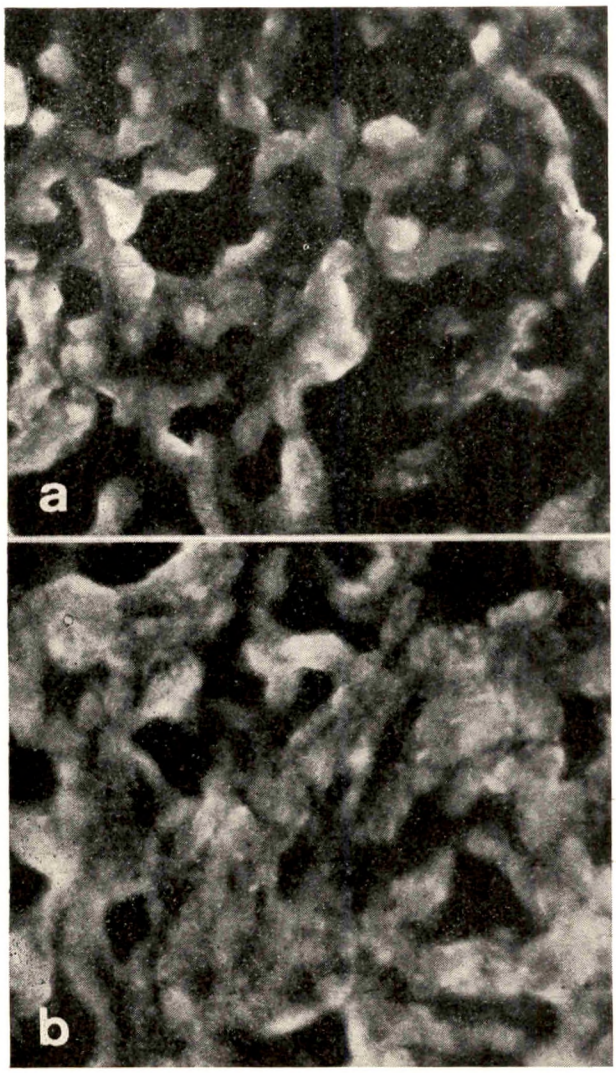


Fig. 2. (*a*) Amyloid deposits in liver AM-Sn reacted with fluorescein-labelled anti-DAM antiserum. (*b*) Amyloid deposits in liver AM-Sn reacted with fluorescein-labelled anti-P-component antiserum.

Areas of bright fluorescence corresponding to deposits of amyloid as observed by polarization microscopy after Congo red stain were discernible in sections of liver (Fig. 2), kidney and spleen from both patients with amyloidosis. No specific differences in the patterns or localization of fluorescence achieved by either anti-P-component or anti-DAM antisera could be ascertained although the anti-P-component antiserum invariably provided brighter and more intense fluorescence of the amyloid deposits than the anti-DAM antiserum. Anti-human IgG antiserum produced weak fluorescence in the tissues of one of the amyloid subjects, AM-Sn (Fig. 2), and in the glomeruli of one of the non-amyloid controls (NL1). Non-specific fluorescent staining was minimal and no fluorescence was visible in the non-amyloid tissues after the addition of anti-P-component or anti-DAM antisera, respectively. We also saw relatively less bright but definite autofluorescence in the amyloid deposits before the addition of specific antisera.

Previous studies using fluorescent antibody techniques suggested that amyloid deposits were composed of immune complexes by virtue of their reactivity with IgG and the C3 component of complement[11,12]; but it was later shown by more precise immunochemical techniques that neither of these proteins is specific for amyloid[13]. This study reveals that amyloid deposits, which by light microscopy appear to be homogeneous, have at least two distinct and specific antigenic determinants, one related to the amyloid fibril and one related to the P-component. Moreover, the availability of monospecific antisera to each of these two antigenic determinants should make it possible to differentiate between the parts these two as yet unrelated proteins play in the pathogenesis of human amyloidosis. It is also hoped that more extensive studies using labelled antisera to amyloid components will provide a new diagnostic method for detecting the presence of amyloid in various biopsy specimens.

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Effect of Skeletal Muscle Native Tropomyosin on the Interaction of Amoeba Actin with Heavy Meromyosin

ACTIN and myosin, recently identified in protozoa and myxomycetes, are presumably responsible for the motility of these organisms[1–5]. Control of this motility is, however, not at all understood. In skeletal muscle, contraction is partly under the control of the protein complex, native tropomyosin which is believed to bind to the actin filaments[6,7]. In the presence of Ca^{2+}, native tropomyosin allows the interaction of actin and myosin filaments to occur whereas in the absence of Ca^{2+} native tropomyosin inhibits this interaction[6], probably by inhibiting the binding of actin to myosin[8]. No protein like native tropomyosin has yet been identified in a primitive contractile system, but we know that *Acanthamoeba* actin activates the ATPase of skeletal muscle heavy meromyosin (HMM) and we have used this fact to investigate the effect of skeletal muscle native tropomyosin on the interaction of *Acanthamoeba* actin with HMM. We have found that native tropomyosin in the presence of Ca^{2+} increases the activation of HMM ATPase by *Acanthamoeba* actin, while in the absence of Ca^{2+} the native tropomyosin inhibits this activation.

Amoeba actin was isolated by the method of Weihing and Korn[4]. After elution of the actin from 'Sephadex', it was concentrated by dialysis against dry 'Sephadex G-200', and then polymerized by dialysis against two changes of 0·1 M KCl–0·5 mM ATP–0·5 mM 2-mercaptoethanol–2·0 mM imidazole (*p*H 7·0). Skeletal muscle actin was isolated by the method of Adelstein *et al.*[9], and F-actin was prepared and taken into solution as described previously[11]. Skeletal muscle myosin and heavy meromyosin were prepared by the method of Kielley and Harrington[15] and Eisenberg and Moos[11] respectively. Native tropomyosin was prepared by the method of Hartshorne and Mueller[12] except that 5 mM 2-mercaptoethanol rather than 2 mM dithiothreitol was used. ATPase activity was assayed using the *p*H-stat as previously described[10]. ATP was purchased from Sigma and ethylene glycol-*bis*-(β-aminoethyl ether)-N,N′-tetraacetic acid (EGTA) was a gift from the Geigy Chemical Co.

Acanthamoeba actin activated the HMM ATPase although it was only about one quarter as effective as skeletal muscle actin under identical conditions (Table 1). Furthermore, as for skeletal muscle actin, in the absence of native tropomyosin, EGTA and Ca^{2+} had no effect on the amoeba actin activation of the HMM ATPase. Conversely, in the presence of Ca^{2+}, native tropomyosin caused a marked increase in the amoeba actin activation of the HMM ATPase. In identical conditions, a similar increase in skeletal muscle actin activation of the HMM ATPase was not observed, but in conditions where the Mg^{2+} concentration was less than or equal to that of ATP, native tropomyosin did induce such an increase in both the actomyosin and the acto-HMM ATPases[8,13].

Table 1. EFFECT OF NATIVE TROPOMYOSIN ON AMOEBA AND RABBIT ACTIN ACTIVATION OF HEAVY MEROMYOSIN ATPase

Native tropomyosin	Activation (μmoles/mg min) EGTA		Ca^{2+}	
	Rabbit actin	Amoeba actin	Rabbit actin	Amoeba actin
−	0·67	0·15	0·64	0·15
+	0·04	0·036	0·54	0·44

All samples contained 2 mM ATP, 4 mM $MgCl_2$, 9 mM KCl, 0·2 mg/ml. of amoeba or rabbit actin, 0·28 or 0·56 mg/ml. HMM and either 1 mM EGTA or 0·1 mM $CaCl_2$. Samples with native tropomyosin contained 0·25 mg/ml. native tropomyosin. The ATPase activity of HMM in the absence of actin is shown in Fig. 1.

Of course, the key effect of native tropomyosin *in vivo* is presumably inhibition of the actin–myosin interaction in the absence of Ca^{2+} (ref. 6), and as can be seen from Table 1, in the presence of the Ca^{2+} chelator EGTA, native tropomyosin does indeed cause a marked decrease in both the amoeba actin and skeletal muscle actin activation of the skeletal muscle HMM ATPase. Because native tropomyosin binds to actin, but apparently does not bind to myosin[14], the present data suggest that skeletal muscle native tropomyosin binds to amoeba actin and affects its activity.

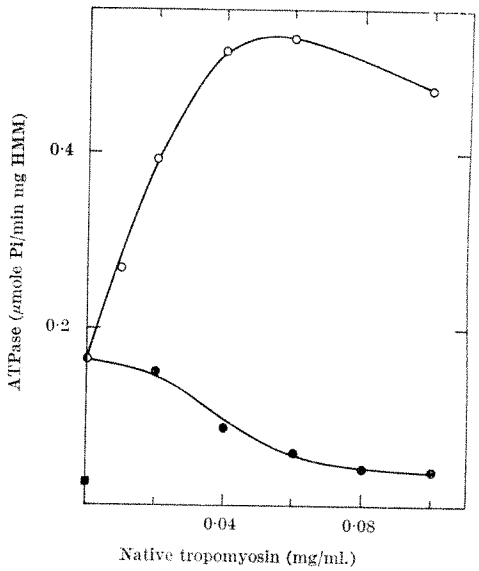


Fig. 1. Effect of native tropomyosin concentration on amoeba actin activation of heavy meromyosin ATPase. All samples contained 2 mM ATP, 4 mM $MgCl_2$, 11 mM KCl, 0·2 mg/ml. amoeba actin, 0·57 mg/ml. heavy meromyosin. ○, 0·1 mM $CaCl_2$; ●, 1 mM EGTA; ■, ATPase of heavy meromyosin in the absence of actin.

Further evidence for this suggestion is demonstrated in Fig. 1 which shows the effect of varying native tropomyosin concentration on the amoeba actin activation of HMM ATPase in the presence of either EGTA or excess Ca^{2+}. Both the activation in the presence of Ca^{2+} and the inhibition in the presence of EGTA level off when the ratio of native tropomyosin to amoeba actin is about 1 to 3 by weight which is similar to the 1 to 2 weight ratio observed with skeletal muscle actin (unpublished data of E. E. and W. W. Kielley). Furthermore, the native tropomyosin inhibition in the presence of EGTA reduces the acto-HMM ATPase almost to the level of that of the HMM alone. Thus skeletal muscle native tropomyosin has a clear effect on amoeba actin activation of skeletal muscle HMM ATPase. Furthermore, because this effect occurs at a relatively low ratio of native tropomyosin to actin it presumably occurs through the specific binding of native tropomyosin to amoeba actin rather than through some non-specific interaction. The clear implication therefore is that amoeba actin has sites for the binding of native tropomyosin. Whether such sites occur on amoeba actin because a protein similar to native tropomyosin occurs in *Acanthamoeba* or whether in fact all actins have such sites even if they do not play a part *in vivo* is not yet known. Attempts are in progress, however, to isolate native tropomyosin-like proteins from *Acanthamoeba*.

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Long Range Coherence and the Action of Enzymes

SOME time ago it was proposed that the energy produced in biological activities is partly stored in various materials through excitation of coherent electrical vibrations (polarization waves). If strong enough, such excitations can be stabilized through non-linear effects leading to various types of deformations[1,2]. R. Ferreira (personal communication) has suggested that such considerations might be of importance for an understanding of the action of enzymes. In fact the properties of a model which I have considered recently (unpublished) seems to support this idea.

The model consists of a system of interacting vibrating electric dipoles suspended in a neutral fluid with whose compressional modes they interact in a non-linear way. This system contains the case treated in appendix I of ref. 1. The electric polarization field P of the dipoles will be (but need not be) restricted to one direction. For weak excitation, this system behaves in the usual way, that is, it exhibits polarization waves whose frequency is very insensitive to the wavelength, and whose energy U is proportional to $\overline{P^2}$, the mean value of P^2. For larger excitations, however, the system shows remarkable properties, the most important being the establishment of a metastable state characterized by a non-vanishing mean value P_+ of P, that is, by a permanent mean displacement as it is found in ferroelectric materials. In the present case, however, this state is metastable; it has a higher energy than the undisplaced ground state though it is stable against small displacements. This metastable state is best reached through excitation of coherent modes which have very different characteristics from non-coherent ones. As a consequence of non-linear properties of the model, excitation of a certain amount of incoherent vibrations in the metastable state will lead as an immediate mechanical consequence to excitation of a coherent mode—quite apart from the further arguments for such excitation given in ref. 2.

Assume now that the model may be applicable to enzyme molecules which would possess metastable excited states in which polar groups are stretched so that the molecule may have a very large dipole moment resembling a ferroelectric case, and in which excitations above this state are partly transformed into coherent vibrational modes. An enzyme molecule so excited will have strong long range interaction with other molecules. This interaction will tend to lift other enzyme molecules into the metastable state, the required energy to be provided from the coherently excited modes. The interaction will also lead to attraction of other types of molecules (substrates) to the excited enzyme molecule, especially when they possess a proper frequency close to that of the coherent modes. It is therefore likely that the total energy of the complex of stretched enzyme and substrate is lower than it would be otherwise and this may facilitate the relevant chemical processes in the substrates.

Some of the properties of our model resemble Koshland's hypothesis of conformational changes[4] but they go much further because they imply a stretching of dipoles throughout the large enzyme molecule rather than a deformation near the active site. A principal consequence is the occurrence of the new long range forces which are likely to dominate the dynamic behaviour.

We thus arrive at the following dynamic picture. Assume the first substrate–enzyme complexes to be formed by fluctuations. The energy liberated by the relevant chemical transformations leaves the enzyme molecules coherently excited near their metastable state; return to the ground state would take a relatively long time. Through the long range forces so established they will (*a*) assist other enzyme molecules to transfer to the metastable state and (*b*) attract further substrates. The energy liberated by the chemical processes so acts as a pumping device. At this first stage the number of activated enzyme molecules—and therefore chemical processes—will consequently increase at an accelerating rate, much as in a chain reaction. This will continue until most enzyme molecules are in the metastable state after which the process will continue in a normal way.

These proposals could be tested in three ways. First, by investigation of the build-up of the reaction rate (first stage; chain-like process) as a function of the enzyme density. Second, by direct observation of the excited coherent modes. These modes are optically inactive. Use of Raman effect would, therefore, be appropriate. Through surface effects weak coherent radiation might, however, be observable. Third, by the detection of increase in the static dielectric constant as a result of the expected large increase in the dipole moment of the enzyme molecules when they are transferred to the metastable state.

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G_{M1}-gangliosidosis Types 1 and 2: Enzymatic Differences in Cultured Fibroblasts

G_{M1}-GANGLIOSIDOSIS occurs in two clinical forms[1,2]. Type 1 resembles Hurler's syndrome somatically, but its psychomotor aspect is apparent from birth and progresses rapidly to cause death by 2 years of age. Type 2 presents no Hurler signs and its psychomotor course, though similar to that in type 1, is neither congenital nor is rapidly progressive; death occurs about 5 years of age. In addition to the accumulation of G_{M1}-ganglioside in neural, and to a lesser extent in extraneural, tissues both types share a profound, generalized deficiency of lysosomal β-galactosidase, with storage and excretion of modified keratin sulphates. Although the clinical and biochemical definition of the type 2 form is very recent[3], failure to observe both clinical types within single sibships is sufficient to suggest that each is caused by a different mutant gene. To test this hypothesis we have examined the gangliosides, glycosaminoglycans and residual β-galactosidase activities in fibroblast cell strains derived from skin in the deltoid area of patients with each type of G_{M1}-gangliosidosis. This communication deals only with the last focus of interest.

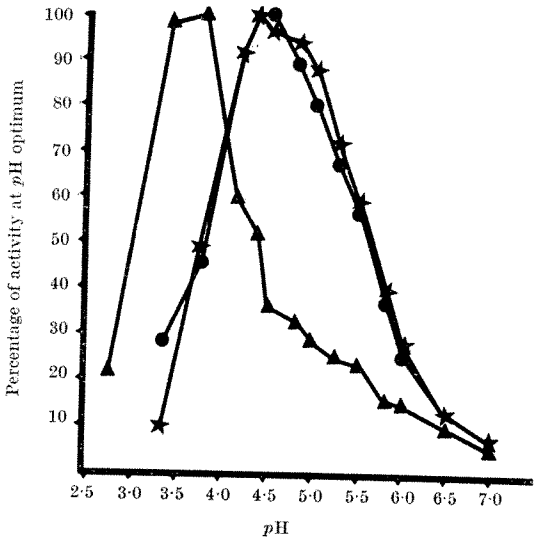


Fig. 1. The *p*H activity curve of β-galactosidase in fibroblasts of a control and both types of G_{M_1}-gangliosidosis. The buffer was 0·1 M citrate-phosphate. Results are expressed as a percentage of the activity at the optimum *p*H for each cell strain. ▲—▲, G_{M_1}-Type 1; ●—●, G_{M_1}-Type 2; ★—★, control.

The cell strains were developed according to Krooth[4] and maintained in Eagle's MEM supplemented with pyruvate, non-essential amino-acids[5], and calf serum (6 per cent foetal and 6 per cent newborn). Monolayers of comparable population density were harvested by successive, short incubations (5 min) in versene and trypsin[6]. The cells ($5–20 \times 10^6$) were then washed twice in 12 ml. of 0·9 per cent NaCl, resuspended in 1 ml. of 0·2 per cent Triton X-100, and sonically disrupted by four 30 s exposures to the 1 cm probe in an MSE sonicator set at 2 μm. After centrifugation of the sonic extract at 20,000*g* for 30 min, the supernatant was used as a source of lysosomal β-galactosidase. The enzyme assay used the fluorogenic substrate, 4-methylumbelliferyl-β-D-galactopyranoside, and was modified from Ockerman[7]. The assay was linear for 1 h at 37° C over the entire range of activities measured, if not less than 120 μg of cell protein was added to the reaction mixture as the source of enzyme. The sensitivity of the fluorometric procedure was essential for dealing with the much reduced β-galactosidase activities in both types of mutant cell strain.

The pronounced deficit of *p*H 5 β-galactosidase activity in both mutant cell strains is shown in Table 1. The greater deficiency in fibroblasts from patients with type 1 G_{M_1}-gangliosidosis is in accord with the relative clinical severity of this form of the disorder. It would be surprising if the major clinical differences between the two types of gangliosidosis were to be reflected only by a relatively slight quantitative difference in the residual β-galactosidase activity of their respective cultured fibroblasts. Indeed, important qualitative differences were soon discovered. Fig. 1 depicts the *p*H dependence of β-galactosidase activity in control and mutant fibroblasts. The activity in type 1 cells had a peak extending from 3·35–3·75, while the activity in type 2 cells closely resembled the control with a peak extending between 4·35–4·5. An equally decisive difference between the two types of mutant fibroblasts was reflected in the thermolability of their respective residual β-galactosidase activities (Fig. 2). Again, the type 2 cells were like the control, whereas the type 1 cells were quite different.

Table 1. β-GALACTOSIDASE ACTIVITY IN FIBROBLASTS OF CONTROLS AND PATIENTS WITH TYPES 1 AND 2 G_{M_1}-GANGLIOSIDOSIS

Acetate buffer, 0·1 M, *p*H 5·0; 37° C

Controls		nmoles*/ mg protein/h
J8	Newborn foreskin	121·7
J4	Newborn foreskin	206·8
GEL	Labia majora (adult)	267·0
AB	Cystic fibrosis	200·2
GL	Unexplained dwarfism	166·8
SC	Foreskin of 5 year old	267·9
J11	Newborn foreskin	263·0
G_{M_1}-gangliosidosis		
Type 1† —KD		0·9
GM		1·5
NAD		0·6
Type 2‡ —WJ		9·4
TL		7·5

* Of 4-methylumbelliferyl-β-D-galactopyranoside (Koch–Light) cleaved.

† KD and GM refer to cell strains developed from the same patient simultaneously but maintained in different laboratories until the assay was performed. NAD represents a different patient.

‡ WJ and TL refer to cell strains developed from the same patient 2 years apart. TL was assayed after the second subculture.

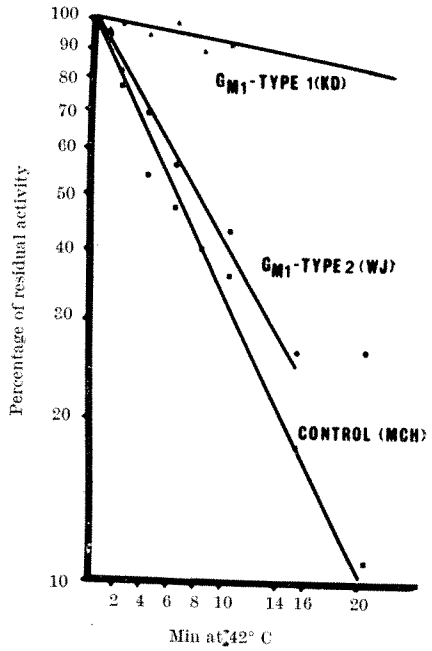


Fig. 2. Thermolability at 42° C of β-galactosidase in fibroblasts of a control and both types of G_{M_1}-gangliosidosis in 0·1 M acetate buffer, *p*H 5·0. Residual activity at each time is expressed as a percentage of initial activity. ▲—▲, G_{M_1}-Type 1; ●—●, G_{M_1}-Type 2; ■—■, control.

We conclude that the two clinical types of G_{M_1}-gangliosidosis are determined by different mutant genes acting to reduce lysosomal β-galactosidase activity, though their precise modes of action remain to be defined. Multiple forms of β-galactosidase have been demonstrated recently within mammalian brain[8], liver[9] and kidney[10] by molecular sieve chromatography or starch gel electrophoresis. The Hurler syndrome seems to be associated with absence of one of these forms in liver[11,12]. It is tempting to suggest that the genes causing each type of G_{M_1}-gangliosidosis differ in terms of their effect on one or more of these forms of β-galactosidase. One difficulty with this approach is that the same form of liver β-galactosidase seems to be absent in three different types of mucopolysaccharide storage disorder: the autosomal recessive Hurler and Sanfillipo syndromes and the *X*-linked recessive Hunter syndrome[12].

To date, five different mutant genes are known to reduce lysosomal β-galactosidase activity in human beings. Comparative studies on cultured fibroblasts expressing one or more of these mutant genes should help to show how these genes act.

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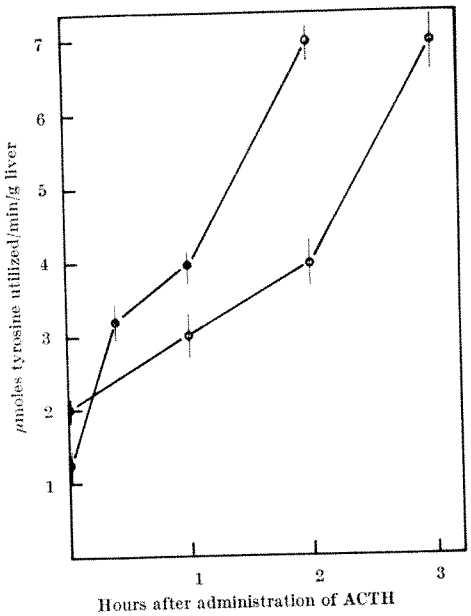


Fig. 1. The age-dependent induction of rat liver tyrosine aminotransferase. Tissue was prepared and enzyme activity assayed as described previously[14]. Each value represents the mean ±s.e. for at least four rats. The intraperitoneal dosage of ACTH was 2·5 units per 100 g of body weight. ●, 2 months old; ○, 18 months old.

Reappraisal of Biological Ageing

LIVING systems depend on adaptive regulation, deterioration in which leads eventually to death. The incidence of most disease increases with increasing age, which suggests that progressive impairment of adaptive response is associated with many illnesses common to advanced age. One biochemical expression of this impairment is the age-dependent increase in the time required to initiate certain enzyme inductions after administration of the appropriate inducing stimulus[1,2] (Table 1).

This biochemical parameter of ageing may provide a unique opportunity to investigate a sequence of events responsible for a particular ageing phenomenon. Recent evidence demonstrated that newly regenerated liver cells in ageing animals exhibit enzyme induction patterns characteristic of untreated animals of the same age[2,10]. So either the age-dependent modification of hepatic enzyme regulation must arise from a genetic alteration of the liver cell which is copied during the cell division

Table 1. REPORTED AGE-DEPENDENT ENZYME INDUCTIONS

Enzyme	Source	Inducing agent	Ref.
Glucokinase	Rat liver	Glucose	1
NADPH ; cytochrome *c* reductase		Phenobarbital	2,3
NADPH oxidase			3
NADPH: neotetrazolium reductase			3
Cytochrome *P*-450			3
Aminopyrine *N*-demethylase			3
Hexobarbital hydroxylase			3
Aniline hydroxylase			3
p-Nitrobenzoic acid nitroreductase			3
Tyrosine aminotransferase	Mouse liver	Exposure to cold	4
Phosphoenolpyruvate carboxykinase	Rat adipose tissue	Fasting	5
NADPH ; malate dehydrogenase		Fasting and refeeding	*
Citrate cleavage enzyme			*
Glucose 6-phosphate dehydrogenase			*
6-Phosphogluconate dehydrogenase			*
Glucose 6-phosphatase	Rat liver	Dexamethasone	6
Fructose 1,6-diphosphatase			6
Phosphofructokinase	Rat prostate, seminal vesicles	Testosterone	7
Tryptophan pyrrolase	Rat liver	Tryptophan	8
Tryptophan pyrrolase	Rabbit liver	Cortisol	9

* Unpublished results of R. W. Hanson.

nduced by partial hepatectomy, or it is not inherent in the liver cell. Two independent observations suggest that the latter is the case. The induction of mouse liver tyrosine aminotransferase by exposure to cold, considered an adrenal steroid mediated process, exhibited an age-dependent lag period, whereas enzyme induction after administration of cortisol was independent of age[4]. Similarly, induction of rat liver glucokinase by glucose feeding, an insulin mediated process, exhibited an age-dependent lag, but enzyme induction by administration of insulin was independent of age (R. C. A., unpublished). Thus the ability of liver cells to respond to hormonal stimulation, unlike that of other cell-types such as adipose cells[11], is not an age-dependent phenomenon[2].

The role of endocrine function in the age-dependent modification of hepatic enzyme regulation is not clear. According to Finch *et al.*[4], the production of corticosterone by the adrenal glands in response to exposure to cold is not influenced by age. So the ageing modification involving tyrosine aminotransferase must occur between adrenal steroid production and hepatic enzyme induction. But the procedures available for accurate determination of functional serum hormone levels are questionable at best. An alternative approach recently developed by Adelman (R. C. A., unpublished) entails stimulation of the appropriate endocrine source and determination of circulating, functional hormone production as expressed by the time course of hepatic enzyme induction. Adrenal corticotrophic hormone (ACTH) administration resulted in a rapid, age-dependent increase in hepatic tyrosine aminotransferase activity (Fig. 1). Eighteen-month old rats required approximately twice as much time as 2 month-old rats to respond similarly to the same body-weight dosage of ACTH. If this enzyme induction was hormone mediated, then the endocrine function in question is influenced by age. It seems unlikely, however, that this induction was mediated by adrenal steroid. Whereas tyrosine aminotransferase activity peaked 4–5 h following administration of cortisol, the ACTH effect was observed in as little as 1 h and terminated after 3 h (R. C. A., unpublished). When the endocrine role in the age-dependent modification of hepatic enzyme regulation is resolved, the influence of sympathetic and parasympathetic innervation and of pituitary and hypothalamic function can be examined.

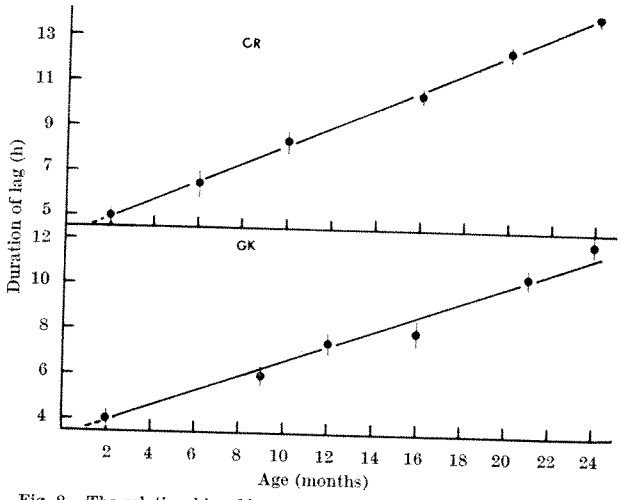


Fig. 2. The relationship of lag period duration of the glucokinase (GK) and NADPH : cytochrome *c* reductase (CR) inductions to chronological age of the rat. Tissue was prepared and enzyme activities assayed as described previously[1,2]. Each value represents the mean ±s.e. for at least four rats.

This general approach not only localizes the tissue of origin of the age-dependent modifications, but also contributes to comprehension of the ageing process(es). The unknown modification which is expressed as an increased lag period of hepatic enzyme induction was observed throughout the entire lifespan of the rat[2]. Looking at the chronological origin of this modification (Fig. 2), it is apparent that the briefest lag period for the inductions of glucokinase[1] and NADPH : cytochrome *c* reductase[2] coincides with the development of adult levels of these enzymes, approximately 15–30 days after birth[12,13]. It is tempting to speculate that the ageing phenomena which are expressed in adult liver and perhaps other tissues are the consequence of developmental phenomena in the foetal neuroendocrine regulatory system.

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Inhibition of Chemical Carcinogenesis in the Hamster Cheek Pouch by Topical Chlorpromazine

NUMEROUS studies have shown that chlorpromazine (CPZ) affects the function of biological membranes[1] and the lysosomes[2]. The effect of CPZ seems to be dose-dependent[3,4], but in most studies it caused a decrease in membrane permeability[5,6], and it has been shown to antagonize lysosomal labilization induced by vitamin A[2]. In previous studies, chemical carcinogenesis in the hamster cheek pouch was promoted by the membrane and lysosomal labilizers vitamin A palmitate[7] and stilboestrol diphosphate[8]. We now report the effect of CPZ on the same experimental model.

The right cheek pouches of thirty male Syrian golden hamsters aged 2 months were painted three times per week with one of the following substances dissolved in liquid paraffin: 0·5 per cent 9,10-dimethyl-1,2-benzanthracene (DMBA) (twelve animals, D group); 0·5 per cent DMBA combined with 1·2 per cent CPZ USP (twelve animals, DC group); or 1·2 per cent CPZ (six animals, C group). A further six animals were treated with paraffin only (P group). Half the animals in each group were killed after 9 weeks, and the remainder after 12 weeks. During the macroscopic examination of the cheek pouches, the tumours were counted, and the nature of these lesions was determined histologically. Multiple sections were also taken from macroscopically non-tumorous areas of the cheek pouches. The neoplastic lesions were classified as benign papillomas, intraepithelial carcinomas, papillomas with intraepithelial carcinoma (atypical papillomas), or invasive squamous cell carcinomas, as defined previously[8]. Papillomas and squamous cell carcinomas could be counted accurately. Because intraepithelial carcinomas did not present tumours macroscopically, the frequency of these lesions had to be estimated from multiple histological sections, and the findings were expressed as − when these lesions were not found, as + when only a few of these lesions were present in some animals of a group, or as + + when many lesions were present in each animal. The cheek pouches in the C and P groups were normal. The results in the other groups are summarized in Table 1.

Generally, the cheek pouch mucosa in the DC group showed mild benign epithelial hyperplasia, hyperkeratosis, and marked fibrosis and inflammation of the lamina propria. The striking finding in this group, however, was a very marked suppression of epithelial neoplasia, with almost complete inhibition of carcinoma formation after 9 and 12 weeks.

The promotion of chemical carcinogenesis in the hamster cheek pouch by membrane labilizing agents encountered in previous studies[7,8] was attributed to the known effect of these substances on cell membranes or lysosomes[9,10]. In this study, tumour inhibition was achieved with the membrane stabilizing agent CPZ. A similar inhibiting effect of CPZ on tumorigenesis has been recorded by others[11,12], but the mechanism of this effect has not been ascertained. The effect of CPZ on carcinogenesis may have been due to decreased permeability of cellular membranes, resulting in less effective penetration of the carcinogen into cells and subcellular structures. On the other hand, decreased permeability of subcellular structures induced by CPZ may have resulted in stabiliza-

Table 1. INCIDENCE OF MALIGNANT AND PREMALIGNANT TUMOURS IN HAMSTER CHEEK POUCHES TREATED TOPICALLY WITH DMBA ALONE OR WITH DMBA AND CPZ

	Group	Animals with carcinoma	Total carcinomas	Average number of carcinomas per exposed pouch	Animals with atypical papillomas	Total atypical papillomas	Average number of atypical papillomas per exposed pouch	Intraepithelial carcinomas
9 weeks	D	3	3	0·5	4	9	1·5	+ +
	DC	1	1	0·2	—	—	—	—
12 weeks	D	6	18	3·0	6	8	1·3	+ +
	DC	1	1	0·2	1	1	0·2	+ *

Each group consisted of six animals.
* In two animals only.

tion of lysosomal membranes and in decreased liberation of lysosomal enzymes, which may play a part in the early stages of cell division and carcinogenesis[10], particularly because CPZ is also known to suppress DNA synthesis in the bone marrow granulocytic series, resulting in delayed cell division[13].

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Fibrin Proteolysis in the Monkey Uterine Cavity: Variations with and without IUD

The uterine tissues of several mammalian species are an abundant source of plasminogen activator[1]. Human menstrual discharge is known to contain high concentrations of plasminogen activator and plasmin but insignificant amounts of fibrinogen and plasminogen[2]. Plasmin, the product of plasminogen activation, proteolytically degrades fibrin and fibrinogen preferentially to other substrates; so a likely physiologic role of plasminogen activator in the uterus would be that of maintaining menstrual blood in a fluid state.

During menstruation, when necrotic endometrial tissue releases its store of activator into the plasminogen rich environment of intrauterine blood, intense fibrinolysis (and fibrinogenolysis) is predictable. The role and magnitude of fibrinolytic activity in the uterus between menses, however, are not clear. During the normal intermenstrual period, endometrial cells are morphologically intact and may not release plasminogen activator. Research on fibrinolytic activity in the cavity or endometrium of the uterus between menses has been hampered by difficulties in collecting adequate quantities of unaltered cavitary fluid or endometrial tissue from unanaesthetized living organisms. Moreover, quantitative fibrinolytic assays on tissue generally do not differentiate between plasmin activity generated by tissue factors and that generated by factors in the blood contained within the tissue. The extraction procedure of Astrup[3], performed on endometrium followed by assay of plasminogen activator, does not necessarily reflect actual plasmin activity *in vivo*; rather it is a measure of the potential for plasmin activity. Fibrinolytic activity other than that of plasmin has apparently been measured in endometrium between menses[4], but the role and extent of such activity *in vivo* are not clear. This report describes preliminary studies of local fibrin proteolysis in the intermenstrual monkey uterus.

In order to investigate *in vivo* proteolytic behaviour, we have used intrauterine incubations of 'Millipore' chambers containing radioactive fibrin. These chambers have a mean pore size of 0·45 μm and have the following properties: they allow free interchange between uterine fluid and chamber of soluble molecules (including proteolytic enzymes, their precursors, and fibrin degradation products); they contain insoluble fibrin polymers; and they exclude cells which might attack fibrin phagocytically. Thus the percentage loss of radioactivity from these chambers following intrauterine incubation was used as an index of proteolytic activity. Because of our interest in the effect of intrauterine contraceptive devices, some incubations were performed on animals wearing such devices (modified Lippes loops).

One preparation of lyophilized ^{125}I-labelled fibrin was used for all experiments (unpublished results of R. W. C. and S. T. S., jun.). This preparation was insoluble in 5 M urea and free of any significant amount of active plasmin, plasminogen, plasminogen activator or other fibrinolytic activity. Each cylindrical 'Millipore' chamber, measuring 1·9 × 13·0 mm, was packed loosely with 10 mg of the fibrin preparation, sealed with silicone rubber, and then hydrated for 2 h in *p*H 7·0 buffer (0·033 M phosphate–0·075 M NaCl) before uterine insertion. *In vitro* experiments on representative chambers showed good retention of fibrin, complete exclusion of red cells and leucocytes from the chamber interior and response to plasmin and trypsin by progressive loss of chamber radioactivity. Degradation of fibrin was not produced by trauma to the chambers as long as there were no grossly visible tears in the nylon backed 'Millipore' membrane. Unanaesthetized, ovariectomized *Macaca mulatta* monkeys artificially cycled with oestradiol and progesterone were used. All animals had chronic surgical fistulas connecting the anterior abdominal surface with the uterine cavity. The fistulas served as a convenient route for accurate insertion of chambers and intrauterine devices. These preparations have been described previously[5,6]. In all cases, the fibrin-containing 'Millipore' chambers were inserted on the twenty-first day of a 24 day menstrual cycle and extracted 18 h after insertion. Chambers were washed in buffer after extraction to eliminate diffusible fibrin degradation products. Determinations of chamber radioactivity were then made in a well-type scintillation spectrometer. Conditions of sample geometry and counting which would assure valid comparisons between chambers were established and maintained. Loss of radioactivity from the chamber over the 18 h incubation was expressed as "per cent lysis" and calculated as follows

$$\text{per cent lysis} = \frac{(C_1 - C_2)\ 100}{C_1}$$

where C_1 = net c.p.m. in chamber before insertion; C_2 = net c.p.m. in chamber after extraction and washing.

Table 1. LYSIS OF FIBRIN IN THREE CONDITIONS OF INCUBATION

Incubation (N)*	Per cent lysis Mean	s.d.
In vitro group (60)	6·1	1·6
In utero group (37)	32·9	10·2
In utero—IUD group (10)	56·7	8·0

* Incubation was for 18 h in all cases. N designates the number of incubations in each group. In the *in utero* groups, N represents the number of animal cycles satisfactorily tested.

Table 1 summarizes the data for three experimental groups. The first group consists of sixty control chambers incubated simultaneously with *in utero* experiments in *p*H 7·0 phosphate–saline buffer at 37° C with gentle agitation. The degree of fibrin lysis measured under these *in vitro* conditions was consistently low with a mean of 6·1 per cent and a standard deviation of 1·6 per cent. In the second group, proteolysis was measured during thirty-seven cycles in eighteen monkeys without intra-

uterine devices. Mean per cent lysis for this group was 32·9 per cent. In the third experimental group, lysis measured during ten cycles in seven monkeys with an intrauterine device (IUD) was 56·7 per cent. The difference in proteolytic activity between the IUD and non-IUD monkey cycles is statistically significant ($P < 0.001$). (Although the compared sample groups appear to be homoscedastic (F test) and results from the t test are significant ($P < 0.001$), the size of the sample population is so small that we have relied on the non-parametric Mann–Whitney U test.) An analysis of intercyclic variation in individual monkeys was done in order to further evaluate possible differences in proteolytic activity caused by intrauterine devices. For this purpose the differences in per cent lysis between cycles were divided into two groups: all consecutively measured cycles without an IUD in individual animals, and all consecutively measured cycles in which an animal wore an IUD during one cycle but not during the other. Data from which these differences are calculated are shown graphically in Fig. 1. Intercyclic differences for the two groups are plotted in Fig. 2. Mean difference in per cent lysis between consecutively measured cycles without an IUD was 6·8 per cent. Nineteen such intercyclic differences in ten monkeys varied from 0·3 to 16·9 per cent lysis. Mean difference between consecutively measured cycles with and without an IUD in individual monkeys was 22·3 per cent lysis. Five such intercyclic differences in four monkeys ranged from 13·4 to 34·9 per cent. The variation in proteolytic activity between the two groups is different statistically ($P < 0.001$).

Harpel and coworkers[7] have measured fibrinolytic activity of mouse uterine fluid after collecting the fluid from distended uterine horns after cervical ligation. They indicated that there is no direct proteolytic effect on fibrin from non-haemorrhagic cavitary fluid of mice. Plasminogen activator has been observed histochemically

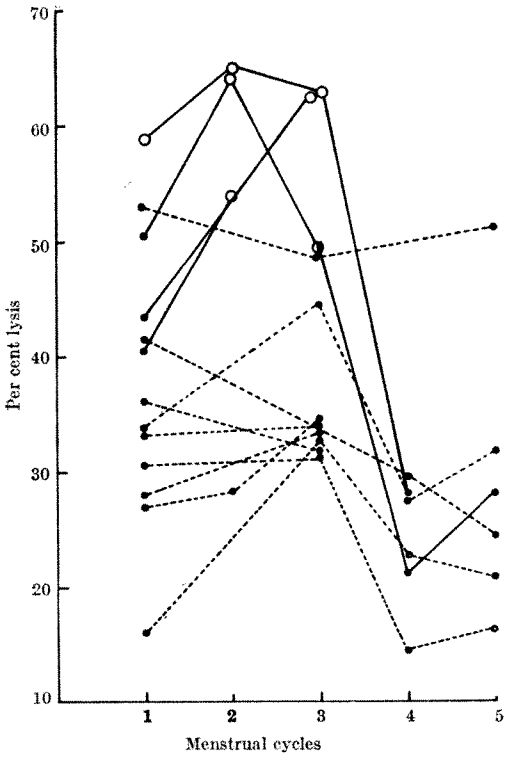


Fig. 1. Per cent lysis of fibrin following 18 h incubation *in utero*. Each incubation begins on the twenty-first day of the menstrual cycle. Open and closed circles represent per cent lysis values while solid and broken lines connect consecutive measurements on individual animals in the following manner: ○, IUD present during cycle measured; ●, IUD not present during cycle measured; —, IUD present during one or more cycles; - - - , IUD not present during any cycles.

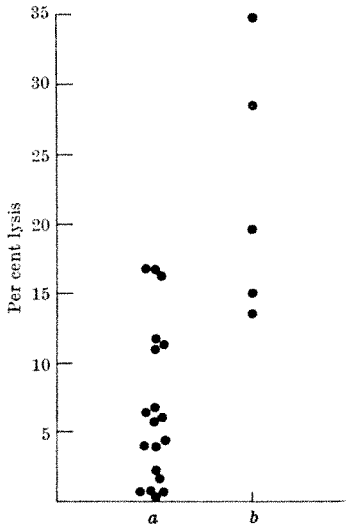


Fig. 2. Intercyclic differences in per cent lysis in (*a*) consecutively measured cycles without an IUD, and (*b*) consecutively measured cycles with and without an IUD.

by other investigators[8,9] in the surface epithelium ar stromal vessels of intact human and rat endometriur but little plasmin or other direct fibrinolytic activity this tissue between menses has been inferred from su techniques. Our work demonstrates direct, and qui active, fibrin proteolysis in the intermenstrual monk uterus.

Because of uncontrolled variables inherent in an *in vi* proteolytic assay, our measurements have probably n quantified enzyme activity in a linear fashion. Tl measurements we have made, however, should rela levels of greater or lesser activity among animal cycle for the same quantity of initial substrate and the san time of incubation were used for each animal cycl Biopsies of endometrium taken immediately after chamb extraction were studied microscopically for histolog alterations caused by the chambers themselves. Althou intracavitary leucocytosis was stimulated, the end metrium was not significantly affected. The possibili that fibrin proteolysis was stimulated primarily l chamber insertion will be investigated in future work.

Several sources of the proteolytic activity we ha measured seem possible. Plasma proteases could enter tl uterine cavity by diffusion from endometrial capillarie Such enzymes could also be released from leucocytes the superficial endometrium or uterine cavity. Perhaps more interesting possibility is the release of plasminog activator from endometrial sources such as the lini epithelium or vascular endothelium. If plasminogen present in the plasma transudate of endometrium ar uterine cavity, plasmin activity could be generated either site by plasminogen activator. Regardless of t characteristics and origin of proteolysis, however, flu in the intermenstrual uterine cavity of monkeys c digest fibrin, and this capability appears to be enhanc by the presence of a plastic intrauterine device.

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Peroxide Production by Mycoplasmas in Chicken Tracheal Organ Cultures

MANY mycoplasmas liberate peroxide *in vitro*[1-3] and this might be an important virulence factor in respiratory infections[1,3,4]. There are, however, few *in vivo* data[5] to support this hypothesis. Recently we showed that several mycoplasma species grew in chicken embryo tracheal organ cultures and that some adversely affected ciliary activity[6]. Subsequent studies have shown that the cytopathic effect of *M. mycoides* var. capri in organ cultures is caused by peroxide secretion[7]. Conversely, damage to cilia caused by *M. gallisepticum* is not (unpublished observations of J. D. C. and D. T-R.). We have now measured and compared peroxide production by several mycoplasmas in tracheal organ cultures and correlated the findings with damage to the ciliated epithelium.

Chicken embryo tracheal organ cultures were prepared as described elsewhere[6]. A group of five cultures was used for each factor investigated. Peroxide was measured by a modification of the aminotriazole–catalase method[8]. In this, catalase combines with hydrogen peroxide to form a complex which then reacts with aminotriazole, so irreversibly inhibiting catalase activity. Because this occurs only when hydrogen peroxide is present, the reaction is a specific test for this molecule. The rate of catalase inhibition is proportional to the rate of hydrogen peroxide secretion, and we have expressed the results not as absolute amounts of peroxide but as a percentage reduction of catalase activity from that originally present. Beef liver catalase (0·2 mg Boehringer), and aminotriazole (3·5 mg Koch Light), were added to organ cultures inoculated 3 to 5 days previously with mycoplasmas. Initially, and at convenient intervals thereafter, 0·05 ml. aliquots were removed from each of five tubes within an experiment group and pooled. To 0·1 ml. of harvested pooled medium was added 0·5 ml. of 2 per cent ethyl alcohol to stop the reaction, the mixture incubated at room temperature for 10 min and diluted to 10 ml. with distilled water. Residual catalase activity was determined by a permanganate titration method[9].

Table 1. THE REDUCTION (PER CENT) IN CATALASE ACTIVITY AFTER INCUBATION OF TUBES WITH AND WITHOUT CHICKEN TRACHEAL SECTIONS MAINTAINED IN MEDIA CONTAINING CATALASE OR CATALASE-AMINOTRIAZOLE (AT)

Duration of incubation (days)	Medium containing catalase and tracheal sections	Medium containing catalase	Medium containing catalase-AT and tracheal sections	Medium containing catalase-AT
1	12	3	62	41
4	35	47	100	100
8	43	47	—	—

We first investigated the stability of catalase and catalase-aminotriazole in the organ culture system. More than 50 per cent of the catalase remained active for at least 8 days in culture tubes whether or not they contained tracheal ring sections (Table 1). Conversely, tubes containing tracheal sections and catalase–aminotriazole lost 62 per cent of their catalase activity in one day and tubes without sections lost 41 per cent of their catalase activity over a similar period. The culture medium therefore contained a substance which on incubation generated peroxide. Because cultures with tracheal sections, in three experiments, lost more catalase activity than cultures without sections it seemed that peroxide was produced by the tracheal rings as well as by the medium.

Table 2. THE COMPARATIVE GENERATION OF PEROXIDE BY SELECTED MYCOPLASMAS, AND TRACHEAL SECTIONS IN THE CHICKEN TRACHEAL ORGAN CULTURE SYSTEM

Duration of incubation (h)	*M. mycoides* var. capri	*M. mycoides* var. mycoides (strain Gladysdale)	*M. gallisepticum* (strain MG2)	*M. gallinarum* (strain W115)	Uninfected culture
4	32				
6	41				
24	98	13	41	10	37
72	100	100	100	86	75

Peroxide is expressed as percentage reduction of catalase activity inhibited. The concentration of each mycoplasma in the medium was 10^7 c.c.u/ml. at time 0 h. Results were obtained in one experiment.

Peroxide generated by several mycoplasmas, including *M. mycoides* var. capri (henceforth called *M. capri*), was measured over a period of time (Table 2). *M. capri* produced peroxide rapidly, virtually all catalase activity being inhibited by hour 24. At this time, some of the other mycoplasma-infected cultures had produced even less peroxide than the uninfected cultures. At hour 72, however, cultures infected by *M. mycoides* var. mycoides and *M. gallisepticum* had produced sufficient peroxide to inhibit all catalase activity, whereas 25 per cent activity still remained in the control cultures. In other experiments (Table 3), only cultures infected with *M. capri*, and the related strain B3, clearly produced more peroxide than uninoculated cultures. Because many of the other mycoplasmas tested liberate peroxide *in vitro*[2] it is surprising that when they were present in the organ culture system we could not demonstrate more peroxide than the greatest amount produced by the tracheal cultures themselves.

Cohen and Somerson[10] noted that the rate of peroxide generation by *M. pneumoniae* was proportional to the number of organisms. The difference in peroxide production between *M. capri* and the other species we investigated was not merely a function of organism concentration. The number of organisms of *M. mycoides* var. mycoides or *M. gallisepticum* (MG2) in the organ cultures was equal to or greater than *M. capri* (Table 3).

Table 3. THE GENERATION OF PEROXIDE BY SELECTED MYCOPLASMAS FOLLOWING GROWTH FOR ONE DAY IN CHICKEN TRACHEAL ORGAN CULTURES

Mycoplasmas and control cultures	Concentration of mycoplasmas c.c.u./ml. of medium	Catalase inhibited (per cent)
M. capri (average of 4 experiments)	10^7	97 (range 89–100)
B3 strain	10^8	83
M. mycoides var. mycoides (Gladysdale) (average of 3 experiments)	10^7	38 (range 13–62)
M. mycoides var. mycoides (T3)	10^8	57
M. gallinarum (W115) (average of 2 experiments)	$10^{8.5}$	9 (range 7–10)
M. gallisepticum (J1)	10^8	17
M. gallisepticum (MG2)	10^7	41
WR1 strain	10^5	45
M. pulmonis (M1)	10^5	33
M. pneumoniae (FH)	10^9	58
M. hominis (73T)	10^8	46
M. laidlawii B	10^8	57
Uninoculated control cultures (average of four experiments)		38 (range 5–62)

Peroxide is expressed as percentage reduction of catalase activity inhibited. The results were obtained from several experiments.

The relationship between mycoplasma peroxide generation and damage of ciliated tracheal epithelium is dependent on many factors. These include the quantity of hydrogen peroxide produced, its delivery to the cellular site, and its effect on the mycoplasmas liberating it. Tissue cell and microbial organism catalase[11] and organism peroxidase[10] must also be considered when evaluating the ultimate results in organ culture or *in vivo*. Of the

mycoplasmas studied, only *M. capri*, strain B3, and the *M. gallisepticum* strains cause rapid ciliary damage of chicken tracheal organ cultures. The damage caused by *M. capri*, which produces a large amount of hydrogen peroxide, can be delayed by adding catalase to the organ culture system, whereas the damage by *M. gallisepticum*, which does not produce a measurable amount of peroxide in the system, cannot be reversed by adding catalase. These findings suggest that peroxide is an important virulence factor in *M. capri* infections, but that a different mediator or mediators should be searched for in *M. gallisepticum* disease.

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Metabolism of D-Alanine in *Rhodospirillum rubrum* and its Bacilliform Mutants

THE photosynthetic bacterium *Rhodospirillum rubrum* affords a particularly simple and versatile system for study of variability in bacterial form. Its morphology

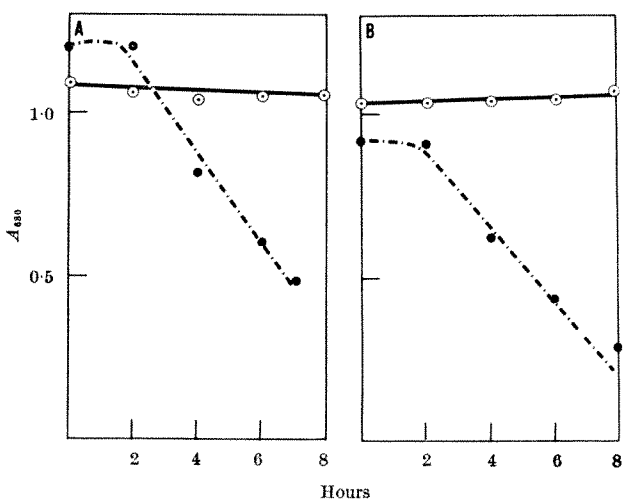


Fig. 1. Lysis of logarithmic phase cells by cycloserine. Strains used were parent *Rhodospirillum rubrum*; strain S_4; a bacilliform mutant; strain S_{10}, another bacilliform strain; and S_4R_1, a spiral revertant of S_4. Cycloserine, 5 μg/ml. added at 0 time, and absorbance of cultures measured at 680 nm (where pigment absorption is minimal). All cultures grown anaerobically in the light in a malate medium[18]. $A.S_4$ bacillus: ○—○; parent spirillium: ●—·—·—●. $B.S_{10}$ bacillus: ○—○; S_4R_1 spiral revertant: ●— — ·—·—●.

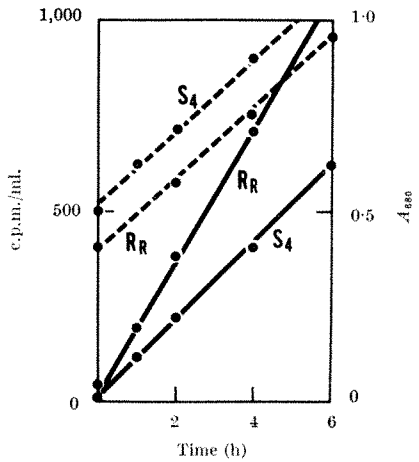


Fig. 2. Incorporation of D-alanine-^{14}C (2 μmoles/ml., 16,500 c.p.m./μmole) into wall fraction by parent spirillum R_R, and bacilliform strain S_4. Alanine added at 0 time to logarithmic phase cultures and the absorbance at 680 nm (●- - -●) and radioactivity incorporated into phenol insoluble (wall fraction) was measured (●—●).

can be altered by nutritional, genetic or chemical means. In addition to spirilla, this organism readily forms rods, vibrios, filaments, circular cells, chains of rods and chains of spirilla[1–3]. Many of these variants have one feature in common: they can be induced either by D-alanine or agents which affect D-alanine metabolism. D-Alanine is a main constituent of bacterial cell walls[4], and the dipeptide derived from it is essential for the terminal cross-linking step in cell wall synthesis[5–9].

It was shown earlier that the wall of curved forms of *R. rubrum* is more extensively cross-linked than that of bacilliform cells, indicating one of the effects of morphological mutation from spirillum to rod. Based on this finding and others, it was postulated that bacilliform mutants were defective in D-alanine synthesis (that is, leaky mutants)[10]. Accordingly, further aspects of D-alanine metabolism in *R. rubrum* have been examined. Evidence is presented for a difference in D-alanine metabolism in parent and mutant strains.

The antibiotic D-cycloserine, an analogue of D-alanine, is a competitive inhibitor of alanine racemase and D-alanyl–D-alanine synthetase[11–14]. Most bacteria are sensitive to this antibiotic and the effects of cycloserine are reversed by D-alanine[15–17]. When added to culture media at sublethal concentrations, cycloserine will convert *R. rubrum* into a vibrio form. At lethal concentrations, cycloserine lysed log phase cells of *R. rubrum* but not stationary phase cells, and this lysis was inhibited by D-alanine. The antibiotic therefore appears to create a lethal unbalanced growth situation[18]. If lysis of *R. rubrum* by cycloserine requires active cell-wall synthesis, treatment with this antibiotic should serve as a sensitive indicator of how much D-alanine the cells need for wall synthesis and cross-linking. A comparison was therefore made of the lytic effect of D-cycloserine on spiral and bacilliform *R. rubrum* strains. The data show that at a lethal concentration of cycloserine, spiral cells are much more sensitive to lysis by this agent than are bacilliform cells (Fig. 1). This result would be expected in a cell making a tightly cross-linked wall, and, consequently, having a greater demand for D-alanine to form precursors for wall closure. At higher cycloserine concentrations, both cell types were lysed; the spirillum at a faster rate.

Assimilation of D-^{14}C-alanine into cell walls of parent and mutant strains was then compared using log phase cultures. The parent spirillum did incorporate more D-alanine into wall material in growth conditions comparable with that of bacilliform cells (Fig. 2).

These results therefore lend support to the hypothesis proposed earlier, that the morphology of *R. rubrum* can be related to the ability of the organism to synthesize

D-alanine necessary to form the requisite precursors for cell-wall cross-linkage. An unbalanced growth situation with respect to D-alanine, whether created genetically or nutritionally, can either alter the morphology of viable cells or, if severe enough, cause lysis. An analogous situation may exist in the L-forms of bacteria in which the levels of wall intermediates are low or nondetectable[20].

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Effect of Ca^{2+} on the Water and Non-electrolyte Permeability of Phospholipid Membranes

THE study of the effect of Ca^{2+} ions on phospholipid membranes by Tobias *et al.*[1-3] has chiefly been devoted to electrical properties and ion fluxes in relation to the hypotheses concerning the axon shifts from the resting to the excited state[4]. Although Leitch and Tobias[2] have shown that Ca^{2+} also induces great changes in the osmotic water flow across these membranes, little attention has been paid to this particular aspect of their behaviour. This report is a study of the modification of the water and non-electrolyte permeability of phospholipid membrane under the influence of calcium. The results obtained suggest the possible role of this ion in the control of cell membrane permeability to non-ionic species.

Phospholipid membranes were made by impregnating 'Millipore' filter disks (estimated pore radius: 250 Å) with 'Cephalin' (Nutritional Biochemicals Co.) as described by Tobias[1]. The membranes were equilibrated for 1 h in either water, NaCl (100 mM) or $CaCl_2$ (50 mM) solutions. The following average composition was obtained: 'Millipore' cellulose material: 7 mg/cm², and cephalin: $2{\cdot}0 \pm 0{\cdot}1$ mg/cm². The water content, measured by drying *in vacuo* at room temperature, was $7{\cdot}5 \pm 0{\cdot}2$ mg/cm² after equilibration in water, $7{\cdot}35 \pm 0{\cdot}2$ mg/cm² in NaCl, and $7{\cdot}1 \pm 0{\cdot}2$ mg/cm² in $CaCl_2$.

The membranes were clamped between two lucite chambers. The hydraulic conductivity, L_p, and diffusion coefficients, ω, for water (^{3}HHO) and for ^{14}C-labelled non-electrolytes were measured in the three media according to methods described elsewhere[5]. Preliminary experiments having shown that 'Cephalin' is not washed out by low pressure (the membranes lose less than 10 per cent of their 'Cephalin' content after 24 h under 40 cm of Hg), L_p was measured under hydrostatic pressures of up to 20 cm of Hg and was found constant for several hours. Hydraulic conductivities, L_p, and water (^{3}HHO) diffusion coefficients, $\omega_{^3HHO}$, were measured on membranes successively placed in water, NaCl and $CaCl_2$ solutions. There are only slight differences in L_p and $\omega_{^3HHO}$ between water and NaCl (Table 1). On the other hand, there is a 100-fold decrease in L_p and a two-fold decrease in $\omega_{^3HHO}$ in $CaCl_2$ solution as compared with NaCl solution. This effect of calcium is reversible. It appears that Ca^{2+} induces an important reorganization of the membrane 'Cephalin'–water medium which takes place without significant changes in composition. It is convenient to translate the phenomenon in terms of membrane porosity and pore radius. Membrane porosity, as measured by the ratio $g = L_p / V_w \omega_{^3HHO}$ (in which V_w is the water molar volume) undergoes a fifty-fold decrease, and the equivalent pore radius of the 'Cephalin' membranes which may be computed from the relation $g = 1 + r^2 RT / 8\eta \bar{V}_w D_w$ (in which η and D_w are respectively the viscosity and the self diffusion coefficient of water) decreases from about 80 Å in NaCl to 12 Å in $CaCl_2$. It must, however, be kept in mind that this simple geometrical translation cannot be taken as a full interpretation of the structural change observed.

Table 1. HYDRAULIC CONDUCTIVITY AND WATER DIFFUSION COEFFICIENTS OF MEMBRANES IN WATER, NaCl AND $CaCl_2$

	Water	NaCl (100 mM)	$CaCl_2$(50 mM)
$L_p \times 10^{11}$ (cm³ dyne^{-1} s^{-1})	$1{\cdot}6 \pm 0{\cdot}1$	$1{\cdot}4 \pm 0{\cdot}1$	$0{\cdot}013 \pm 0{\cdot}003$
$\omega \times 10^{15}$ (mole dyne^{-1} s^{-1})	$2{\cdot}0 \pm 0{\cdot}15$	$1{\cdot}80 \pm 0{\cdot}13$	$0{\cdot}75 \pm 0{\cdot}05$
$g = L_p / V_w \omega_{^3HHO}$	435 ± 5	428 ± 5	$10 \pm 1{\cdot}5$

These figures are the averages obtained from data on five different membranes. In spite of the relatively large variation from membrane to membrane, g values are remarkably constant.

Table 2. DIFFUSION COEFFICIENTS OF NON-ELECTROLYTES

	NaCl (100 mM)		$CaCl_2$ (50 mM)		ω_{CaCl_2}
	K_s	$\omega \times 10^{15}$	K_s	$\omega \times 10^{15}$	ω_{NaCl}
Water	1	$1{\cdot}80 \pm 0{\cdot}1$	1·0	$0{\cdot}76 \pm 0{\cdot}05$	$0{\cdot}41 \pm 0{\cdot}02$
Ethanol	$1{\cdot}55 \pm 0{\cdot}02$	$1{\cdot}48 \pm 0{\cdot}1$	$1{\cdot}54 \pm 0{\cdot}02$	$0{\cdot}49 \pm 0{\cdot}05$	$0{\cdot}33 \pm 0{\cdot}01$
sec-Propanol	$1{\cdot}84 \pm 0{\cdot}02$	$1{\cdot}30 \pm 0{\cdot}1$	$1{\cdot}86 \pm 0{\cdot}02$	$0{\cdot}37 \pm 0{\cdot}03$	$0{\cdot}28 \pm 0{\cdot}005$
Formamide	$1{\cdot}31 \pm 0{\cdot}02$	$0{\cdot}90 \pm 0{\cdot}05$	$1{\cdot}31 \pm 0{\cdot}02$	$0{\cdot}14 \pm 0{\cdot}01$	$0{\cdot}16 \pm 0{\cdot}005$
Acetamide	$1{\cdot}40 \pm 0{\cdot}02$	$0{\cdot}60 \pm 0{\cdot}05$	$1{\cdot}39 \pm 0{\cdot}02$	$0{\cdot}059 \pm 0{\cdot}005$	$0{\cdot}10 \pm 0{\cdot}005$

These figures are the averages obtained from four sets of data on four different membranes. In spite of the relatively large variations from membrane to membrane, the ratio $\omega_{CaCl_2}/\omega_{NaCl}$ for a given solute seems remarkably constant.

Table 2 lists the diffusion coefficients, ω, of water and four small non-electrolytes—ethanol, *sec*-propanol, formamide and acetamide—measured successively in NaCl and $CaCl_2$ solutions. In every case, ω is markedly smaller in $CaCl_2$ than in NaCl. The ratios $\omega_{CaCl_2}/\omega_{NaCl}$ decrease with the solute size. Furthermore, this ratio is two to three times lower for amides than for alcohols. The solute partition coefficients K_s, measured between the membrane water and the water of the bathing solutions, are listed in columns 2 and 3 of Table 2. There are no significant differences between the K_s measured in NaCl and $CaCl_2$ solutions. The effect of calcium on ω cannot be ascribed to a diminution of the solute K_s and must therefore be related to the decrease in the radius of the aqueous paths. The great difference in sensitivity to the change in a geometrical parameter of amide and alcohol diffusion may be explained by two considerations: because of their greater lipid solubility, alcohols exhibit higher K_s than amides. This could partially explain the small sensitivity of alcohols, which can diffuse both through water and lipids. The computation of the frictional coefficients, however, which takes into account the partition coefficient, shows that a difference in lipid solubility alone cannot account for the phenomenon

observed. The difference in the behaviour of amides and alcohols is more completely explained by the different hydrogen-bonding ability of the two chemical functions. It has been shown[5] that the diffusion rate of non-electrolytes decreases with their increasing H-bonding ability. This effect is greater in dense membranes, in which the solute-membrane frictions are the preponderant factor controlling the diffusion, than in more porous membranes, in which solute–water frictions, less sensitive to H-bonding, predominate.

The effect of Ca^{2+} on the phospholipid membrane model suggests that this ion may have an action not only on ionic permeability, as shown in particular by Whittam[6], but also on water and non-electrolyte permeability. The model seems particularly relevant to the study of specialized cells such as the epithelial cells of the urinary bladder. The 'Cephalin model' mimics remarkably well the great permeability changes observed in this structure under the influence of neurohypophyseal hormones. Leaf and Hays[7,8] have shown on the toad bladder that these hormones produce a forty-fold increase in the L_p of the luminal membrane of the cells and only a 70 per cent increase in water diffusion. Furthermore, the increase of the diffusion rate of non-electrolytes under the influence of the hormones depends critically on their chemical nature, and is much greater for amides than alcohols[8]. Leaf and Hays[8] and more recently Hays[9] have proposed a model based on a system of two barriers in series, in order to explain their findings on the toad bladder. On the basis of the present results, however, it may be considered that the great porosity change of a single barrier can influence the diffusion of non-electrolytes in a selective way according to their chemical function. In addition, the action of Ca^{2+} on phospholipid membrane models suggests the possibility that a molecular mechanism comparable with the shift from the resting to the excited state of axon membranes will apply in the case of these great permeability changes induced by hormones. Experiments are in progress to test this possibility.

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Effects of Salts and *p*H on Fish Chemoreceptor Responses

SEVERAL studies have been reported on the effect of *p*H on the taste responses to salt and other taste stimuli in different species. It is suggested that *p*H dependent receptors exist for NaCl[1–3]: in the case of rat and blowfly, the response to NaCl tended to be inhibited below *p*H about 3. I demonstrate here that addition of NaCl or other salts at relatively weak concentrations to HCl solutions increases the response of carp (*Cyprinus carpio*) palatal chemoreceptors.

The summated responses of multifibre strands of the palatine nerve supplying the palatal chemoreceptors were recorded from eleven preparations, using a technique described previously[4]; the stimuli were mixed solutions containing 0·05 M NaCl and varying concentrations of HCl. In each preparation, application of 0·05 M NaCl elicited a very small response. Its magnitude was a fifth of that, for example, of 0·001 M HCl or even smaller. Addition of NaCl at the same concentration to 0·001 M HCl, however, resulted in an enhancement of the response, the magnitude of the response being on average 1·9 times as large as that for the single acid solution. The effect of NaCl was tested also for 0·0005, 0·0025, 0·005 and 0·01 M HCl, and it was found that the combined effectiveness was strongest between 0·001 M to 0·0025 M HCl concentrations, yielding a saturation response–concentration curve in each preparation similar to that illustrated in Fig. 1. The effect of varying concentrations of NaCl is also shown. The magnitude of the response to each of the mixed stimuli containing different quantities of NaCl approaches that for a single acid solution as the concentration of the HCl in each solution is raised to 0·01 M. At this molarity all the curves for these stimuli attain the same magnitude, within experimental error, though they show differences at lower concentrations of HCl. In general, a further increase in concentration did not bring about marked increase in magnitude of the response to the single HCl solution; in this, the carp differs from the rat[5]. In some fish including the carp, it is known that there exist in the palatal or facial chemoreceptor system specific receptors responsive to a limited dilute concentration range of electrolytes (refs. 6 and 7, and I. H. and T. Ogawa, unpublished data). It was therefore suspected that the concentrations of HCl used here might stimulate this specific receptor. It has also been shown that this specific receptor system is depressed by dilute concentration of divalent salts, such as $CaCl_2$ and $MgCl_2$, or by relatively concentrated monovalent salt; solutions for example, about 0·01 M or higher concentrations of NaCl[6,7]. Consequently, the effect of 0·0005 M $CaCl_2$ in varying concentrations of HCl was investigated in five preparations. No noticeable depression of the response was observed over the concentration range of HCl (Fig. 1). $CaCl_2$ also showed an enhancing effect close to that of NaCl of the same normality (for 0·001 M HCl, see Table 1). By contrast with the results with 0·0025 M NaCl, 0·0005 M $CaCl_2$ did not influence the response very much. $CaCl_2$ by itself at the same concentration did not elicit a noticeable response, so it may be concluded that the participation of the specific receptor system in the summated response to HCl was little,

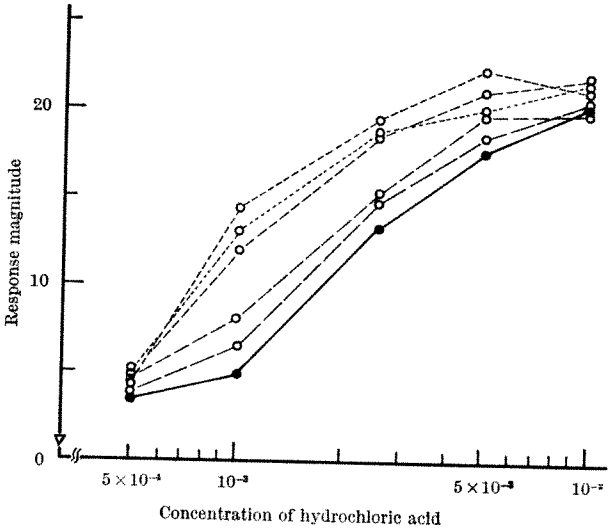


Fig. 1. Summated responses of the carp palatine nerve to mixed solutions of HCl with 0·0005 M $CaCl_2$, and with varying concentrations of NaCl plotted in arbitrary units against molar concentration of HCl. The response to 0·2 M NaCl is also shown (▽). ●—●, HCl; ○——○, HCl+0·0005 M $CaCl_2$; ○— — —○, HCl+0·0025 M NaCl; ○– – –○, HCl+0·0125 M NaCl; ○ · · · ○, HCl+0·05 M NaCl; ○· · · ·○, HCl+0·2 M NaCl.

if any. Thus the results suggest that the enhancement of the response by NaCl—by itself very weak in stimulative effectiveness over the concentration range used—may be related to the activity of the acid receptor rather than the salt receptor.

Table 1. EFFECTIVENESS OF VARIOUS SALTS IN HYDROCHLORIC ACID SOLUTION ON THE CARP CHEMORECEPTOR

Salts	Response to HCl + salt	Salt	Salts	Response to HCl + salt	Salt	Salts	Response to HCl + salt	Salt
NaCl	1·7	0·2	KCl	1·7	0·2*	Na_2SO_4	0·9‡	0·2
NaBr	1·7	0·2	LiCl	1·8	0·2*	$MgSO_4$	1·0‡	0·2*
$NaNO_3$	1·5	0·2	RbCl	1·7	0·2*	$MgCl_2$†	2·0	0·3*
$NaClO_4$	1·5	0·3	Choline-Cl	1·9	0·3	$CaCl_2$†	1·9	0·4*
			NH_4Cl	1·9	0·3*			

* Mean for two preparations.
† Data are presented for 0·025 M.
‡ 0·0025 M HCl; pH = 3·0.

Magnitude of summated responses to 0·001 M HCl + 0·05 M salts and to 0·05 M salts expressed relative to that to 0·001 M HCl. Mean values for three preparations.

Similar enhancements were observed with several chloride salts having different monovalent or divalent cations and sodium salts of different monovalent anions (Table 1). Na_2SO_4 and $MgSO_4$ did not enhance the response over the concentration range of HCl 0·0005 M to 0·01 M. The pHs of the mixed stimuli containing 0·05 M Na_2SO_4 or $MgSO_4$ were found to be slightly higher than those of the corresponding single acid solutions (by a factor of 0·2–0·3); for example, the pH of the mixture containing 0·0025 M HCl and 0·05 M Na_2SO_4 or $MgSO_4$ was nearly the same as that of the single HCl solution at 0·001 M. Both the solutions yielded close response magnitudes as seen in the table. These results do not show whether the enhancement arises from cation or anion effects (except for the sulphate). But some of these salts differ in their effectiveness in salt receptor stimulation, especially when salts with different cations are used[8–12]. To distinguish between effects on the two sorts of receptor, experiments with single fibres were carried out. Fibres were found which did not respond well to NaCl but responded to its mixed solutions with HCl of various concentrations in spike frequencies higher than those noted in responses to each of the single solutions (Fig. 2).

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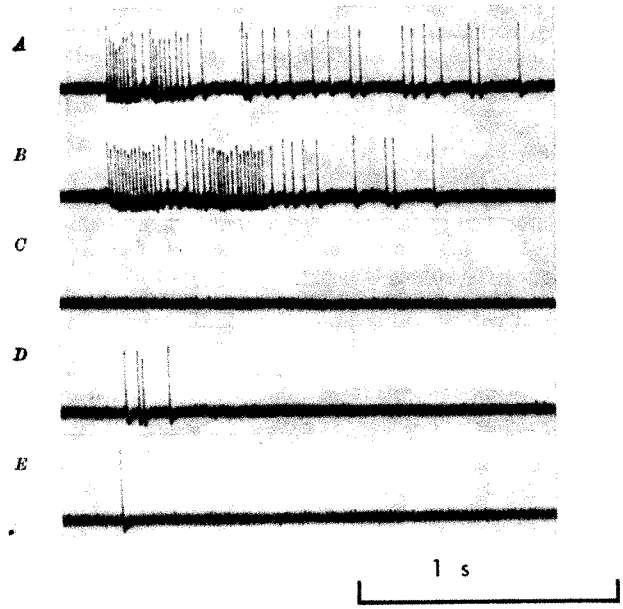


Fig. 2. Responses of a single unit to (*A*) 0·001 M hydrochloric acid, (*B*) mixture of 0·001 M HCl and 0·05 M NaCl, (*C*) 0·05 M NaCl, (*D*) 0·2 M NaCl, and (*E*) 0·0005 M sodium ferrocyanide.

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Unusually High Taste Acuity for Phenylthiocarbamide in Two Formosan Aboriginal Groups

FOR this study of acuity of taste for phenylthiocarbamide (PTC), 100 individuals were selected from each of two aboriginal groups living in Taiwan: the lowland-dwelling Ami, and a branch of the Atayal. Acuity was measured by asking the subject to discriminate between distilled water and a PTC solution in a sorting test in which the probability of a correct answer from guessing was 0·125[1]. The solutions comprised a series integrally numbered from zero, solution n containing $2{\cdot}60 \times 2^{-n}$ g PTC/l. The highest value of n at which the subject could still taste the PTC was called his DSN (discriminated solution number) and a continuity correction of +0·5 yields the most likely value for the threshold. We also corrected for the effects of age and sex, taking the 32 year old male as standard subject[1]. Most of the subjects we used were schoolchildren, which meant age-effect corrections lay mostly between −0·7 and −1·3.

Few of the Ami or Atayal subjects had DSNs of less than 6 and many were of 19 and over. This has been found for several other Mongoloid groups, presumed "pure", from which samples of fifty individuals have been tested[2,3]. But whereas in these other samples the incidence of these high DSNs ranged from 4 per cent to 12 per cent, it was 17 per cent in the Ami sample and 30 per cent in the Atayal one. Furthermore, the range of high DSNs was much greater than has been found before. Nine Ami and eleven Atayal subjects had DSNs between 23 and 25; two Atayal had DSNs of 26; one Ami had a DSN of 27; two Atayal had DSNs of 29; and one 11 year old Atayal boy had a DSN of 42. Seventeen of these twenty-six subjects described the taste at the DSN as other than bitter or qualified-bitter. Most of these values have special significance and will be discussed briefly later.

It should be pointed out that solution 42 contains only about $0{\cdot}6 \times 10^{-12}$ g PTC/l. and is about 4×10^{-15} M. If Moncrieff's[4] estimate that 0·05 ml. of solution (we use 2·5 ml.), properly applied, is sufficient for taste-testing is correct, the subject with a DSN of 42 must have been able to taste as little as 10^5 molecules of PTC. In this study, three of the subjects with scores of 25 and all with greater scores, except for one with a score of 26, were checked without their knowledge by adding a repeat series to the end of the test series. Only two of these subjects (one with a DSN of 26, and one with a DSN of 29) failed the repeat trial, and the boy with a DSN of 42 successfully completed two repeat trials.

Frequency distributions of taste acuity for PTC in human populations were earlier held to justify the

hypothesis[5,6] that three genotypes, TT, Tt and tt, are involved, but only two phenotypes, for the so-called "tasting" gene, T, was believed to be completely dominant over the "non-tasting" allele, t. Dominance, however, may be incomplete[7]. It has been contended more recently from consideration of data accumulated from thirteen samples each of 50, drawn mostly from separate ethnic groups, that the overall distribution calls for the recognition of at least five but more likely six modes[2,3]; and in a hypothesis (my work, unpublished) based on these data a case has been made for replacing the classical T, t gene-pair by an allelic series, T_1, T_2 – – – T_6, with possibly an additional allele, T_7, appearing in Japan. In terms of this hypothesis, I suggest that gene T_6 is present in both groups discussed in this communication but at greater frequency in the Atayal. Gene T_7 is present and at much the same frequency (of the order of 0·05) in both groups. There are indications that a gene, T_8, may be present, at least in the Atayal, and I feel there can be little doubt about the presence in this group of a further allele, T_x, though one may speculate at this stage about the value of x.

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Temporal Bounds of Post-ingestive Glucose Induced Satiety in Man

Most studies of the short term effects of the intake or administration of nutrients on hunger in man have been based on verbal reports. Whether these purport to describe the intensity of the desire for food—appetite itself—or the various sensations the subject habitually experiences when wanting food, the validity of such reports ultimately rests on what the subject wishes or does not wish to eat. We[1], in common with some other workers[2], have found it quite practicable to observe meaningful variations in the spontaneous size of meals taken when the subject is introduced to the experimental foods with little or no manipulation of his feeding behaviour before the experimental session. We wish to report here a study of the satiating effects which glucose produces after it has passed the mouth. It is intended to complement our previous study of the satiating effects of protein[1].

Twelve volunteers (one female) took their lunch and afternoon snack with us on 1 or 2 days per week when their mental and physical activities were not unusual. The primary foodstuff was a cooked cornflour and milk protein preparation, which was given as a savoury spread on crispbread and as a fruit-flavoured dessert (blancmange)[1]. A subject came at his usual lunchtime, was given a fixed amount of savoury and a hot drink and then allowed to eat as much dessert and drink as much water as he wished. He was counted as having finished when he stopped eating for at least 5 min, but he was kept for 30 min however soon he finished his lunch. The subject returned 3 h later to have as much dessert and water as he wished, staying for 15 min however soon he finished this supplementary snack. After the snack he was asked to describe that morning's breakfast and whether he had eaten a supper of unusual size or experienced any untoward reactions after any previous experimental day.

In the sessions reported here, the subject was given an additional 100 ml. drink at lunchtime. On some occasions this load contained 50 g of D-glucose, 0·5 ml. of 0·4 per cent quinine hydrochloride and 1 ml. of saturated citric acid solution. On other occasions a control load was given containing 2 ml. of 10 per cent (w/v) sodium saccharine, 2 ml. of saturated sodium cyclamate and 1 ml. of saturated citric acid. All solutions were at room temperature (21°–23° C). Subjects were instructed to gulp these drinks from the back of the throat; some did not even realize that the solutions were sweet. The drink was given either 20 min before or immediately before the beginning of lunch or immediately after finishing the meal according to our criterion. Sequences of conditions were counter-balanced between subjects.

Table 1. EFFECT OF GLUCOSE ON INTAKE PARAMETERS

Load	Timing relative to lunch	Food intake (kcalories) lunch	Food intake (kcalories) supplement	Water intake (ml.) lunch	Water intake (ml.) supplement	Duration (min.) lunch	Duration (min.) supplement
Glucose	20 min before	211*	131†	385	98	14·3	5·3
	Immed. before	295†	201	313	52	10·7	10·7
	Immed. after	331	158	309	100	19·2	9·5
Control	20 min before	322	177	305	80	15·8	8·4
	Immed. before	352	194	283	74	16·5	9·3
	Immed. after	335	201	295	58	15·3	12·0

Reliability of difference from relevant control condition, by correlated t test: * $P < 0·01$, † $P < 0·02$.

Table 1 shows the mean intakes for these six conditions. Glucose taken 20 min before lunch reliably depressed caloric intake relative to control load at both lunch and the supplementary snack (the depression of lunch calories relative to glucose after the meal was also reliable at $P < 0·01$). The intake of water at lunch was not increased reliably. Glucose taken just before lunch gave a detectable satiety at that meal, but half the size of that induced by the earlier load. Neither load given with lunch affected supplementary intake appreciably. The mean lengths of meals followed the pattern of variations in caloric intake but the differences between conditions were not statistically reliable.

The caloric content of the breakfast preceding a test was not found to differ reliably between conditions. On the occasions included in these data, no untoward reactions or unusually large or small suppers were reported. The caloric content of the experimental lunches were in the range 35–138 per cent of reported normal lunches (mean 87 per cent).

We conclude that there are gastrointestinal or post absorptive effects of glucose which induce a satiety from about 15 min after ingestion, which probably has largely disappeared less than 3 h later—which is 3–5 h before the next meal in these subjects. It seems therefore that the post-absorptive effects and gastrointestinal chemoreceptor and mechanoreceptor action of concentrated glucose solution of caloric value more than half the spontaneous intake do not act soon enough to account for the initial satiety which ends a meal in man. Reactions to the oral qualities of the foodstuffs available must constitute the principal factor in the first 20 min or so. It also seems that the glucogenic components of a meal are not dominant in maintaining satiety in the later phases of the inter-meal interval: we have already presented evidence that protein contributes more than carbohydrate or fat to keeping hunger at bay[1].

It should be noted that the post-ingestive satiety induced by glucose is not necessarily regulatory. Ingestion of glucose (188 kcalories) 20 min before the meal resulted in an approximately equicaloric reduction in total intake relative to saccharine ingestion at the same interval

before the meal (157 kcalories, Table 1). Glucose immediately before or after the meal, however, produced little net satiety (50 or 47 kcalories, respectively). Admittedly the supplementary sampling of satiety was limited to a brief period in mid-afternoon, but it is unlikely that 20 min difference in the timing of the load should shift compensatory reduction of intake so completely away from a period 3 h later. Rather, such a rapid completely behavioural caloric compensation may depend on the absorption of glucose load before the meal. In other circumstances it seems that either the behavioural regulation of body weight (or energy reserves[3]) takes more than a few hours or there is regulation by reduction of metabolic efficiency. Examples of lack of behavioural compensation even in the long term for energy-yielding loads have been seen in dogs[4] and rats[5].

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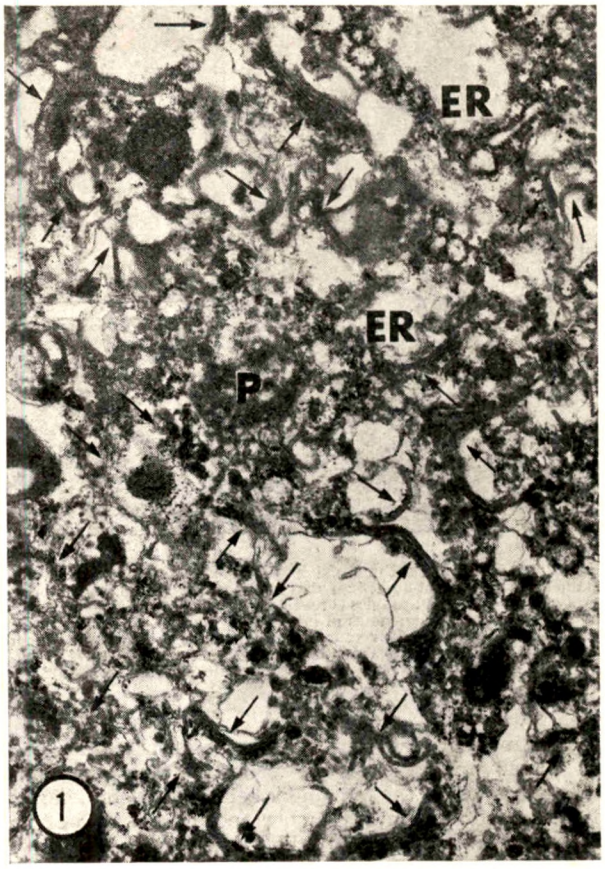


Fig. 1. Thin section of Golgi apparatus-rich fraction from rat mammary gland showing numerous stacks of parallel cisternae, dictyosomes (arrows) surrounded by small vesicular profiles. The plate-like portion of a cisterna (P) is seen in a dictyosome sectioned tangentially. Some endoplasmic reticulum (ER) is present in the fraction. (×20,000.)

Lactose Synthesis by a Golgi Apparatus Fraction from Rat Mammary Gland

Lactose synthetase (UDP galactose : D-glucose 1-galactosyl transferase, EC 2.4.1.22) catalyses the final step in the synthesis of lactose of milk and is composed of two subunits, both of which are required for activity[1]. The A subunit is membrane bound and, in the absence of the B subunit, transfers galactose from UDP galactose to N-acetylglucosamine with the formation of N-acetyllactosamine[2–4]. The B subunit is identical to the milk protein α-lactalbumin, and modifies the acceptor specificity of the A subunit to include glucose[2,3]. Galactosyl transferase activities have been specifically localized in Golgi apparatus from rat[5] and bovine[6] livers. Because the localization of lactose synthetase activity has been the subject of much speculation[8], it was of interest to extend these studies to mammary tissue. We show here the A protein of lactose synthetase to be concentrated in a Golgi apparatus fraction from rat mammary gland.

Inguinal and thoracid mammary glands were recovered from first lactation Holtzman rats (Holtzman Co., Madison, Wisconsin) 2–6 weeks post-partum. Approximately 10 g of tissue was rinsed with ice-cold homogenization medium[5], minced and homogenized for 2 min in fresh medium using a Polytron '20 ST' homogenizer[5]. Cell fractions rich in Golgi apparatus, rough endoplasmic reticulum and plasma membrane as well as supernatant fractions were obtained from this homogenate as described previously[5,9–11]. Fractions were identified by electron microscopic examination of both negatively stained and sectioned material[9]. Lactose and N-acetyllactosamine synthetase activities were determined as described previously[5] by the method of Palmiter[4] in conditions of linear kinetics with respect to protein and time. Protein was estimated according to Lowry *et al.*[12] with bovine serum albumin as the standard.

Lactose synthetase, when assayed in the presence of exogenous α-lactalbumin, was concentrated in the Golgi apparatus fraction (Table 1). This activity was much lower or absent from fractions rich in plasma membrane and endoplasmic reticulum as well as from the microsomal supernatant. The reaction was dependent on added glucose and was stimulated by the addition of α-lactalbumin. The N-acetyllactosamine synthetase activity of the preparations was dependent on N-acetylglucosamine but was inhibited by α-lactalbumin as shown previously by Brew *et al.*[3]. Results in Table 1 are representative of mid-lactation animals. Although the lactose and N-acetyllactosamine synthetase activities were considerably lower for animals in the latter stages of lactation, the activity of the Golgi apparatus fraction was always approximately ten times that of the total homogenate.

When examined in the electron microscope, thin sections of pellets of the Golgi apparatus fractions showed fields containing stacks or dictyosomes comprised of parallel flattened cisternae surrounded by small vesicular profiles (Fig. 1). In negatively stained preparations, the Golgi apparatus were identified by the complex system of peripheral tubules (Fig. 2). These tubules correspond to the small vesicular profiles seen in thin section[9]. Rough endoplasmic reticulum and occasional mitochondria were the only contaminants detected in the Golgi apparatus fraction.

Except for the presence of more endoplasmic reticulum, the appearance of the Golgi apparatus fractions from mammary tissue was similar to those obtained from rat liver[9]. Measurements taken from a series of fifteen

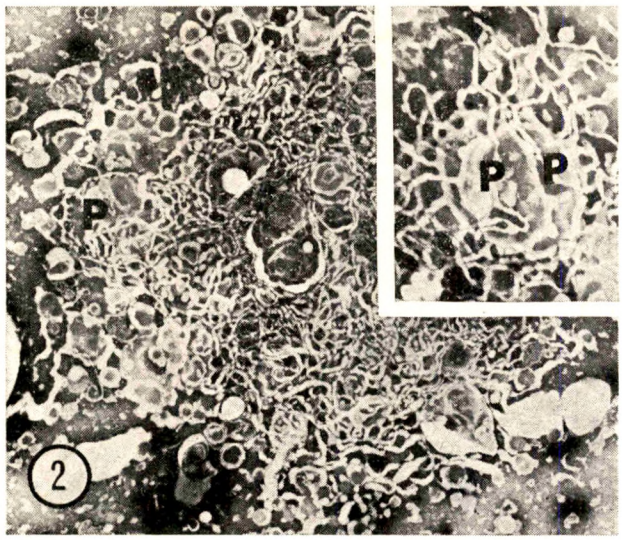


Fig. 2. A portion of the Golgi apparatus-rich fraction negatively stained with 1 per cent potassium phosphotungstate, *p*H 6·5. A complex array of plates (P) and tubules is revealed in the larger fragments which appear to represent aggregates of intact dictyosomes. ×16,000. Inset shows details of cisternal structure in a fragment of a dictyosome which was partially unstacked to show the system of peripheral tubules which are continuous with the plate-like central portions (P) of the cisternae. (×24,000.)

electron micrographs through a pellet centre suggested that 70–85 per cent of the pellet volume was Golgi apparatus, most of the remainder being rough endoplasmic reticulum. So the much higher specific activity of the Golgi apparatus fraction (Table 1) is a consequence of the presence of Golgi apparatus rather than a contaminating cell fraction although the true specific activity of Golgi apparatus membranes may be considerably higher than the present data indicate.

Table 1. LACTOSE AND N-ACETYLLACTOSAMINE SYNTHETASE ACTIVITY OF RAT MAMMARY CELL FRACTIONS

Fraction	Reaction mixture	Specific activity Lactose	Specific activity N-acetyl-lactosamine
Golgi apparatus	Complete	563·7	516·7
	−Acceptor	1·1	0·0
	−α-lactalbumin	34·8	1,457·0
Total homogenate	Complete	62·5	30·6
	−Acceptor	14·7	2·8
	−α-lactalbumin	21·7	123·7
Endoplasmic reticulum	Complete	6·2	76·1
	−Acceptor	2·6	0·0
	−α-lactalbumin	0·0	
Plasma membrane	Complete	0·0	
	−Acceptor	0·0	
	−α-lactalbumin	0·0	
Microsomal supernatant	Complete	19·4	
	−Acceptor	0·9	
	−α-lactalbumin	13·9	4·9

* The unit of specific activity is nmoles/h/mg protein.
For lactose synthetase the acceptor was glucose and for N-acetyllactosamine synthetase the acceptor was N acetylglucosamine. Assay conditions were identical to those used in a previous study[5] except that in this case complete reaction mixtures also contained 0·1 mg (6·5 nmoles) α-lactalbumin.

Our results confirm the suggestion of Brew[8] that the A protein of lactose synthetase is associated with the Golgi apparatus although we do not have evidence that it is exclusively localized there. Its occurrence in the Golgi apparatus has been suggested as a logical extension of the events known to occur during the secretion of milk constitutents[8,13]. During lactation, α-lactalbumin is synthesized on ribosomes and transferred to the lumen of the endoplasmic reticulum[14]. Secretory proteins are normally transported through endoplasmic reticulum to the Golgi apparatus where they are accumulated in secretory vesicles[15]. If a similar export route is assumed for α-lactalbumin, this would bring the α-lactalbumin into contact with the A protein of the Golgi apparatus membranes, creating the conditions necessary for lactose synthesis. It is therefore not unreasonable to assume that lactose may accumulate, along with proteins, in secretory vesicles of the Golgi apparatus and be d charged together into the gland lumen (see ref. 13).

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Mounting Behaviour in the Female Cat

MOUNTING behaviour of the kind associated with t sexual activities of males has been observed in fema of a number of mammalian species, and has been exte sively investigated in the guinea-pig, rat and do Mounting by females has been observed in the cat, b it is said to be rare, and the conditions in which it occ have not been adequately defined[2]. This report provid data on the mounting behaviour of female cats kept social conditions in a laboratory, and on some of t factors determining its occurrence.

Observations were made on eleven multiparous fema cats, all over 16 months old, over a period of seve months. These cats lived as members of a larger gro of adult females and adolescents of both sexes, in indoor room 3 × 4·8 m, connected by way of cat-doo with an outdoor wire-fenced enclosure of the same si The outdoor enclosure had a common wire fence bounda with a similar enclosure which housed male cats. the observations were made in the outdoor enclosu with which the cats were thoroughly familiar.

The females were screened daily for signs of oestro behaviour, namely, crouching, flattening of the ba and hind-limb treading, sometimes accompanied vocalizations. The relationship of these behaviou signs to the ovarian and vaginal cycles is well esta lished[3]. The screening took place in the outdoor enclosu Sexually experienced males were present in the adjace enclosure separated from the females by a wire fence that social interaction between males and females w possible but mounting could not take place. In the conditions, any display of oestrous behaviour by the te females in the absence of physical contact with oth cats or with the observer was noted. Then, those fema not showing oestrous behaviour in the absence of conta stimulation were subjected to manual stimulation of t

rump and flanks by the observer, as a further test for the presence of lordosis. On the basis of this two-stage screening procedure, the females were selected for further observation on oestrous and anoestrous test days, defined as follows: (*a*) oestrous test day—oestrous behaviour in the absence of contact stimulation was observed for the third consecutive day; (*b*) anoestrous test day—all signs of oestrous behaviour, both in the absence of contact stimulation and in response to manual stimulation by the observer, were absent for the fourth consecutive day. Each female was given mounting tests on four oestrous test days, each from a different oestrous cycle, and four anoestrous test days. For these tests, the female was placed for 1 h with a group of fourteen cats, of whom three to five were oestrous females, six to eleven were females showing no signs of oestrous behaviour, and none to three were adolescent males and females between three and seven months old. If the required number of oestrous females was not naturally available in the colony, oestrogen-treated females showing the full pattern of oestrous behaviour were used. During the mounting tests, the performance of any components of male-like mounting behaviour by the test females was recorded, together with details of the age, sex and oestrous condition of the cat towards which the mounting behaviour was directed.

Ten of the eleven females in the group performed elements of the male mounting sequence. With the exception of the "chirp" call given by males before mounting, all the components which lead to intromission by the male were seen[4]—that is, neck-grip, partial mount, full mount, forelimb rubbing, hindlimb stepping, arching of the back, stamping on the mounted cat's rump (a pattern seen in males which increases the elevation of the female's perineal region during lordosis), and pelvic thrusting. Four of the females never progressed beyond the neckgrip and partial mount. Pelvic thrusting was seen in only three females, and had a lower frequency than is usual in males. There was considerable inter-individual variation in mean mount durations (3–414 s).

The orientation of the behaviour with respect to the mounted cat was sometimes bizarre, and in such cases the position of the "neck-grip" varied considerably. Mounting from the side and front was seen, as well as the normal posterior mounting. In mounts from behind, the mounting cat often had its pelvic region well forward on the back of the mounted cat, and occasionally during prolonged mounts the mounting cat would slide off and lie on its side, maintaining a neck-grip and continuing to clasp the mounted cat with its four paws. These poorly orientated mounts are reminiscent of descriptions of mounting by male cats in which the dorsal nerves of the penis have been sectioned[5].

There was a clear relationship between mounting behaviour and the oestrous condition of the mounting cat. The twenty-two test periods in which mounting occurred were all oestrous tests, and for the group as a whole there was a highly significant tendency for mounting to occur in oestrous rather than anoestrous tests ($P = 0{\cdot}002$, two-tailed sign test[6]). Furthermore, of the thirty-seven cats which were mounted, thirty-one were in natural or induced oestrus, five were females showing no sign of crouching or treading and one was an adolescent male. For the group as a whole, there was a highly significant tendency for mounting cats to choose to mount oestrous females ($P = 0{\cdot}001$, one-tailed sign test). The mounting female was an adequate stimulus to produce the full pattern of crouching, treading and tail-deflexion in the oestrous cat being mounted, but no after-reactions were observed, presumably because they are specific to vaginal stimulation resulting from intromission.

The predominant selection of oestrous females by the mounting cats may simply reflect the fact that other females will not tolerate being mounted, for it is known that the female cat will accept only a male at the height of oestrus[3]. Alternatively, stimuli from an oestrous cat may be specifically responsible for eliciting mounting by other females. During the tests, groups of oestrous females commonly consorted together, sniffing at each others' perineal regions, and engaging in mutual head and neck rubbing while treading. Pairs of oestrous females would roll on the ground, clasping each other, and would engage in prolonged bouts of mutual genital stimulation by crouching back-to-back, treading and rubbing their perineal regions together. Mounting episodes were frequently seen during these periods of head-rubbing and genital stimulation. Furthermore, a mounting female was frequently immediately mounted by the cat she was mounting, or by another oestrous female.

On the basis of these observations it appears that, in appropriate conditions, mounting behaviour forms a prominent part of the spectrum of social behaviour among female cats. It occurs at oestrus and is directed preferentially towards other oestrous cats. The conditions appropriate for the appearance of homosexual mounting behaviour in females have previously been investigated in detail in the guinea-pig, rat and dog. It seems that the cat may be somewhat more selective in its choice of objects to mount than are rats[7] and dogs[8], and, unlike these species but in common with the guinea-pig[9], it shows a tendency to restrict homosexual mounting behaviour to periods when circulating ovarian hormones are at a high level.

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Lack of a Long-term Effect of LSD on Y-maze Learning in Mice

It has recently been reported that lysergic acid diethylamide-25 (LSD) has an inhibitory effect on T-maze learning in mice[1]. The animals were tested 2 min after an intraperitoneal injection of LSD, in view of the observations of Uyeno and Benson that the effect of LSD on agonistic behaviour approaches a maximum 5 min after injection[2]. Because of the controversy regarding potential long-term psychological effects of LSD, we had undertaken an examination of the effect on learning a shock avoidance task by repeated treatment of mice with increasing quantities of LSD over a long period. We report here the effect of this regimen on Y-maze learning in mice.

Twenty-four male mice of the NIH strain, 1 month old initially, and weighing 25 g, were given a series of sixteen subcutaneous injections of LSD (Sandoz batch 65001) at evenly spaced intervals over a period of 2 months. The first dose, administered when the mice were 30 days old, was 2·5 μg of LSD per mouse dissolved in 0·1 ml. of isotonic saline solution. The dosage was gradually increased to a maximum of 12·5 μg per injection, so that each mouse received a total of 111·25 μg of LSD. Twenty-four

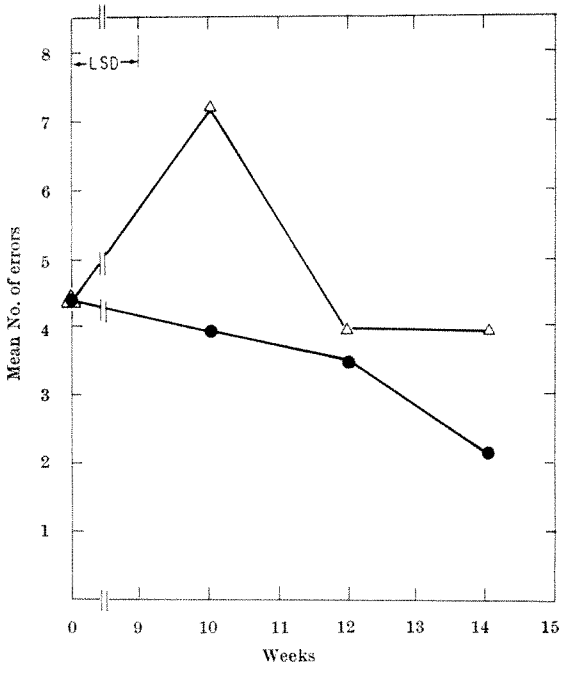


Fig. 1. Mean of errors in Y-maze learning of LSD-treated (△) and saline injected control (●) groups of mice.

age-matched controls were simultaneously injected with 0·1 ml. of saline solution. The mice were kept continuously from the beginning of the injection period in individual plastic cages and fed a standard commercial diet (Purina Lab Chow pellets) with water *ad libitum*. The mice which received LSD showed striking behavioural changes compared with the control animals. They were hyperactive for a period of several hours following each injection of the drug. They burrowed and tunnelled vigorously, climbed onto the spouts of the water bottle, ran inverted along the tops of the cages, and were found to be extremely aggressive in the presence of other mice.

Before administration of LSD, all forty-eight mice were tested for their ability to learn an avoidance task in the standard Y-shock apparatus described by Cardo[3]. This apparatus is a three-armed enclosure with hinged doors and a 1 cm × 1 cm wire grid over the wooden floor. The voltage applied to the grid is adjusted by a variable transformer and the operator can control the choice of exit arm by an electrical switching device, to eliminate the effect of possible bias on the part of the test animal. The mean number of errors at the time of original testing was $4{\cdot}3 \pm 0{\cdot}6$ s.e. (Fig. 1). The mice were then divided into two groups of equal learning ability and injected with LSD in saline or saline alone as described. The mice were re-tested 1 week after cessation of administration of LSD, by which time the signs of hyperactivity had disappeared. The mice which had received LSD showed an increase in the mean number of errors in the avoidance task to $7{\cdot}0 \pm 1{\cdot}03$ s.e., whereas the mean of the errors of the saline control group had decreased slightly to $3{\cdot}9 \pm 1{\cdot}09$ s.e. The difference between these groups is significant ($P = 0{\cdot}02$–$0{\cdot}05$) (ref. 4). The mice were tested 2 weeks later, when the mean number of errors of the LSD-treated group was $4{\cdot}0 \pm 0{\cdot}9$ s.e.—quite similar to that of $3{\cdot}5$–$0{\cdot}7$ s.e. for the control group. A subsequent test 5 weeks after discontinuing LSD injections indicated that the mean of the errors in the LSD-treated mice was still $4{\cdot}0 \pm 1{\cdot}1$ s.e. while that in the saline control group had decreased to $2{\cdot}2 \pm 0{\cdot}9$ s.e. This difference between the two groups is not statistically significant ($P = 0{\cdot}2$–$0{\cdot}3$).

The data obtained in the present experiment are consistent with the previous report[1] of an acute impairment of learning in mice as a result of administration of LSD. But the ability of the LSD-treated mice to learn an avoidance task had returned to the level of the control mice by 3 weeks after the chronic administration of LSD. We therefore conclude that there is no evidence of significant long-term impairment of learning attributable to LSD.

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Inhibitory Action of the Amygdala on the Lateral Hypothalamic Area in Rats

THE mammalian lateral hypothalamic area (LH) and the ventromedial hypothalamic nucleus (VMH) are involved in feeding and satiety function, respectively[1]. There is also reciprocity of electrical activity between the two[2]. Anatomically related to these areas, the amygdala has been shown to be involved in the same functions. Lesions of the basolateral nucleus of the amygdala results in hyperphagia[3], while its electrical stimulation arrests feeding behaviour[4]. The stimulation of the amygdala also produces changes in the spontaneous discharge pattern of LH units, suggesting an inhibition or an activation–inhibition sequence between the LH and the amygdala[5,6]. We report here evidence that the inhibitory effect of the amygdala on the LH neurone is due to a long lasting inhibitory postsynaptic potential (IPSP) exerted through the stria terminalis.

While rats were under light ether anaesthesia[6], intra and extracellular potential recordings were made from neurones in the centre of the LH[6] by means of glass microelectrodes filled with 3 moles of KCl (d.c. resistance, 20–80 MΩ). While intracellular recordings were being made, polarizing currents were applied across the cell membrane through the recording electrode by means of a modified constant current device[7]. Bipolar stimulating electrodes (40–80 kΩ) were always placed in the amygdala[6] and in the stria terminalis (A, 4·38; L, 3·50; H, +7·70, after König and Klippel[8]). Stimulating pulses lasted 0·1 ms and were less than 0·2 mA in intensity.

Single pulse stimulation to the amygdala produced a characteristic evoked potential in the LH consisting of an initial negative deflexion followed by a positive deflexion (N and P waves respectively, upper left of Fig. 1*A*). Mean latency and peak time of the N wave were about 6 and 12 ms respectively. The P wave lasted about 30 ms, and the N wave always appeared at a lower threshold than the P wave. Furthermore, the depth profile of the N and P waves within the LH differed in distribution—P waves were recorded almost equally throughout the dorso-ventral extent but N waves mostly in the ventral half. Thus each wave seems to be mediated by a separate pathway.

The evoked potentials produced by paired stimuli to the amygdala (Fig. 1*A*) showed the inhibitory nature of the P wave on the N wave. As the interstimulus interval was decreased, the amplitude of the N wave to the second stimulus became progressively suppressed, as

shown by the plot (Fig. 1*B*) of the amplitude of the N wave as a decreasing function of interstimulus interval from 90 to 10 ms. The P wave, on the other hand, remained virtually unchanged. Thus the inhibition caused by the P wave might be due not to its recurrent nature, but to its postsynaptic or presynaptic nature. The latter could be excluded after the depth profile of the P wave had been considered in comparison with the P wave produced by presynaptic inhibition[9]. On single unit recordings, the LH neurones responded with one to two driven spikes about 10 ms after stimulation of the amygdala (Fig. 1*C* and *D*). The latency corresponded to the peak time of the N wave. This was followed by a long lasting suppression of spontaneous firing for up to about 100 ms (Fig. 1*C*). Driven discharges, which would be produced by the second stimulation at various times after the first stimulation, were also inhibited for about 100 ms (Fig. 1*D*), although the latency of the first driven discharge was shortened at the stimulus interval by about 2 ms (second recording from the top in Fig. 1*D*). This was probably a summation of the N wave (to be discussed later). The time course of the suppression of the unit discharges corresponded to that of the N wave.

These findings suggest that the N and P waves reflect excitatory and inhibitory postsynaptic potentials respectively. We have proved this to be correct. First, temporal summation of the first two N waves was observed as a result of repetitive stimulation of the amygdala at 300 Hz; then the third N wave was decreased by the following P wave. The summation of P waves to the first three pulses of 150 Hz was also observed. Second, the post-tetanic potentiation was observed, for example, an increase in the amplitude of the N wave 1·8 times after 1·3 s of a tetanic stimulation (threshold intensity for N wave, 30 Hz for 6 s); the P wave could also be potentiated 1·5 times after 1·3 s (1·5 times threshold, 25 Hz for 2·5 s).

The hypothalamus, including the LH and VMH, receives fibres from two anatomically distinct pathways of the amygdala: the stria terminalis and the direct amygdalohypothalamic tract (DAH)[10]. An acute lesion of the VMH, regarding the reciprocal activity between it and the LH, made by passing a strong d.c. current (5 mA for 30 s), produced no change in the response of the LH, but that of the stria terminalis resulted in the disappearance of the P wave and the prolongation of the N wave. These results may indicate that the N wave was elicited through the DAH, and the P wave through the stria terminalis.

Intracellular recordings, which were consistent with this interpretation, revealed a similar excitatory–inhibitory sequence of potentials; that is, an excitatory postsynaptic potential (EPSP) followed by an IPSP. Although the IPSP lasted about 100 ms while the duration of the P wave was 30 ms, the difference can be attributed to differences in intracellular potential and extracellular current fields[11].

Intracellular recordings are shown in the upper trace of each record in Fig. 2*A*. The EPSP and IPSP lasted about 15 and 100 ms respectively. Each lower trace is an extracellular record made soon after the withdrawal of the electrode from the cell, and the initial positive

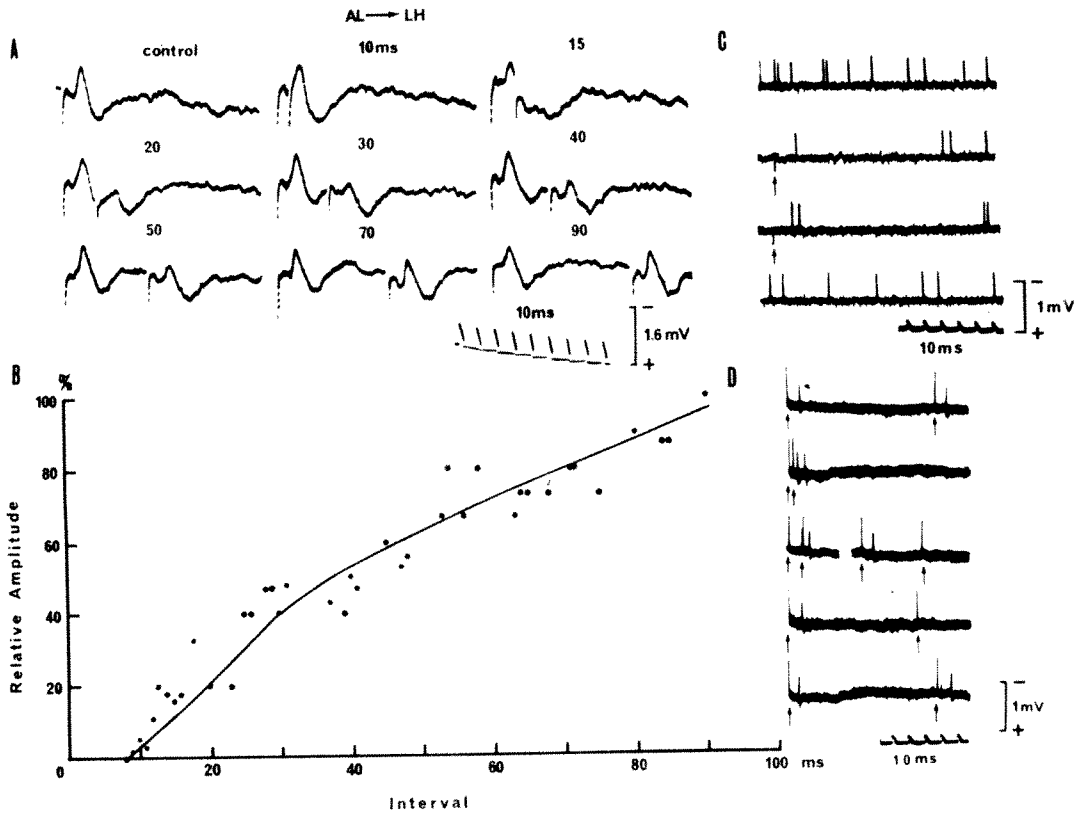


Fig. 1. Extra-cellular records from LH neurones in response to single stimulations of the amygdala. *A*, Time course of inhibition of the N waves during a conditioning P wave. The top record on the left shows the control N and P wave sequence produced by a single stimulation pulse. Test stimulations were applied at various intervals, indicated by values (in ms) above each record. Stimulus intensity was more than twice that of the threshold. *B*, Sizes of test N waves are plotted against conditioning-test intervals as percentages of the control. Each point is the mean value of result of ten trials. Note the inhibition of the N wave lasting about 100 ms in comparison with the time course of the P wave lasting about 30 ms. *C*, The uppermost record shows spontaneous unit discharges of a LH neurone; in the second and third records, driven unit discharges (one or two spikes) with 10–20 ms latency are followed by an inhibition of spontaneous unit discharges lasting about 100 ms; the lowermost record shows the spontaneous discharges 1·25 s after the third record. *D*, Similar experiments to those in *B*, but involving unit discharges. Test stimulations of the amygdala were applied at various intervals. One driven unit discharge appeared at the latency of about 10 ms. Arrows indicate stimulus artefacts. The top and bottom records served as control. The latency became shortened when the stimulus interval was about 2 ms (second record from top). In the third record (left), the driven discharge overlapped on the stimulus artefact. The test stimulation could not produce the driven discharge at intervals of up to about 100 ms.

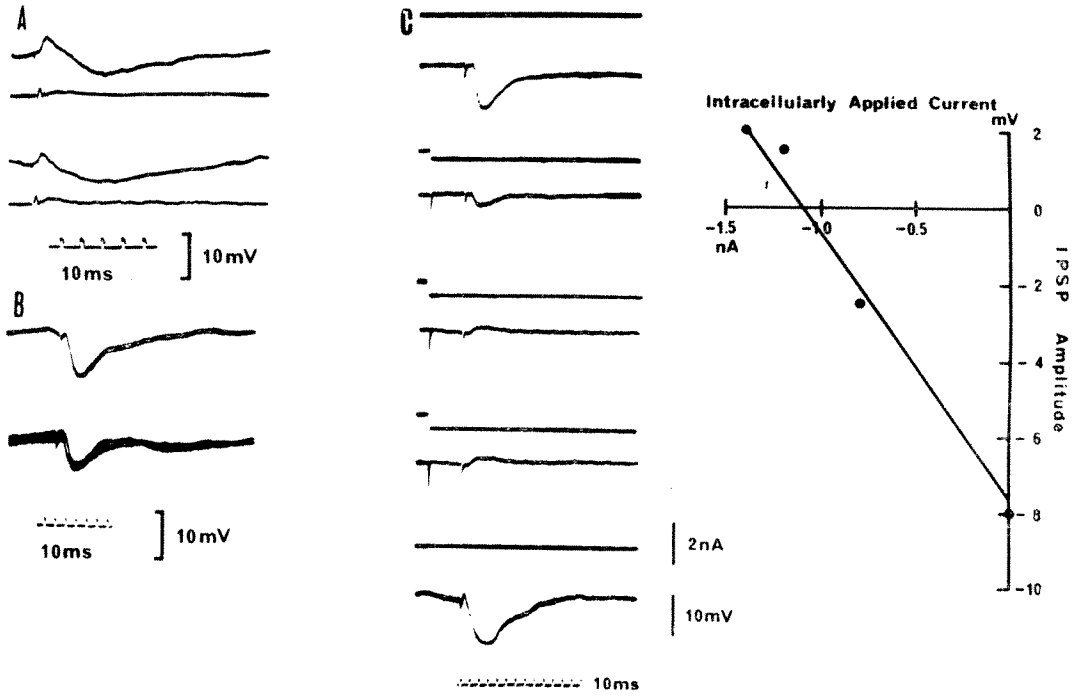


Fig. 2. *A*, Intracellular recordings of synaptic potentials in two LH neurones in response to stimulation of the amygdala. The upper trace in each pair is EPSP followed by a long-lasting IPSP being about 100 ms. The lower trace is an extracellular recording elicited by the same stimulus intensity, immediately after withdrawal of the electrode from the same cell. The small brief positive spike just after the stimulus artefact may represent the action potential from the presynaptic terminals. *B*, Shows the intracellular IPSP in two LH neurones. The upper record is a single trace of IPSP without association of preceding EPSP; the lower records, four traces were superimposed. Note the delayed IPSP with smaller amplitude. The resting potential of these neurones was about −50 mV in *A* and *B*. *C*, Reversal in the polarity of IPSPs recorded intracellularly in a LH neurone. Inward currents were applied through the same recording electrode filled with 3 M KCl. On the left, the upper traces of each record indicate the intensity of the inward current. The top and bottom recordings were obtained 1·25 s before and after the application of current. Right, relationship between the intensity of the inward current and the amplitude of IPSPs. Reversal point is at 1·1 nA. The resting potential was −50 mV.

deflexion may represent presynaptic activity. Other examples showed only IPSPs without the preceding EPSP (Fig. 2*B*). The IPSP was readily reversed in polarity when inward current was applied intracellularly (Fig. 2*C*, left). On the right of Fig. 2*C* the amplitudes of the IPSPs are plotted against the injected current. The inward current at the reversal point of the IPSP was $1{\cdot}1 \times 10^{-9}$ A.

This powerful action of the postsynaptic inhibition may account for the effective suppression of the feeding behaviour and also of the spontaneous activity of the LH neurone.

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Alpha Rhythm and Fine Eye and Eyelid Movement

The source of the alpha rhythm of the EEG was originally attributed to activity within the central nervous system, and, in particular, suppression of the rhythm to processes of vision[1,2], attention[3], or cognition[4,5]. But failure to relate the waveform measured at the scalp to the behaviour of cortical neurones has recently provoked discussion of an alternative generator[6,7]. Lippold has proposed that the alpha rhythm represents a modulation of the corneo-retinal potential caused by ocular tremor[8,9]. In order to test this hypothesis we have investigated the correlation between ocular tremor and alpha activity, as suggested by Good[10].

The subjects were healthy males aged 18–45, in whom the alpha rhythm had been found in earlier experiments to persist while the eyes were open (p-types)[11]. EEG recordings were made from positions C_3–A_1, C_4–A_2, O_1–O_2 P_z–A_2 (ref. 12) using a Beckman dynograph. Simultaneous recordings of eye movement were obtained by the following photoelectric method, modified from Stark and Sandberg[13]. Subjects wore a pair of spectacle frames to which were attached a source of red light and a single phototransistor. The latter monitored light reflected from the lower temporal quadrants of the right eye. Because the average brightness of this region changes as the margin of the iris moves, the output of the phototransistor provides an index of ocular activity. This output was calibrated with respect

to vertical and horizontal movements, though sensitivity is not linear for all vectors of movement. Recordings were made while subjects, seated in a darkened and sound isolated chamber, fixated a dimly illuminated point source.

Simultaneous recordings of EEG and eye movement are seen in Fig. 1. It is clear that bursts of alpha activity can and do occur without detectable eye tremor at these frequencies (Fig. 1*a*). At other times slower high amplitude ocular movements appear to be "notched" at alpha frequency while the EEG shows only characteristic alpha activity at about 10 Hz (Fig. 1*b*).

The photoelectric sensor responded also to movement of the eyelid when the eyes were closed. Fig. 1*c* shows that fairly long periods of correspondence occurred between eyelid flutter and alpha rhythm of the EEG. In these conditions eyelid movement may reflect activity of the levator palpebrae or passive movement from ocular tremor. If the latter is the case, then ocular tremor at alpha frequency must have increased in amplitude relative to other frequencies while the eyes were closed. This is not accompanied by any increase in the amplitude of the alpha rhythm of the EEG. Alternatively, the sensor may record merely the characteristic tremor of skeletal muscle; in this case, of the levator palpebrae.

Saccades of less than 2° occurred without appearing to contaminate the EEG and, in fact, ocular tremors of 10° or more would be necessary to produce an artefact of alpha rhythm in the manner suggested by Lippold. Such tremors were not observed.

Where ocular tremor contained activity at alpha-like frequency, five second samples of the simultaneous recordings were subjected to time series analysis on a Noratom ISAC computer[14]. Alpha rhythm was present in the EEG record for all samples. Fig. 2 shows that its peak occurred at approximately 10 Hz both when the eyes were open or closed. By contrast, the eye sensor channel shows a peak at 5 Hz. This was prolonged as a plateau to 10 Hz when the eyes were closed, representing the faster component of eyelid flutter (Fig. 2*c*). It is notable that even in these records, selected for maximum content of alpha frequency in the eye movement record, 10 Hz is not the predominant frequency. If ocular tremor generated the alpha rhythm the peak frequencies should be the same in both records.

The autocorrelations in Fig. 3 indicate largely random eye movement and eyelid flutter, whereas all three EEG traces show a pronounced rhythmicity at 10 Hz. In the first records, Fig. 3*a*, there is no indication of 10 Hz

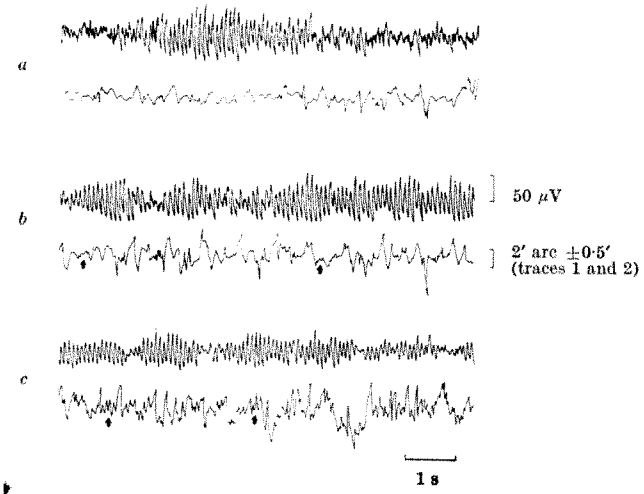


Fig. 1. Simultaneous records of EEG (P_z–A_2) (upper trace of each pair) and eye movement (lower trace). *a*, Recording made with eyes open. Note the large burst of alpha activity unaccompanied by corresponding 10 Hz activity in the sensor trace. *b*, Recording made with eyes open; arrows indicate periods when eye movement appears to be notched at alpha frequency. *c*, Recording made with eyes closed. Sensor reveals eyelid flutter. Signs of activity at alpha frequency in eyelid flutter are even more pronounced.

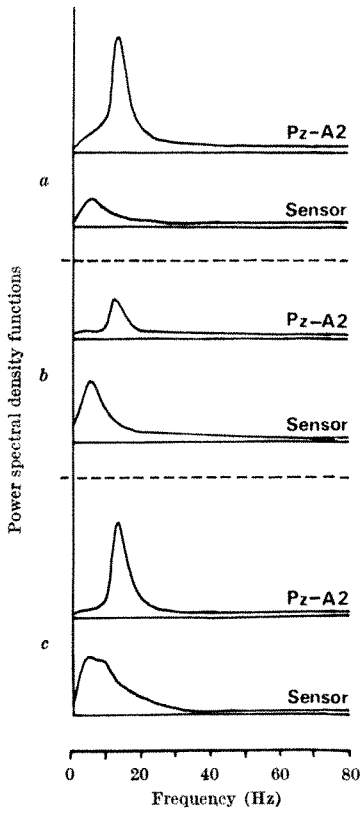


Fig. 2. Power spectral density of EEG and eye movement/eyelid flutter obtained during periods of high alpha-like activity in the sensor channel. *a* and *b*, Eyes open; *c*, eyes closed.

rhythmicity in the autocorrelation of the eye movement, and cross correlation with the EEG fails to reveal any relation between them. In the second record, Fig. 3*b*, there is again no evidence of rhythmicity at 10 Hz in the eye movement but cross correlation reveals a common component. This stands up to the test of normalization by calculation of the cross products of the standard deviations of the two autocorrelations. After normalization, however, common 10 Hz activity is greater between eyelid flutter and the EEG (Fig. 3*c*) than between ocular tremor and the EEG (Figs. 3*a* and *b*). This result might have emerged if the 10 Hz component of the EEG had been stronger when the eyes were closed. But of the samples selected, alpha activity was most powerful in the first record taken while the eyes were open (Fig. 3*a*) and no cross correlation was evident.

These findings are inconsistent in a number of respects with the view of ocular tremor as a generator of alpha rhythm. The predominant frequencies of ocular tremor are markedly different from those of simultaneously recorded alpha rhythm. The random components of ocular movement contrast with the redundancy of the alpha rhythm; one would expect coherent signals originating in the orbit to be obscured by superimposition of neuronal activity when measured at the scalp. In fact, the reverse is observed. Even if, in the present study, the eyeball were oscillating at alpha frequency under the closed lid, such movement is evidently not essential for the appearance of alpha rhythm at the scalp. In view of the relationship between the amplitude of saccades and the artefacts[15,16] they induce in the EEG, one would expect to record very conspicuous tremor of the eyeball when alpha rhythm occurred with the eyes open. This was not the case.

Although these findings seem to preclude an orbital origin for the alpha rhythm, the cross correlations of Figs. 3*b* and *c* argue for a weak interaction in the opposite direction. That is to say, eye movement may be modulated by alpha rhythm. This will occur when conscious control of fixation wanes or is interrupted by eye closure. In

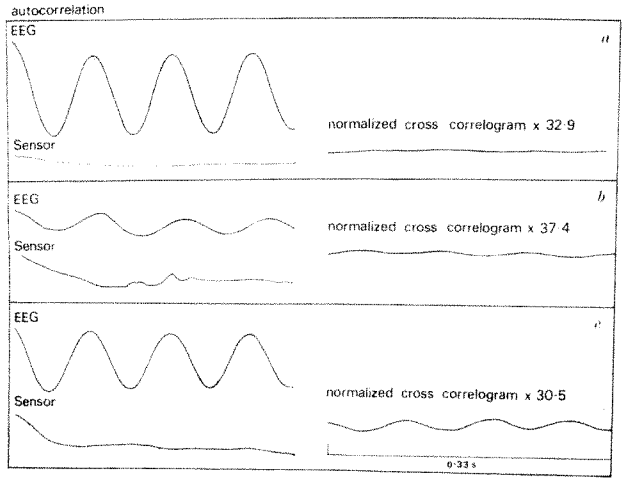


Fig. 3. Auto and cross correlograms of EEG and eye movement. In each group of traces, the upper left trace shows the autocorrelogram of the EEG; lower left: autocorrelation of the eye movement/eyelid flutter; right hand trace: cross correlogram of EEG and eye movement/eyelid flutter. *a* and *b*, Eyes open; *c*, eyes closed. The numeric factors (obtained from the cross product of the standard deviations of the autocorrelation functions) necessary to normalize each cross correlation are indicated above the respective cross correlograms. The phase difference between cross correlograms *b* and *c* has no biological significance.

these conditions the oculomotor, abducens and trochlear nuclei are deprived of information for the servo control of fixation and may become more susceptible to extraneuronal current flow at alpha frequencies. The greater correlation between eyelid flutter and EEG may reflect a similar process operating on that part of the oculomotor nucleus not directly involved in fixation.

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Paired Comparison Technique for measuring Stimulus Control Functions in the Pigeon after Training with Discrete Trials

Since the work of Skinner and his associates[1], free responding techniques, in which an organism can respond repetitively without altering the stimulus situation, have commonly been used in studying discrimination learning in the pigeon. Guttman and Kalish[2] developed a procedure for obtaining reliable measures of response strength at different "values" along a "stimulus dimension" (for example, wavelength of a patch of light); from these, "stimulus control functions" could be plotted. This procedure has been used by Hanson[3] to obtain post-discrimination stimulus control functions, and later workers have used a wide variety of experimental designs and stimulus dimensions[4]. In a free responding situation however, as Jenkins has pointed out[5], the assessment of stimulus control may be subject to error from confounding effects—for example, a reinforcement within a stimulus presentation may in itself lead to an increased rate of responding on that presentation. He suggests that these effects can be overcome most easily by the use of a discrete trial training procedure, in which the organism's response terminates the trial. But the free responding Guttman and Kalish procedure for measuring stimulus control functions then becomes inappropriate.

This testing procedure has been developed after unsuccessful attempts to measure stimulus control functions in a discrete trial situation using various dependent variables, for example, latency of response, number of responses to extinction. It involves a paired comparison test in which a test trial consists of the presentation of two stimuli occupying different values on the stimulus dimension tested. A single response by the pigeon to one of these two stimuli terminates the trial and over successive test trials all possible combinations of pairs of stimuli from a given set of test stimulus values are presented, as in conventional uses of the paired comparison technique. Stimulus control functions are then obtained with number of choices of test stimulus values as dependent variable. David[6] has developed a range of statistical tests for use with paired comparison data in this form. It is also possible to obtain scale values in terms of one or more of the theoretical models developed for paired comparisons and to assess the goodness of fit of data to model[7].

As the test involves a choice between two stimuli there are complexities in interpretation in intra-dimensional designs (that is, in designs where both training stimuli have values along the test stimulus dimension) in that the bird's response may be based on the relationship between the two stimuli, rather than the absolute value of the reinforced stimulus. Thus the test is most suitable for use in interdimensional designs (designs where only one training stimulus has a value on the test stimulus dimension) and in extradimensional designs (designs where neither training stimuli have values along the test stimulus dimension), where such relational explanations are not possible.

In order to produce a high resistance to extinction so that a large number of test trials can be used in the absence of reinforcement, pigeons are given extensive training at a low probability of reinforcement for pecks at the positive training stimulus, S^+ (typically 0·125 or 0·1). In this way reliable stimulus control functions have been obtained after single stimulus training, successive go/no-go and simultaneous discrimination training.

As an example of theoretical interest, results obtained from a simultaneous discrimination using an interdimensional design are cited. Four experimentally naive 2–3 year old cock pigeons were maintained at 80 per cent of their free-feeding weight. They were trained in a sound attenuated chamber on one wall of which were two 1 inch diameter response keys with 1·5 inch between their centres and at the same height. In training a trial started when both keys were trans-illuminated, one key showing S^+ (a red circular disk), the other showing S^- (a grey line at 45° to the horizontal, with red circular surround—the red and grey parts being equated for brightness by a human observer). A single peck at S^+ darkened both keys, gave access to 4 s reinforcement of mixed grain and initiated an inter-trial interval of 10 s. A single peck at S^- darkened

both keys and initiated the inter-trial interval. Any inter-trial responding delayed the onset of the next trial until 10 s after the last peck. Such inter-trial responding was rare. S^+ appeared with equal frequency on left and right keys, as determined by Fellows[8] sequences. Stimulus presentation and reinforcement contingencies were controlled automatically by conventional electromechanical devices in a separate room from that containing the training chamber. Pigeons were run in daily sessions of forty-eight reinforcements until at least 90 per cent correct responding occurred on three successive sessions. The probability of reinforcement for pecking at S^+ was then dropped successively to 0·5 (two sessions of forty-eight reinforcements), 0·25 (two sessions of forty-eight reinforcements) and finally 0·125 (ten sessions of twenty-four reinforcements each). A correction procedure was used throughout such that a peck at S^- on a trial when reinforcement was available on S^+ led to S^+ only being presented, in the same position, on the next trial. Pigeons were then tested on three successive sessions. A total of sixty test trials were run per session.

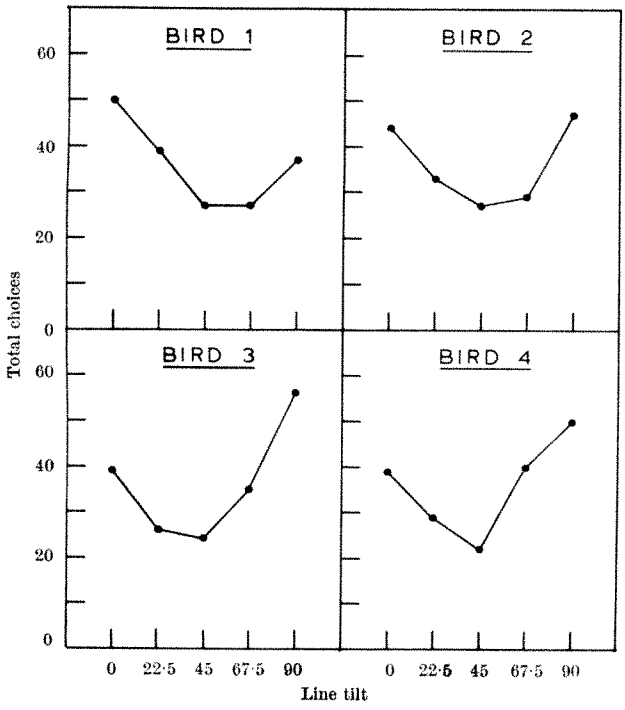


Fig. 1. Total number of choices of different line tilts during paired comparison testing after training on a simultaneous discrimination with S^+ a red disk and S^- a grey line tilted 45° clockwise from the vertical, with red surround. All pigeons show the U-shaped function with minimum at the trained S^- tilt, indicating stimulus control by S^-.

Pairs of stimuli taken from a set of five having grey lines tilted at 0°, 22·5°, 45°, 67·5°, and 90° to the vertical, each with red surround, were presented. Each block of twenty trials contained all possible combinations of test stimuli presented in randomized sequence. All test trials were run in extinction, all birds responding on all test trials. Test trials were preceded by five reinforcements at a probability of reinforcement of 0·125 for response to S^+ and followed by nineteen reinforcements in the same condition.

Fig. 1 shows the results of these tests. Statistical analysis[6] shows differences between the overall totals of choices of different line tilts for individual birds at $P < 0{\cdot}01$ for all pigeons. The U-shaped stimulus control functions indicate that S^- has attained control over responding. Thus the assertion made by Bloomfield[9] that simultaneous discriminations are explicable purely in terms of the rule: go to S^+, is incorrect. It has frequently been suggested[10–12] that the peak shift effect[3] occurs if, and only if, S^- has developed stimulus control. As peak shift has not been found in the simultaneous discrimination situation (ref. 13 and unpublished results of R. L. Rudolph), this finding of control by S^- in a simultaneous discrimination casts doubt on the generality of this suggestion.

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GENERAL

Occupational Orientations at Bradford and Warwick Universities

IT is well established that occupational attitudes can be shaped by educational experiences[1], but comparatively little is known about the specific effects of a British university education. Becker *et al.*[2,3] have differentiated the effects of higher vocational and non-vocational education in the United States. They found attitude changes to occur, but whereas, in the former, students came to see themselves as members of their prospective professions, in the latter, they tended to identify with their subjects. Various official reports[4,5] in recent years have taken the view that university education in Britain chiefly promotes identification of the second kind.

These reports are expressions of a climate of opinion which has greatly influenced the development of university education in recent years. For example, the technological universities have been established with the aim of providing an education related to industrial needs.

Studies among scientists[6,7] have shown that they can be characterized in terms of the relative importance they attach to professional goals (for example, advancing knowledge by fundamental research) and organizational goals (for example, producing results useful to employers). The two orientations are not, of course, mutually exclusive, but most scientists probably emphasize one set of goals or the other. How this bias develops is little understood, but the view of the Jones and Swann Committees[4,5] seems to be that pure science courses influence students in the direction of professional goals. Given the distinctive aims of applied science courses, they might be expected to produce an organizational emphasis.

Goldberg, Baker and Rubenstein[8] have devised scales for assessing the relative strengths of professional and organizational orientations, and these have recently been refined and modified for use among British students by Child and Musgrove[9]. The questionnaire asks subjects what weight they would give to a number of factors in judging the worthwhileness of a project.

At the University of Bradford, in 1967, a longitudinal study was started among science and engineering students entering the university for the first time in an attempt to shed some light on the assumption of occupational identities. Among the questionnaires administered was the Child–Musgrove questionnaire. The same students were approached again in January 1970, when they were in the third year of their four year courses. The initial response rate was good—86 per cent—but in the follow-up study only 41 per cent of those who had reached the third year returned usable questionnaires. Engineering students who responded on this second occasion were somewhat more organizational on entry than non-respondents (means: respondents 25·3, non-respondents 23·9, $t=2·07$, $P<0·05$), but otherwise the 1970 respondents were representative of the sample as a whole.

At the University of Warwick a longitudinal study of the development of occupational attitudes was started in January 1969, among students who were in their first year. Like the Bradford study, this survey included the Child–Musgrove questionnaire and, to provide some comparative data, it was also given to a sample of third year students. Percentage returns were consistent among the various groups giving an overall figure of 64 per cent.

Data from the Bradford and Warwick studies are presented in Table 1. The group of applied scientists at Bradford comprises students from the Schools of Applied Physics and Industrial Chemistry; the group of pure scientists at Warwick comprises students from the science faculty reading the nearest equivalents to the Bradford disciplines. The sample of engineering students at Bradford includes students from three schools—chemical, electrical and mechanical engineering—and these are compared with students at Warwick reading engineering science.

Table 1. PROFESSIONAL AND ORGANIZATIONAL ORIENTATIONS OF FIRST AND THIRD YEAR STUDENTS AT BRADFORD AND WARWICK

University and field of study	Professional scores 1 N	Mean	s.d.	3 N	Mean	s.d.	Organizational scores 1 Mean	s.d.	3 Mean	s.d.
Bradford										
Applied science	44	21·3	4·5	14	20·2	4·2	23·1	4·4	19·8	5·1
Engineering	191	20·3	3·6	42	20·5	3·6	24·2	3·1	23·0	4·1
Warwick										
Pure science	58	22·0	3·8	51	20·3	4·0	20·1	5·1	19·5	5·4
Engineering	40	20·5	3·7	19	19·8	4·4	22·4	3·7	22·8	3·6

The data of Table 1 show that the two universities seem to be attracting students with different occupational orientations. Both the Bradford engineers ($t_1=2·34$, $P<0·02$) and the Bradford applied scientists ($t_1=3·18$, $P<0·01$) were significantly more organizational on entry than their Warwick counterparts. (t_1 = significance of difference between independent means). First year science students at Warwick were significantly more professional than organizational ($t_2=2·42$, $P<0·02$). (t_2 = significance of difference between correlated means.) But on entry the Bradford applied scientists ($t_2=1·63$, $P<0·15$) like the engineers at both Bradford ($t_2=7·12$, $P<0·001$) and Warwick ($t_2=2·09$, $P<0·05$) tended to be more organizational than professional.

Comparing first and third year data, few changes seem to have occurred among the engineering students. Their professional scores remained constant at both universities, and Warwick third year students were as organizational as those in the first year. Bradford engineering students, however, became less organizational over two years ($t_2=4·11$, $P<0·001$) so that among third year students organizational orientations in the two universities were similar.

The professional scores and organizational scores of scientists in the two universities also converged. The Bradford applied scientists, who were the more organizational at the outset, became significantly less organizational over two years ($t_2=2·55$, $P<0·05$). Conversely, at Warwick, third year pure science students were significantly less professional than first years ($t_1=2·27$, $P<0·05$).

These results contain elements which were anticipated, but also elements which are altogether surprising. Research in the United States has indicated that different universities characteristically attract different student populations. Astin[10], for example, found that student choice and institutional selection resulted in the enrolment of students whose "educational and vocational plans are remarkably suited to the curricula offerings of the institutions". In our study, the organizational emphasis of students entering the University of Bradford matches the publicized aims of the technological universities.

American research has also revealed the different occupational orientations of both student[11] and practising[12] scientists and engineers and these also appear in our study.

But what was wholly unanticipated was the convergence of the occupational orientations of applied scientists at Bradford and pure scientists at Warwick. In spite of the different intentions of the courses, it seems that their outcomes in this respect were similar. This raises two important considerations.

First, the contention of the Jones and Swann report does not appear to hold. The study of the pure sciences at university does not seem to cause students to espouse professional and eschew organizational goals, at least when the whole attainment range is considered. Gannicott and Blaug[13] have recently reached a similar conclusion from a quite different line of evidence.

A second doubt concerns the distinctive contribution of courses like applied physics and industrial chemistry. This has been challenged before, by Wyatt[14], for example, who showed that some applied science courses differed little from their pure equivalents apart from the compulsory periods of industrial training. While this is probably not generally true, and there may be considerable differences in content, the pure sciences and their applied variants are alike in being specified by the empirical phenomena studied. In this they differ from engineering and other technologies which are organizations of knowledge around practical problems[15]. The pattern of occupational orientations reported here suggests that the pure and applied sciences may lie within the same, and engineering within a different, "boundary of influence"[16].

We thank Professor F. Musgrove, who is in overall charge of this work, for advice.

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Book Reviews

WHERE ECCLES ERRED

Policy and Practice

The Colleges of Advanced Technology. By Tyrrell Burgess and John Pratt. (LSE Studies on Education.) Pp. xvi+236. (Allen Lane, The Penguin Press: London, November 1970.) 105*s*.

THE expansion of higher education in Great Britain from the mid-1950s on is associated above all with the enthusiasm and dynamic energy of one remarkable man, now Lord Eccles, who was Minister of Education for two periods during a critical time, and his team of devoted civil servants. The expansion was based on three points of view, all of which turned out to be incorrect. The first was that the rise in the number of students to be expected largely arose from the increase in the size of the age-groups entering higher education, chiefly as a result of the post-war birth boom. In fact, it related to an increase in the proportion of the age-group staying on at school. Even in the Robbins report there was a considerable underestimate of the likely numbers who would be coming forward. Provision for higher education expansion, relatively lavish though it may have seemed, has therefore always been too little and too late. In this respect the British experience, it must be admitted, is no different from that elsewhere.

Second, it was assumed that the major sector of the expansion, other than that in teacher training, should be in places for science and technology. This ran counter to the student demand, with the consequence that not only do some science and technology departments stand relatively empty in parts of the country, but also the qualifications for admission to those courses have been significantly lower than the qualifications for admission on the arts and social sciences sides. Even today the universities, with 55 per cent of their existing provision for science and technology, are planning to make 55 per cent of their new provision in the quinquennium 1972–77 in the same field. It is likely that some of these expanded departments, especially in physics and chemistry, will become museums to the history of ideas rather than places where students are actually taught.

Third, it was assumed without argument that the expansion in the supply of trained scientists and technologists would automatically lead to economic growth and, indeed, would be required by it. In the event, there is little evidence and less systematic argument to connect the growth of the economy with the development of science and technology. Indeed, it is almost certain that the growth of research in science, in particular, is a consequence rather than a cause of the growth of the national income. So powerful, however, is the desired association of ideas, the faith that the rate of growth of the national income depends on the expansion of industry, and the expansion of industry on the recruitment of engineers, and the training of engineers on the development of the basic sciences, that the nation continually goes on pouring resources into this area in spite of the evidence to the contrary. (Rolls-Royce's story, it may be said, is no accident.)

In this process, a number of technical colleges were developed by Lord Eccles as Colleges of Advanced Technology, and the Colleges of Advanced Technology, following Robbins, then became universities. Burgess and Pratt's story is largely the story of this conversion. They tell the tale very well, though it must be said that there is a fundamental distinction between history which reveals its sources and journalism which conceals them. Recent history falls heavily between these two stools, and there is reason to feel that another author might have told another story, and there is little available evidence to check which story was true.

There is no doubt the authors are correct about the chief features of the story. The conversion to university status was accompanied by a slowing down in the rate of growth which was also the result of the decreasing desire to study science and technology. The Colleges of Advanced Technology turned out to be very expensive per student place, and the process of conversion to university status was no easy matter. Burgess rather ruefully predicts that, given the class structure of English society, it is probable that the polytechnics will pass along the same road. If so, there are indeed many warnings in his book which need to be heeded.

JOHN VAIZEY

RACE RELATIONS

Race and Racialism

Edited by Sami Zubaida. (Explorations in Sociology.) Pp. xiii+185. (Tavistock: London and New York, October 1970.) 36*s* boards; 18*s* paper.

RACE relations research in Britain has been characterized until recently by the absence of a specifically "sociological" orientation. In the studies of prejudice and discrimination, racial conflicts were usually seen as the consequence of cultural clashes between the "host" and "immigrant" communities, and this tension was expected to disappear as the immigrants were gradually assimilated. But the exacerbation, rather than the lessening, of racial conflicts has undermined the validity of such assumptions, and necessitated a radical re-examination of the study of race relations.

This re-examination is the chief aim of *Race and Racialism*, a collection of seven conference papers by British sociologists. Sami Zubaida, in an excellent critical introduction, argues that research on racial and ethnic groups should define "their relationship to the wider social structure and the historical development of this relationship". One of the major problems is the definition of the key concepts in such an analysis, and the contributors themselves disagree in their exposition, for example, of "racism" and "racialism". Michael Banton, in his lucid paper, argues that because "racism" originated as a biologically based doctrine, sociologists mislead when they now use it to describe culturally based theories of racial inequality.

John Rex and David Lockwood both examine whether race relations situations demand a specific set of analytical concepts, or whether they may be defined as "class" situations using the categories of stratification theory. Rex, in his stimulating paper, constructs a typology of stratification situations in colonial and metropolitan societies, involving racial and ethnic groups. Whether or not these situations come to be defined as racial depends, he argues, on the existence of deterministic belief systems. Why "racist" ideologies develop where they do, however, still remains a question for future research.

Lockwood discusses the significance of the concept of

the "plural" society. Though one may disagree with some of the distinctions he draws between class conflict on the one hand and racial or ethnic conflict in plural societies on the other, his paper greatly illuminates the whole question of racial versus class consciousness and conflict.

Among the empirical contributions, that by Sheila Allen provides a good example of the integration of research in race relations with that in industrial sociology. She shows that the failure of the trade unions in Bradford to involve the lower skilled workers quickly became transformed into a racially defined conflict when the workers concerned were immigrants.

This book indicates that future research will move into more politically relevant areas—as social and economic structures become the objects of analysis. It also promises a more theoretical orientation in a field previously dominated by the British empiricist tradition. This book can be recommended to all who welcome such developments. JENNIFER HURSTFIELD

CARE OF SCHIZOPHRENICS

Institutionalism and Schizophrenia

A Comparative Study of Three Mental Hospitals 1960–1968. By J. K. Wing and G. W. Brown. Pp. xiii + 260. (Cambridge University: London, September 1970.) 75*s*.

THE authors of this book set out to evaluate the effects of the changing social milieu on hospitalized female schizophrenics. They describe their attempt as a search for "hard and obstinate facts" rather than for novel theories, and they have been successful. It is clear, as the authors point out, that all the polemic and propaganda for changes in hospital regimes and the real improvements that have occurred have been necessary from the point of view of humanity alone; but it is also pertinent to ask whether these changes have also resulted in the reduction of illness handicaps and in the encouragement of personal improvement. Their chapter on this area is a useful review of previous work.

Methodologically, the investigation rests on the firm basis of techniques the reliability and validity of which have been tested in earlier research. Assessments are made of the clinical condition of the patients and of the ward environments in which they live in three British hospitals. An attempt is made to show that certain "negative" aspects of the clinical condition, such as social withdrawal, are associated with the poverty of the social situation in which the patient finds herself. This hypothesis and numerous subsidiary ones are supported by a "cross-sectional" analysis of the patients and conditions in the three institutions. Advantage has then been taken of changes which occurred in the organization of their treatment regimes, during periods of four and eight years, to illuminate the problems of cause and effect in the associations established. Useful evidence, pointing to the efficacy of a richer social environment in evoking more positive behaviour, has certainly been forthcoming.

The work is liberally illustrated with clinical anecdotes and descriptive chapters on the wards studied. This produces a somewhat uneven texture in the book which is offset by the gains in understanding of the institutions and personalities concerned.

This is an essential text for anybody involved in the management of institutional care of patients. It gives a balanced view of the interaction between possible biological and social factors in the evolution of this particular condition but, more than this, it indicates the possibilities of stepping into natural experimental situations, and, with quite simple methods and analysis, achieving a rational assessment of the effectiveness of treatment ideologies. J. A. CLARKE

BUTTERFLY BEAUTIES

A Field Guide to the Butterflies of Britain and Europe

By Lionel G. Higgins and Norman D. Riley. Pp. 380 + 6((Collins: London, September 1970.) 42*s*.

AMATEUR and professional entomologists alike will we come this book for its authority, its thoroughness an at the same time its simplicity which enables even th beginner to identify rapidly the "rare" butterfly whic he finds in his garden. Furthermore, it encourages th young collector to want to know more about the way i which the scientific classification of species is organize(All the technical terms used are explained in a glossar and there is a separate check list of all the species as we as an index of English names. The last section contair nearly four hundred miniature maps indicating th localities in Europe where each species may be found.

The introduction, which contains general hints o collecting, might be thought rather old-fashioned by som experts, but, if so, it is to be looked on as a touch (old-world charm which serves to indicate the immens pleasure which two authors of riper years have derive from a lifetime spent in the study of butterflies. A speci feature of this book is the excellence of the illustration: Every species and many of the subspecies have a coloure illustration of both upper and underside, and of bot sexes. So often in natural history books one sees illus trations in which the artist has painted not so much wha he actually sees as what he thinks he ought to see an the result is often disappointing. In the young an talented artist Brian Hargreaves, the authors have a illustrator who attains the highest standard of butterfl pictures I have seen. Fellow worshippers of these gloriou insects will pray that he may be persuaded to devote hi life's work to painting the butterflies of the whole world Such a work would indeed be a major contribution t natural history.

In the text, each species receives about half a pag into which are tucked a description which notes all specia distinguishing features, the distribution and range, cater pillar food plants and the type of country where it i most likely to be found. Similar species which are mos likely to be confused are also noted.

Even for those with only a passing interest, this bool is a "must". E. R. LAITHWAITE

BREATHING REFLEXES

Breathing

Hering–Breuer Centenary Symposium. Edited by Rutl Porter. (A Ciba Foundation Symposium.) Pp. xiv + 402 (Churchill: London, 1970.) 80*s*.

IN April 1868, Hering described, with full acknowledg ment, work performed by his pupil Breuer entitled "Self steering of Respiration through the *Nervus vagus*". Late that year, Breuer published the full account of his worl on the reflex effects upon breathing which occur when th lungs of certain animals are inflated and deflated. Th inhibition of breathing which follows inflation of the lung has since become known as the "Hering–Breuer reflex" This was the first convincing demonstration in biology and perhaps in any discipline of what today would be called "feedback". It made a great impression on physiology and medicine and disturbance of this reflex was later to be invoked to explain a wide variety of respiratory disturb ance in disease. It is only in the past decade, however, that the reflex has been studied directly in man and its signifi cance and limitations in disease clarified. New reflexes arising from different receptors within the lungs have also been identified and their possible role in disease in place of the Hering–Breuer reflex considered.

The original aim of this Ciba symposium was to review and discuss this new work and its relations to other aspects of respiratory physiology and disease. But when it was appreciated that the meeting would coincide with the centenary of the publication of the Hering–Breuer reflex, the content and direction of the symposium were expanded and a most valuable biographical review and translation into English of the Hering and Breuer papers were added.

It is appropriate that the opening scientific contribution to the symposium compares the role of inflation reflexes in man and other animals. The remainder of the first section contains contributions on afferent nervous pathways including the sensory innervation of the airways and other reflexes from the lungs which are probably responsible for many of the effects which have previously been attributed to the Hering–Breuer reflex. These include receptors responding to changes in pulmonary interstitial pressure and others to mechanical deformation of the bronchi and bronchioles.

The second section of the book contains three contributions devoted to central interactions and peripheral motor mechanisms which consider the origin of the respiratory rhythm, neurological control in relation to metabolic and behavioural requirements and the variation of respiratory motor activity in relation to varying sensory inputs. The third section concerns respiratory sensation, the stimuli, receptors and pathways involved and possible psychological aspects.

The final section concerns the relationships of these physiological aspects to clinical problems including possible mechanisms of some respiratory symptoms, the relationship of respiratory frequency to breathlessness and the effects of vagal block on the sensation arising from pulmonary disease.

Although I found the symposium a most rewarding experience, it was also salutary to discover on reading the proceedings how much one had failed to appreciate at the time and how much more valuable it is to read the proceedings at leisure and digest the wealth of information and ideas that were discussed during those three days.

J. B. L. Howell

DERMATOLOGY OR BIOLOGY?

An Introduction to the Biology of the Skin

Edited by R. H. Champion, T. Gillman, A. J. Rook and R. T. Sims. Pp. x+450. (Blackwell (Scientific): Oxford and Edinburgh, 1970.) 85*s*.

For some years now there has been a growing tendency to introduce the word "biology" into all sorts of contexts where thirty years ago it had never set foot. A host of things which previously just existed are now credited with "biological significance", symposia and books dedicated to the biology of this, that and the other are currently fashionable, and we are even witnessing the germination of a crop of new bio-subjects. It is to be hoped that the widening and freer use of the word "biology" will amount to something more than lip-service and better than prostitution. I prefer to think it represents a public expression of a widespread sense of longing to get away from the lifeless world of test-tubes and slide-rules and "back to nature", which of course is not the same thing as "back to the trees". So intense is this longing that sometimes, as in this book, it gets ahead of itself and shades into wishful thinking.

This book is based on a postgraduate course of lectures which has been given at the University of Cambridge each year since 1963. As I have implied, the reason for its being written may be more encouraging than the book itself, which is unlikely to introduce anybody to the biology of anything, though one hopes it will not actually put anybody off. There are a few outstanding exceptions to the generally arid level of the book, particularly the chapters by Gillman on "Dermo-epidermal Interplay" and by Herbertson on "Inflammation", and the book should be read if only for the sake of these contributions. They and a few others are written about living processes as living processes, by authors whose approach to their subjects is that of the classically enthusiastic naturalist, a breed to which all true biologists have belonged and still must. These chapters and the motivation behind them would have been as welcome and comprehensible to Harvey or Pasteur or Darwin as they are to us. But these golden exceptions are few and far between in an otherwise lifeless desert of technological fluff and hack banality.

A number of the authors write as if they had just attended the course rather than given it. Some sound to be less than enthusiasts for their subjects, which they seem only to have mugged up for the purpose of filling the book. But the saddest group of contributions are those whose authors give the impression that their answer to the question "how does a seed grow into a tree?" would consist largely of a series of chemical formulae for the synthesis of chlorophyll and a page or two of equations. One can only assume that these two groups of non-biocontributors were press-ganged into the book because of shortage of local bio-talent.

The award for the most non-biological topic goes to chapter one on "Aspects of Mensuration", featuring a commendation of the new (SI) system of units (1 c.c. = 10^3 mm^3, and all that). The system is fairly thoroughly pilloried by its use throughout the rest of the book. If this kind of thing had to be included in a book about biology, surely its place was at the back of an appendix rather than as the opening chapter. The gold medal for the most unbiological remark in the book goes to the opening comment in chapter eleven: "Hair . . . was evolved to protect the skin against physical trauma, cold and ultra-violet light". No reference is given for the source of this instant, three-in-one resolution of a fascinating and perennially insoluble problem of cutaneous biology. Like all truly great gaffes this one's stature grows in the marvellous absurdity of its implications the more it is pondered: evolution by natural[3] selection[3] according to the SI system.

In short, this book might more truthfully have been entitled "A Young Person's Guide to Modern Investigative Dermatology". But the fact that it was called "An Introduction to the Biology of the Skin" shows that the authors wished it was. And full marks for that wish.

Ian W. Whimster

ASPECTS OF STEROIDS

Advances in Steroid Biochemistry and Pharmacology

Vol. 1. Edited by M. H. Briggs. Pp. xi+517. (Academic: London and New York, October 1970.) 150*s*; $21.50.

This volume, the first in a series, contains ten chapters devoted to various specialized aspects of the chemistry, biochemistry, physiology, pharmacology and analysis of steroids. It is not often that up to date reviews on such varied aspects of steroids are all found in one volume and for this reason alone the book will be a most welcome addition to a library or private collection. The editor has skilfully selected the topics so that the metabolism of steroids in man, animals, arthropods, marine invertebrates and plants is discussed.

There is much of interest here for the specialist in a particular field as for the beginner. Drs Vinson and Whitehouse review comparative aspects of adrenocortical function and, by way of introduction, devote some pages to a discussion of the methods utilized in a study of biosynthetic pathways, and explain how to interpret data

derived from kinetic studies—important topics which are often brushed aside too casually. The control of adrenocortical steroidogenesis is also considered and the possible mode of action of ACTH, the renin/angiotensin system and other regulatory mechanisms is discussed. Not least, this chapter concludes with an extensive table in which are collected a mass of data concerning adrenal steroid formation in a large number of species. Clearly, the authors are to be congratulated on their ability to review some 750 papers on adrenocortical function which have appeared during the past five years.

For many steroid laboratories the question "to automate or not to automate" is becoming increasingly important to answer. Drs Grant and Hall discuss the pros and cons of automation and give a critical survey of the various methods available for steroid analysis, pointing out which of these can be modified for automation. Dr R. D. Bulbrook reviews the mass of data now accumulated concerning the prognostic value of steroid assays in human breast cancer—a particularly welcome contribution in the light of the recent controversy concerning the value of the urinary discriminant. Other valuable chapters are concerned with the biosynthesis of sterols, the mode of action of insect hormones, the sterols of marine invertebrates and plants, the effects of steroids on tryptophan metabolism, cervical mucus and vaginal epithelium and the control of reproduction in farm animals.

It is to be hoped that the second volume in this series will attain the same high standard as this volume.

D. B. Gower

MOLECULAR IMMUNOLOGY

Biochemistry of Antibodies

By Roal'd S. Nezlin. Translated from the Russian by Michel C. Vale and edited by Fred Karush. Pp. xiii + 381. (Plenum: New York and London, 1970.) $25; 234*s*.

Anybody involved in teaching "molecular immunology" must have been faced with the difficulty of recommending suitable textbooks. This book is not the complete answer but it does provide a useful addition to the limited bibliography. No textbook in such a rapidly advancing field can hope to be up to date. In this case there is the added delay involved in translation of the original Russian text. The English edition has been updated by the author and also the original commentary has survived the test of the time lag very well. The methodological sections are lacking in current details; this in part must reflect the isolation of Russian science. But there is a good discussion of the methods covered. The physicochemical properties and structure of antibodies are well reviewed. The longest chapter on antibody biosynthesis provides a most welcome condensation of an extensive body of data and phenomena. From this background must eventually come an understanding of the immune response at a macromolecular level. This review could prove of value to the biochemist newly approaching antibody biosynthesis. The book closes with brief clear accounts of genetics and theories of antibody production.

There is, throughout, extensive referencing. Because of its origin the book contains many references to the Soviet literature which provide an interesting window on immunological studies in the Soviet Union even if those in Russian will be unhelpful to the majority of readers.

The translation is pleasing with an easily readable style and few ambiguities in the scientific English. The rare slip such as: "The allelic exception is also known not to apply to hemoglobulin" will cause more amusement than confusion. The general production standards deserve praise, particularly the clear attractive type.

This volume should prove of value to students, teachers and researchers embarking on, or engaged in, the field of immunology.

A. R. Williamson

RECENT SOIL STUDIES

Écologie Microbienne du Sol

By Yvon Dommergues and François Mangenot. F viii + 796. (Masson: Paris, 1970.) 180 francs.

This volume provides a thorough, up to date and int national coverage of information which is only otherw available from numerous monographs, journal artic and symposium reports. The 800 closely printed pag are grouped into five unequal sections. First, a systema account covers very briefly the principal groups microorganisms (bacteria, actinomycetes, fungi, alg protozoa and viruses). Then there is a long section deali with the effect of microorganisms on chemical process and cycles in the soil. This is followed by sections on t influence of physio-chemical factors on the microflo on interactions between microorganisms and vegetati and, finally, on interactions between the different grou of microorganisms.

The last section (reflecting, of course, today's inadequa understanding) is somewhat superficial and disjointe especially the brief sections on interactions with the s fauna. The penultimate section (interactions with veget tion) reflects the current preoccupation with *Rhizobiu* studies but is extremely brief on mycorrhiza. There i however, a useful section of about 50 pages on the prope ties of the rhizosphere.

The major biochemical section (part two) is writte chiefly by Dommergues and deals in turn with cycl of the major chemical elements (through carbon an nitrogen to iron, manganese and the like). Details a given of the principal intermediary compounds involve in decomposition processes, and there follows a fairl full discussion of recently explored topics such as humidif cation, the soil atmosphere, organo-metallic compoun and the effects of microbes on soil structure. Thus, most useful summary of some of the chief researc interests of the Nancy CNRS laboratory is placed in general context and made available to a wider audienc

The fact that I was periodically frustrated by th terseness of the treatment is less a reflexion on the write of the book than on the spectacular growth of soil micr biology over the past few decades and of the magnitud of the task undertaken. The authors have successfull assimilated the international literature and have provide an entry to the field for all who are interested in th ecology of soil. As a textbook of this kind it would hav benefited from a much fuller and more informative inde but it certainly deserves a wide readership and woul merit translation into other languages.

A. Macfadyen

OVERPRODUCTIVE LAKES

Eutrophication in Large Lakes and Impoundments

Compiled by C. P. Milway. (Uppsala Symposium, Ma 1968.) Pp. 560. (OECD: Paris; HMSO: London, 1970. 70*s*; 46 francs; $10; 37.50 Sw. francs; 30.40 DM.

The problems caused by the excessive enrichment o waters by fertilizers from sewage, industry and agricultur are world-wide. It is at last admitted by almost everybod that even the largest lakes should not be accepted a limitless repositories for rubbish. The Organization fo Economic Cooperation and Development (OECD) has bee collecting and collating facts about the problem for som years. This report is a valuable account of the situation i certain large lakes in Europe and North America, and in the Oslo Fjord. The first part, in English and French, dis cusses the aims and achievements of the symposium research and management, and matters discussed durin the meeting, together with synopses of facts about th lakes which form the case studies described in Englis

in the second part of the report. The lakes are Mälaren, Sweden; Neagh, Northern Ireland; Maggiore, Italy (with more than half the drainage basin in Switzerland); Constance (Bodensee) in the Federal Republic of Germany, Switzerland and Austria and the Laurentian Great Lakes of America. There are also shorter sections on Lake Lugano, Italy, and lakes in the Netherlands, and a note on the proposed Morecambe Bay Barrage.

Though much remains to be done, the scope of the investigations now under way and of the proposals under review offer hope for the future. Sometimes there seems to be a confusion of ideas and the verbosity of some parts of the report does not help. The book contains many signs of hasty production. From the title it seems that it is a compilation, which may explain the lack of editorship. Though rapid publication was needed, the secretariat of OECD should have done better in 18 months, especially with the help of the preprints it had before the symposium started. There are too many careless slips. Tables and figures may or may not be numbered and may or may not have captions. Photographs of lakes, some of which are very poor, appear once or twice without any indication of what parts of them and their surroundings are shown. In the text of the article on Lough Neagh references are given to published papers and unpublished documents in the usual way, but there is no bibliography at the end of the article.

J. W. G. Lund

WATER MEASUREMENT

History of Hydrology

By Asit K. Biswas. Pp. xii+336. (North-Holland: Amsterdam and London, 1970.) 117*s*; Hfl. 50; $14.

It is surprising that, for a science so basic as hydrology, this is the first book dealing solely with its development. Not that attention to the subject has been lacking, for there are quite a number of papers dealing with certain periods of progress and several books that attempt to cover the history of hydrology in a chapter or two. Now much of this material, and more unearthed by the author himself, has been brought together in a single volume. Here theories about the origins of springs and rivers can be traced from the age of Aristotle to the seventeenth century when Perrault, Mariotte and Halley scotched the concept of a subterranean cycle and, by experiment, confirmed the correctness of the pluvial theory. Ideas about the assessment of flow in open channels can be followed from the Romans' assumption that cross-sectional area of channel was the only control of discharge, to the last century when Manning devised his formula. Something can be learned of the development of instruments for determining rainfall and evaporation, and about the foundations of hydrogeology which are covered through mention of the works of Darcy, Dupuit and Forchheimer.

The book is well produced, written in an easy style and is adequately illustrated. More space could have been devoted to the period during the last century when the first country-wide instrument networks were being installed, and the role of the meteorologist in this development might have been mentioned. On the other hand, it is difficult to find errors of fact and omissions of references. One that could have been brought in was the answer by Barrington[1] to Heberden's (not Herberden) rainfall results from Westminster Abbey. Then there are some minor points like the Bishop of Llandaff becoming Llandoff, and hydrologic replacing hydrological, for which the author can be forgiven. But one thing that he should be aware of is the irritatingly short chapters that appear almost as inserts here and there and the rather arbitrary way that the chapters divide up rather than bring together the material he is presenting. Nevertheless, Dr Biswas should be congratulated for producing a volume of interest to all scientists concerned with water. He should also be congratulated in producing it since 1964—others might have spent a lifetime over the same task and achieved less.

John C. Rodda

[1] Barrington, D., *Phil. Trans. Roy. Soc.*, **59**, 359 (1769).

SUPERNOVAE BEFORE PULSARS

Supernovae and their Remnants

Edited by Peter J. Brancazio and A. G. W. Cameron. (Proceedings of the Conference on Supernovae, held at the Goddard Institute for Space Studies, NASA, 1967.) Pp. viii+240. (Gordon and Breach: New York and London, January 1970.) $21; 175*s*.

This book reports a conference which took place on November 2–3, 1967. In one respect this was a very unlucky piece of timing, just before the discovery of pulsars which are now widely accepted as supernovae remnants; but from another point of view this report is singularly important as a landmark in astronomy, neatly packaging the pre-pulsar knowledge of supernovae, and offering a chance of comparison between the theories held at the end of 1967 and the observations of new phenomena from pulsars which have been flowing in ever since.

The surprise which the discovery of pulsars caused is brought back to mind by A. G. W. Cameron's paper on the expected properties of neutron stars in which he concludes that "a neutron star will become a dead object rather rapidly", with the corollary that they would probably be undetectable.

The Crab Nebula is, naturally, the subject of a large portion of this volume, but there is a thorough discussion of other remnants in our galaxy and of supernovae seen in other galaxies, as might be expected from contributors such as Zwicky and Minkowski. The papers on theoretical models of supernovae explosions remain largely unaffected by recent observations, and still show some wide discrepancies between theories and observations. But the volume is of a generally high standard; all of the contributors are well known in their field, and the editorial work seems to have been unusually painstaking and accurate, many of the papers having been updated to some extent during the preparation of the book.

Although the gap between conferences and the publication of their proceedings seems to be increasing (and some proceedings would be better left unpublished), the unique position of this volume in the context of the revolutionary impact made on astronomy by pulsars ensures its historical importance, and its excellent presentation will make it widely used as a source guide of basic importance to both theoretical and observational astronomers.

John Gribbin

SPINS AND SOURCERY

Particles, Sources and Fields

By Julian Schwinger. Pp. iv+425. (Addison-Wesley: Reading, Mass., and London, September 1970.) 140*s*.

Professor Schwinger is well known, not only as the man whose Nobel prize-winning calculation of the anomalous magnetic moment of the electron is one of the great triumphs of relativistic quantum mechanics, but also as a lecturer of great flow and persuasiveness. His book reproduces on the printed page those carefully constructed sentences long familiar in the lecture hall, and the audience might be said to be represented by the odd character, Harold, who pops up from time to time to ask a conveniently leading question.

The eloquence is directed towards presenting an approach to relativistic quantum mechanics (elsewhere called by its author "sourcery"), which he believes is more satisfactory than either operator field theory on the one hand or analytic S-matrix theory on the other. It is better than field theory because it operates at the phenomenological level and does not pretend to know what the fundamental field structures are. Its superiority to S-matrix theory lies, it is claimed, in its taking space-time structure seriously with its notion of localized sources. The motto of the book is explicitly given as "If you can't join 'em, beat 'em". Nevertheless one cannot help feeling that in the end Schwinger has, in his own special way, joined 'em. The source picture is very similar to aspects of Feynman's space-time approach to quantum mechanics and it would require the arts of a mediaeval disputant to split the difference between a scattering skeleton and a Feynman diagram. All sensible relativistic quantum mechanics, whether field theoretic or S-matrix, keep this diagrammatic picture in view as a guide to their thinking, without believing that it is in literal detail the fundamental physical theory. There is a good deal more common ground than Schwinger suggests.

The book may be judged not only as an exposition of Schwinger's philosophical point of view but also as an introduction to relativistic quantum mechanics. In its favour from this point of view are the detail and clarity of the exposition. Less satisfactory is the insistence on the sourcery phraseology and the total lack of references. The book will be a useful source of information on higher spin formalisms and on gravitons. But (and here I fulfil a prediction by Harold that reviewers would complain of things left out) it is a pity that no hint of the power of dispersion theoretic methods is given. The complex plane has been a remarkable discovery (see p. 36).

J. C. POLKINGHORNE

COMPLEX FUNCTIONS

A First Course on Complex Functions

By G. J. O. Jameson. (Chapman and Hall Mathematics Series.) Pp. xii + 148. (Chapman and Hall: London, September 1970.) 30*s*.

THE author of this book has aimed at producing a thoroughly rigorous introduction to complex function theory, suitable therefore for undergraduates interested in mathematical analysis for its own sake rather than for its applications. Formally, the subject is attractive and easy, the difficulties chiefly arising from topological problems concerning the nature of the domains to which Cauchy's theorem applies. To turn these difficulties the theorem is proved for a triangle and extended to a star-shaped open set; this suffices for many purposes. To keep the book small, more advanced topics which are not immediate consequences of Cauchy's theorem, such as analytic continuation, Riemann surfaces, and the general mapping theorem, are excluded.

There are three sections. Basic theory comprises: the complex number field, making use of the concepts of a metric space; power series and the exponential function; and the definition of integration. Differentiable functions have a section which includes Cauchy's theorem, the residue theorem and the Taylor and Laurent series. The final section is chiefly concerned with the calculus of residues and particularly with the evaluation of real integrals by contour integration.

The undergraduate coming to this text may well have had some earlier heuristic and geometrical acquaintance with the complex variable. The bracing discipline of this lucid if austere account will, if he is to be a serious student of analysis, set him firmly on his road.

T. A. A. BROADBENT

Short Notices

Innocent Killers. By Hugo Van Lawick-Goodall and Jane Van Lawick-Goodall. Pp. 222 + 33 plates. (Collins: London, October 1970.) 45*s*.

THE Van Lawick-Goodalls have taken the unusual step of publishing original scientific research in the form of a book written specifically for the general public. It is unfortunate that this laudable attempt to bridge the gap between popular and scientific writing is not altogether successful. Lack of quantitative data and anthropomorphisms such as descriptions of animals as "resentful" will make the scientific reader uncomfortable. The standard of writing is patchy. Although the chapters on wild dogs and jackals, which contain straightforward accounts of observations of the behaviour of these animals, are very readable, the initial "background" chapter fails to be so. Nevertheless, in spite of these drawbacks, this book, with its exciting photographs, contains much of great interest about the lives and behaviour of some little-known animals.

Man's Ancestors: An Introduction to Primate and Human Evolution. By Ian Tattersall. (Introductory Studies in Biology.) Pp. 64. (Murray: London, October 1970.) 20*s* paper.

PALAEOANTHROPOLOGY may not be everybody's idea of a fast moving subject. Nonetheless, new discoveries of fossils and developments in dating techniques are leading to continual reappraisals of the steps of man's evolution, and up to date summaries of current thinking from time to time certainly help to link the new finds together. This new book, by Ian Tattersall, is a good example of the review required and students, in particular, should find it useful. Its one disadvantage is that the author seems a little unsure of his potential readership. He assumes only slight knowledge of animal classification, evolutionary processes and the geological timescale and therefore has introductory chapters on these aspects. On the other hand, without a glossary of terms or a labelled drawing of a human or primate skeleton or of a skull, he expects his readers to be acquainted with some quite complicated anatomical terminology. The large size of the book (29·5 × 21·5 cm) may make it awkward for the bookshelf, but it does allow for a more adventurous layout than is usual and photographs (of which there are plenty) and line drawings are shown to good advantage.

Carbon Fibres. Edited by Eric A. Smith. (Design Engineering Series.) Pp. 79. (Morgan-Grampian: West Wickham, Kent, 1970.) 55*s*.

IN any rapidly developing technological field there is often an acute lack of any overall state-of-the-art publication suitable both for the professional and the interested layman. This book usefully combines the two needs and covers many aspects of the production and use of carbon fibres and fibre reinforced materials. Each of the eleven chapters is written by authorities on carbon fibres drawn from industry, the world of engineering journalism, and the government departments and research establishments which have been connected with carbon fibre development. The editorial introduction points out that there is an inevitable overlap between chapters, but this is on the whole desirable because the reader is reminded from time to time of the important basic facts and figures. The appearance of rather unfamiliar abbreviations could, however, make it rather difficult to dip randomly into the book if one is a complete stranger to the subject. A useful bibliography is provided, together with a list of principal British manufacturers and suppliers of both carbon fibres and composites.

Correspondence

ESRO X-ray Astronomy Satellite

SIR,—I would like to comment on the proposal by the ESRO Mission Definition Group for a Lunar Occultation Satellite for high resolution studies of cosmic X-ray sources (*Nature*, **228**, 756; 1970). The authors imply that in order to determine positions of X-ray sources to arc seconds it is necessary to achieve inertial stability of the spacecraft to arc seconds. However, this is not the case; in fact, the positional accuracy achieved is typically a small fraction of the vehicle stability. As an example, for Sco X–1 an arc minute position was obtained from a sounding rocket whose pointing accuracy and stability were measured in degrees.

Formally the requirement to achieve high angular resolution is that one be able to reconstruct the inertial attitude of the vehicle on the same angular scale as desired for the X-ray observations. As an example, on NASA's HEAO, which will be rotating at a rate of 30 arc minutes per second, we expect to be able to determine instantaneous orientation to several arc seconds and locate X-ray sources to the same precision (*Nature*, **228**, 603; 1970).

There is a second requirement which has to do with the effective observing time per source; namely, that the vehicle pointing accuracy be sufficient so that the source can be acquired within the field of view of the X-ray instrument. This is readily achieved. Modulation collimators can be made to have fields of view of many degrees, and even focusing X-ray telescopes, unless they are designed to function at energies in excess of several keV, can have fields of view of a degree or more.

The techniques for accomplishing high angular resolution X-ray measurements are neither expensive nor complex, the principal requirements being large apertures and time resolution. The same considerations apply to high spectral resolution measurements as well.

Yours faithfully,

HERBERT GURSKY

Director, Space Research Division,
American Science and Engineering,
11 Carleton Street,
Cambridge, Massachusetts 02142.

Nocturnal Songbird Migration

SIR,—In their recent communication (*Nature*, **228**, 476; 1970) Gauthreaux and Able have reopened the question of whether passerine nocturnal migrants compensate for wind drift. They have shown that, in the southern United States, on a selection of seven nights in spring and five in autumn, birds flew almost exactly down-wind. Furthermore, on two nights when wind direction changed by about 70°, the migrants' flight directions also changed, so that they still flew down-wind. They propose that radar evidence[1] (including my own), which disagrees with the hypothesis that birds are drifted from a constant heading, refers not to songbirds but to shorebirds and/or waterfowl.

In England, there is no question of confusion between the radar echoes from small passerine migrants and those from waders and waterfowl. The former fly slowly (about 20 knots) and depart some 40 minutes after dark from the whole land-mass; the latter fly much faster (at least 30 knots) and depart 1–2 h before dark from discrete localities, usually on the sea-coast. If our interpretation of the radar evidence is incorrect, it is not because we have misidentified the major groups of bird species concerned.

Regrettably, Gauthreaux and Able's presentation does nothing to resolve the question of whether birds fly on chosen tracks or chosen headings. In the situation they have described—that of down-wind flight—these possibilities cannot be distinguished. Furthermore, it would have been useful to know what their migrants did on nights other than the twelve they selected. From my own data, it would also be quite easy to select twelve nights on which the wind coincided in directions with the migrants' tracks; but on many other nights the migrants' tracks followed similar directions even though the wind was partly cross or opposed to these. It was, in part, on this evidence that I concluded that migrants were not drifted from a fixed heading.

Finally, I remain to be convinced that the two instances of change in mean migration direction during the night with change in wind direction disprove the hypothesis that passerine migrants usually compensate for wind drift. Firstly, different birds are involved at different times of night. Might they not be different species with different preferred tracks? Secondly, we are not told exactly where the observations were made. If the spring observations refer to Lake Charles, Louisiana, then birds departing from the coast of the Gulf of Mexico at dusk and flying NE (some passing the observation point at 02.40 GMT) would all have passed well before the second set of observations were made at 06.20 GMT; so it may not be surprising that a different mean flight direction was seen at the later hour as it could have involved a very different group of migrants.

Yours faithfully,

P. R. EVANS

Department of Zoology,
University of Durham,
South Road,
Durham.

[1] Nisbet, I. C. T., and Drury, W. H., *Bird Banding*, **38**, 173 (1967).
[2] Bellrose, F. C., *Proc. Fourteenth Intern. Ornithol. Cong.*, 281 (1967).
[3] Evans, P. R., *J. Zool.*, **150**, 319 (1966).
[4] Lack, D., *Ibis*, **111**, 253 (1969).

Biochemical Theology[1]

SIR,—

I have isolated from murine
Urine,
Collected from the Church or House
Mouse,
A substance which induces, by injection,
Reflexion,
Verbalization
And prognostication
(Sagacious
And, of course, efficacious).
Is this what makes Dr Crick
Tick?
If not,
What?

Yours faithfully,

A. G. OGSTON

Trinity College,
Oxford.

[1] Crick, F., *Nature*, **228**, 613 (1970).

Obituary



James Fisher

JAMES FISHER, the ornithologist whose outstanding achievements as a writer, broadcaster and publicist did so much to raise the science of ornithology to its present status and popularity, and who was a prodigious worker for wild life conservation, died in a car accident on September 25, 1970. He was fifty-eight.

He was the son of a well known headmaster of Oundle School, Dr Kenneth Fisher, a keen ornithologist himself, and was educated as a King's Scholar at Eton and at Magdalen College, Oxford, where he graduated with first class honours in zoology. From 1936 to 1939 he held a notable post as assistant curator with the Zoological Society of London under his former teacher Julian Huxley. Throughout his career he was attracted to numerical data and maps and this, together with his interest in seabirds, led him on several expeditions to study the distributions of the Atlantic and Arctic seabirds. He led a team to assess the population of the Atlantic gannet and made a major study of the spectacular increase and spread of the fulmar from a sole colony on St Kilda to an extensive breeding range round the British coasts. His investigations took him repeatedly to remote islands and it was typical that he should have contrived to be lowered by helicopter on Rockall when he accompanied a naval party taking formal possession of the island for the British Crown.

It was the example he set by these studies and his first book *Birds as Animals* (published in 1939) that changed the ways of many bird enthusiasts and helped to make field ornithology the scientific discipline that it is today.

During the war his work took on national economic importance. In particular, his survey of the rook was valuable to the agricultural industry. Afterwards he turned to publishing and was associated with the production of the 'New Naturalist' publications, an outstanding series of works in natural history. He himself contributed *The Fulmar* (1952). He also became a publisher in his own right and was an advisor to and director of various publishing houses.

Of the many offices that he held in the field of nature conservation, he was at one time vice-chairman of the Royal Society for the Protection of Birds; he was honorary secretary of the British Trust for Ornithology during its formative period; and at the time of his death he was deputy chairman of the Countryside Commission and a member of the Survival Service Commission of the International Union for the Conservation of Nature.

Physically, James Fisher was a large man; he was a fine oarsman in his youth, winning the Silver Sculls in 1934. He was also a man of generous nature, never sparing himself either in his own pursuits or in helping others. His failing was a tendency to undertake more than anyone could achieve. He leaves his wife Margery, whom he married in 1936, their three sons and three daughters.

Announcements

University News

Mr M. J. Hamlin has been appointed to the newly established chair of water engineering in the **University of Birmingham.**

Sir F. Elwyn Jones has been elected president of **University College, Cardiff,** and **Professor L. A. Moritz** has been appointed vice-president (administration).

Mr Charles N. Myers, formerly executive associate with Education and World Affairs, has been appointed executive director of the Center for Studies in Education and Development, **Harvard University,** in succession to **Mr William R. Charleson.**

Dr F. D. Gunstone and **Dr. D. H. N. Spence** have been appointed to personal chairs in chemistry and botany, respectively, in the **University of St Andrews.**

Dr A. L. Walpole, a member of ICI Limited (Pharmaceutical Division), has been appointed visiting professor in the Department of Biochemistry, **University of Strathclyde.**

Miscellaneous

Dr D. Küchemann, head of the Aerodynamics Department, Royal Aircraft Establishment, has been awarded the **Ludwig-Prandtl-Ring** by the **Deutsche Gesellschaft für Luft-und-Raumfahrt.**

Dr J. H. Pearn, University of Queensland, has been awarded a **Florey fellowship,** to study the genetic aspects of some inherited children's diseases and to work on the fundamental pathophysiology of muscular diseases in children.

A **European Branch** of the **Environmental Mutagen Society** was founded earlier this year at Neuherberg and a council formed of representatives from various countries. Further information can be obtained from the secretary, Professor E. M. Cohen, Medical Biological Laboratory TNO, PO Box 45, Rijswijk 2100, The Netherlands.

Academician A. N. Tupolev, Sir Robert Cockburn and **Dr C. S. Draper** have been awarded honorary fellowship of the **Royal Aeronautical Society.** The British gold medal for aeronautics for 1970 has been

awarded to **Mr P. A. Hufton**, Royal Aircraft Establishment, and the British silver medal to **Mr M. J. Brennan**, Hawker Siddeley Aviation. **Mr Walter Tye**, Air Registration Board, has received the gold medal of the Royal Aeronautical Society, **Mr E. E. Marshall**, BAC, has received the Society's silver medal and **Mr N. F. Harpur**, BAC, the bronze medal.

Applications are invited from workers actively engaged on cancer research within a university department or research institute for travel fellowships to be awarded by the **International Agency for Research on Cancer** during 1971–72. The fellowships are intended to promote collaboration and consultation between institutions and are available for a maximum of three months, which may be spent at one or several different places. Further information can be obtained from the chief of the Research Training and Liaison Unit, International Agency for Research on Cancer, 16 avenue Maréchal-Foch, 69 Lyon 6e), France.

International Meetings

April 5–8, 1971, **Atomic and Molecular Physics,** York (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

April 14–16, 1971, **Electrical Conduction in Organic Solids,** Nottingham (Dr M. R. Willis, Department of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD).

April 26–28, 1971, **Frequency Control,** Atlantic City (J. M. Stanley, Solid State and Frequency Control Division, US Army Electronics Command, Fort Monmouth, New Jersey 07703, USA).

May 3–5, 1971, **Bioengineering,** Fort Collins, Colorado (Rocky Mountain Bioengineering Symposium Inc., PO Box 59, USAF Academy, Colorado 80840, USA).

June 3–4, 1971, **Molecular and Cellular Repair Processes**, Baltimore (Dr C. J. O'Donovan, Vice-President for Scientific Affairs, Miles Laboratories Inc., Elkhart, Indiana 46514, USA).

July 25–31, 1971, **Pure and Applied Chemistry Congress,** Boston (A. T. Winstead, c/o American Chemical Society, 1155 Sixteenth Street NW, Washington DC 20036, USA).

July 26–31, 1971, **Physics of Electronic and Atomic Collisions,** Amsterdam (Louise Roos, FOM-Instituut voor Atoom- en Molecuulfysica, Kruislaan 407, Amsterdam-Watergraafsmeer, The Netherlands).

August 1–6, 1971, **Immunology,** Washington (Secretariat, First International Congress of Immunology, 9650 Rockville Pike, Bethesda, Maryland 20014, USA).

August 2–4, 1971, **Olfaction and Taste,** Munich (Dr D. Schneider, Max-Planck-Institut für Verhaltensphysiologie, 8131 Seewiesen, Germany).

August 2–7, 1971, **Stochastic Point Processes,** New York (Dr P. A. W. Lewis, Mathematical Sciences Department, IBM Research Center, PO Box 218, Yorktown Heights, NY 10598, USA).

August 2–6, 1971, **Regulation of Food and Water Intake,** Cambridge (Dr J. T. Fitzsimons, Physiological Laboratory, Downing Street, Cambridge CB2 3EG).

August 16–19, 1971, **Spectroscopy,** Clayton (Dr J. E. Kent, Department of Chemistry, Monash University, Clayton, Victoria 3168, Australia).

August 17–19, 1971, **High Frequency Generation and Amplification—Devices and Applications,** Ithaca (Professor Lester Eastman, Cornell School of Electrical Engineering, Phillips Hall, Ithaca, New York 14850, USA).

British Diary

Monday, December 14

Education and the Heavy Electrical Industry (6 p.m.) Professor G. H. Rawcliffe, Institution of Electrical Engineers, at the Queen's Building, The University, Bristol.

Electrical Research on British Rail (7 p.m.) Mr L. L. Alston, Institution of Electrical Engineers, at the College of Further Education, Crewe.

Materials for Piles (5.30 p.m., informal discussion) Institution of Civil Engineers, at Great George Street, London SW1.

Optical Studies of Adsorbed Layers at Interfaces (two-day symposium) Faraday Society, at the Royal Institution, 21 Albemarle Street, London W1.

Railway Breakdown Services (5.30 p.m.) Mr K. R. M. Cameron, Institution of Mechanical Engineers, Railway Division, at 1 Birdcage Walk, London SW1.

Tuesday, December 15

Application of Linear Induction Motors to High Speed Transport Systems (6 p.m.) Professor J. M. Barwell, Institution of Electrical Engineers, at the Carlton Hotel, Edinburgh.

Chocolate Fats (6.15 p.m.) Dr G. R. Howat, Society of Chemical Industry, Food Group, jointly with the Oils and Fats Group, at 14 Belgrave Square, London SW1.

Drive and Control Systems for Large Satellite Tracking Antennae (6.15 p.m.) Mr B. R. Tuppen and Mr J. B. Owens, Institution of Electrical Engineers, jointly with the Institution of Mechanical Engineers, at Lanchester Polytechnic, Eastlands, Rugby.

Electrode Kinetics and Processes Involving Solid Electrolytes (one-day meeting) Society for Electrochemistry, Solid State Sub-Group, in the Metallurgy Department, Imperial College, London SW7.

Micro-circuits, Noise, Sequential Control and Mechanised Assembly (6.30 p.m.) Mr A. Pugh, Institution of Electrical Engineers, at the University, Nottingham.

Noise—Some Aspects on its Measurement and Control (2 p.m., symposium) Society of Environmental Engineers, in the Mechanical Engineering Department, the University, Birmingham.

Severnside of the Future (5.30 p.m.) Mr A. V. Hooker, Institution of Civil Engineers, at Great George Street, London SW1.

The Polar Ionosphere and the Magnetosphere (10 a.m., discussion meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

The Role of the Buying Activity and Buying Policy in the Process Engineering Industry (6 p.m., discussion) Institution of Mechanical Engineers, Process Engineering Group, at 1 Birdcage Walk, London SW1.

Thyristor Engineering (6.30 p.m.) Mr D. B. Corbyn, Institution of Electrical Engineers, at Hastings College of Further Education, St. Leonards on Sea, Sussex.

Wednesday, December 16

Designing War Ships for a Cost Effective Life (6 p.m.) Vice-Admiral R. G. Raper, Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1 (Charles Parsons Memorial Lecture).

Distribution (symposium, 2.30 p.m. to 8.30 p.m.) Institution of Electrical Engineers, at Lanchester Polytechnic, Eastlands, Rugby.

Modern Developments in Titanium (7.30 p.m.) Mr G. Willison, Oil and Colour Chemists' Association, at the Carlton Hotel, North Bridge, Edinburgh.

Telemetry (7.30 p.m.) Mr R. E. Young, Institution of Electrical Engineers, at Inverness Technical College, Inverness.

The Design of Amplitude-Phase Modulated Pulse Sequences for Radar (6 p.m.) Mr M. H. Ackroyd, Institution of Electronic and Radio Engineers, Aerospace, Maritime and Military Systems Group, at 9 Bedford Square, London WC1.

Thursday, December 17

Additives in Iron Ore Pelletizing, Dr D. F. Ball, Mr P. R. Dawson and Dr J. T. Fitton; **Estimating the Stability of Excavated Slopes in Opencast Mines,** Professor E. Hoek (5 p.m.) Institution of Mining and Metallurgy, at the Geological Society, Burlington House, Piccadilly, London W1.

511th Meeting (two days) Biochemical Society, jointly with the Association of Clinical Biochemists, at the Royal Postgraduate Medical School, Hammersmith Hospital, London W12.

Friday, December 18

164th Meeting (two days) Genetical Society, in association with the Population Genetics Group and the Fisher Memorial Committee, in the Botany Lecture Theatre, University College London, Gower Street, London WC1.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Education and Science. Student Numbers in Higher Education in England and Wales—a Projection, with some Discussion of Its Cost Implications. Pp. vii + 50. (London: HMSO, 1970.) 12*s* (60*p*) net. [2110

Royal Observatory Bulletins. No. 158: Proper Motions in the Field of the Open Cluster NGC 6939. By R. D. Cannon and A. G. Purcell. Pp. 69–81. (Herstmonceux: Royal Greenwich Observatory, 1970.). 5*s* net. [2110

This is Man's World, with David Attenborough, Liz Dyer, John Faulkner, David Goulder, Rick Jones, Sandra Kerr and Cyril Tawney. Stereo Record. Issued in conjunction with the World Wildlife Fund. (London: The Decca Record Company, Ltd., Argo Division, 1970.) [2210

Farming and Wildlife: a Study in Compromise. Edited by Derek Barber. Pp. 93. (Sandy, Bedfordshire: Royal Society for the Protection of Birds, The Lodge, 1970.) [2210

Building Research Station Digest, No. 122: Security. Pp. 8. (London: HMSO, 1970.) 9*d* (4*p*). [2310

Flora Zambesiaca: Mozambique, Malawi, Zambia, Rhodesia, Botswana. Pteridophyta. By E. A. C. L. E. Schelpe. Edited by A. W. Exell and E. Launert. Pp. 254. (London: Crown Agents for Oversea Governments and Administrations, 1970.) 35*s*. [2310

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 259, No. 832 (22 October, 1970): Studies on Pathogenic and Non-Pathogenic Small Free-Living Amoeba and the Bearing of Nuclear Division on the Classification of the Order Amoebida. By B. N. Singh and S. R. Das. Pp. 435–476+Plates 28–30. (London: The Royal Society, 1970.) 27*s*; $3.50. [2310

Proceedings of the Conference on Veterinary Education organized by The Royal College of Veterinary Surgeons. Pp. 150. (London: Royal College of Veterinary Surgeons, 1970.) [2310

Kelvin's Instruments and the Kelvin Museum. By George Green and John T. Lloyd. Pp. 68+19 plates. (Glasgow: University of Glasgow, 1970.) 20*s*. [2610

Agricultural Research Council. Third Report of the Research Committee on Toxic Chemicals. Pp. vi+69. (London: Agricultural Research Council, 1970. Obtainable from HMSO.) 5*s* (25*p*). [2710

Report by the Post Office Users' National Council on the Post Office's Proposed Increased Postal Tariffs. Pp. 35. (London: Post Office Users' National Council, 1970.) [2710

Report of the Gastroenteritis Survey, Dublin City, 1964–1966. Pp. 97. (Dublin: Medical Research Council of Ireland, 1970.) [2710

The Wood We Grow. (Report of the Tenth Discussion Meeting of the Society of Foresters of Great Britain, Edinburgh, 20–22 March, 1970.) Pp. 96. (Supplement to *Forestry*.) (London: Oxford University Press, 1970.) 20*s*. [2810

PEP Broadsheet 521: Women's Careers—Experience from East and West Germany. By Dr Ingrid Sommerkorn with R. Nave-Herz and Miss Christine Kulke. Pp. vi+132. (London: Political and Economic Planning, 1970.) 24*s*. [2810

Department of Education and Science. Circular 17/70: Grants to Recognized Students Attending Approved Courses of Teacher Training. Pp. 30. (London: HMSO, 1970.) 4*s* (20*p*) net. [2910

The Zoological Record. 1967, Vol. 104, Section 3: Porifera together with Archaeocyatha. Compiled by S. Ware. Pp. 20. 12*s* 6*d*. 1968, Vol. 105, Section 1: Comprehensive Zoology. Compiled by S. Markowski. Pp. 27. 15*s*. (London: The Zoological Society of London, 1970.) [2910

Microscope on Performance. By A. Desmond Burridge. Pp. 40. (London: De Beers Industrial Diamond Division, 7 Rolls Buildings, Fetter Lane, 1970.) *Gratis*. [3010

Medical Research Council. Monitoring Report No. 17: Assay of Strontium-90 in Human Bone in the United Kingdom—Results for 1968. Pp. iii+26. (London: HMSO, 1970.) 3*s* 6*d* (17.5*p*) net. [3010

Department of Education and Science; Scottish Education Department; Northern Ireland Ministry of Education; University Grants Committee. Education Statistics for the United Kingdom 1968. Pp. xxvii+57. (London: HMSO, 1970.) 30*s* net. [3010

Other Countries

India. Annual Report of the Central Building Research Institute for 1969. Pp. vii+80+16 plates. (Roorkee, U.P.: Central Building Research Institute, 1970.) [2010

Smithsonian Contributions to Zoology, No. 55: Atlantic Deep-Sea Calanoid Copepoda. By Ellsworth H. Wheeler, Jr. Pp. 31. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) $0.45. [2010

National Hydatids Council. Tenth Annual Report and Statement of Accounts, year ended 31 March 1970. Pp. 24. (Wellington, NZ: National Hydatids Council, 101 Willis Street, 1970.) [2110

Annals of the New York Academy of Sciences. Vol. 170, Article 2: International Conference on Bioelectrical Impedance. By S. E. Markovich and 68 other authors. Pp. 407–836. (New York: New York Academy of Sciences, 1970.) $26.50. [2110

Smithsonian Contributions to Zoology, No. 64: Zoracallida, New Order, and *Doraster constellatus*, New Genus and Species, with Notes on the Zoroasteridae (Echinodermata : Asteroidea). By Maureen E. Downey. Pp. 18. (Washington, DC, Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) $0.30. [2110

US Department of the Interior: Geological Survey. Bulletin 1312-A Portable Refraction Seismograph Survey of Gold Placer Areas near Nor Alaska. By H. Gary Greene. Pp. iv+29. $0.60. Water-Supply Pa 1923: Surface Water Supply of the United States 1961–65. Part 8: Weste Gulf of Mexico Basins. Vol. 2: Basins from Lavaca River to Rio Gran Pp. ix+786. $4. (Washington, DC: Government Printing Offi 1970.) [21

India: Council of Scientific and Industrial Research. Index to Literat on Science of Science, Vol. 6, Nos. 5 and 6, May and June 1970. Pp. (New Delhi: Research Survey and Planning Division, CSIR, 1970.) [22

Science Council of Canada. Special Study No. 10: Agricultural Science Canada. (Background Study for the Science Council of Canada.) Pp. 1 (Ottawa: Queen's Printer, 1970.) $2. [22

US Department of the Interior: Geological Survey. Water-Supply Pa 1924: Surface Water Supply of the United States 1961–65. Part 9: Colora River Basin. Vol. 1: Colorado River Basin Above Green River. Pp. vii 488. (Washington, DC: Government Printing Office, 1970.) [22

National Museum of Canada—National Museum of Natural Scienc Publications in Biological Oceanography, No. 1: Studies on the Caprellid (Crustacea, Amphipoda) of the American North Pacific. By Diana Laubitz. Pp. vii+89. $2.25. Publications in Palaeontology, No. Tyrannosaurs from the Late Cretaceous of Western Canada. By Dale Russell. Pp. viii+34 (4 plates). No. 2: A Postglacial Muskox (*Ovibos Moschatu* from Grandview, Manitoba, and Comments on the Zoogeography of *Ovibo* By C. R. Harington. Pp. vi+13. Publications in Zoology, No. 4: Fauna Sable Island and its Zoogeographic Affinities. By H. F. Howden, J. E. Martin, E. L. Bousfield and D. E. McAllister. Pp. 45. (Ottawa: Queen Printer, 1970.) [22

Australia: Commonwealth Scientific and Industrial Research Organiz tion. Research Report of the Division of Mineral Chemistry, 1969/197 Pp. 20. (Port Melbourne: CSIRO, 1970.) [22

Canada: Department of Energy, Mines and Resources. Geologic Survey of Canada. Paper 69-2A: Age Determinations and Geologic Studies. K-Ar Isotopic Ages, Report 9. By R. K. Wanless, R. D. Steven G. R. Lachance and R. N. Delabio. Pp. vi+78. $1.50. Paper 70–11: Rapid Determination of Total Organic and Inorganic Carbon in Shales an Carbonates. A Rapid Determination of Total Sulphur in Rocks and Mineral By A. E. Foscolos and R. R. Barefoot. Pp. v+14. $1.50. Paper 70–4 Preliminary Report on the Copper Deposits, Coppermine River Area, Distri of Mackenzie. By E. D. Kindle. Pp. v+13. $0.75. (Ottawa: Queen Printer, 1970.) [22

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Smithsonian Contributions to the Earth Sciences, No. 3: Minor and Trac Elements in Meteoritic Minerals. By Brian Mason and A. L. Graham. Pp 17. $0.30. Smithsonian Contributions to Paleobiology, No. 5: Catalogu of the Illustrated Paleozoic Plant Specimens in the National Museum c Natural History. By Arthur D. Watt. Pp. 53. $0.60. Smithsonian Con tributions to Zoology, No. 45: Bredin-Archibold-Smithsonian Biologica Survey of Dominica—The Crane Flies (Diptera : Tipulidae). By Charles P Alexander. Pp. 59. $0.60. No. 67: A Partial Revision of the Orient Isomyia of the Viridaurea Group (Diptera : Calliphoridae). By Maurice T James. Pp. 14. $0.25. (Washington, DC: Smithsonian Institution Press 1970. For sale by US Government Printing Office.) [221

Commonwealth of Australia: Department of Supply. Australian Nationa Antarctic Research Expeditions. ANARE Scientific Reports. Series B (II Zoology. Publication No. 110: Some Aspects of the Ecology of a Calanoi Copepod, *Pseudoboeckella brevicaudata* Brady 1875, on a Sub-antarctic Island By A. J. Evans. Pp. 100. (Melbourne: Antarctic Division, Department o Supply, 1970.) [231

International Atomic Energy Agency, Vienna. Nuclear Techniques fo Studying Pesticide Residue Problems. (Proceedings of a Panel organize by the Joint FAO/IAEA Division of Atomic Energy in Food and Agricultur and held in Vienna, 16–20 December 1968.) Pp. 84. (Vienna: IAEA London: HMSO, 1970.) 78 schillings; 25*s*; $3. [231

Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin–Dahlem Bibliographie der Pflanzenschutz-Literatur/Bibliography of Plant Protec tion. Neue Folge, Band 4, Heft 1. Bearbeitet von Dr W. Laux, unte Mitarbeit von Dr W. Sicker und Dr D. Blumenbach. Pp. xxx+161. (Berlin-Dahlem: Biologische Bundesanstalt für Land- und Forstwirtschaft, 1970. 16 DM. [231

US Department of the Interior: Geological Survey. Professional Pape 411-I: Theory of Dispersion in a Granular Medium. By Akio Ogata Pp. iv+134. $0.45. Professional Paper 491-D: Evapotranspiration by Woody Phreatophytes in the Humboldt River Valley, Near Winnemucca, Nevada. By T. W. Robinson. Pp. iv+41. $0.55. Professional Pape 562-H: Flume Width and Water Depth Effects in Sediment-Transport Experiments. By Garnett P. Williams. Pp. iii+37. $0.55. (Washington, DC: Government Printing Office, 1970.) [2310

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Putting Down More Aircraft

THE first thing to say about the summary of the report of the Roskill Commission on the siting of the third London airport is that the Minister of Trade and Industry, Mr John Davies, has been sensible in persuading his officials to make the decision known before the report in full could be published and that, to judge from the small sample of its work now available, the Roskill Commission has been as thorough and lucid as its earlier publications have tended to suggest. The second thing to say is that the decision, to build an airport at Cublington, nearly 50 miles north-west of London, is probably wrong. Indeed, the summary of the report now made available suggests that the commission found it hard to decide between Cublington and the quite different site at Foulness, farther away from London to the east on the Thames estuary. One of its members, Professor Colin Buchanan, has actually chosen to dissent from what the majority of his colleagues recommended, and has plumped for Foulness as the most advantageous site. Most probably, the government will be hard pressed to know precisely what to make of the Roskill recommendation. Plainly there is at least a chance that the recommendation to build at an inland site will be discarded.

The commission says that there is no ideal site for a new London airport but that, of course, is a truism. In the siting of all kinds of public facilities, from supermarkets to sewage works, decisions must be compromises. Inevitably, airports bring noise to people living nearby, many of whom never leave the ground. Airports also bring traffic, from which local inhabitants suffer without profit. Without careful planning control, airports can also become foci for rapid industrial development, with the result that isolated parts of the country can be turned into eyesores. The list of complaints against airports is indeed so long and easily extended that it is tempting to wonder how they are ever built. The truth is, of course, that they are necessary as well as being potential nuisances. Civil aircraft are not merely for the jet set. Without them, commerce would be greatly hampered; without easy access to them, prosperity would be diminished. Judge Roskill and his team have made it plain that their choice must be an unpleasant compromise and they reject what might be called the Luddite view of modern airports—that they should be done without. That is a good beginning.

The commission seems also to have rejected the argument that its objective should be to devise a strategy for the development of an airports policy for the United Kingdom as a whole and, to the extent that this view is to be accepted in its broadest sense, the commission is right. Instead, it claims to have been guided alternately by the doctrine that the airport should be sited in the way that makes the best economic use of the investment which its construction will entail and the view that it should be put where it will be most helpful for the forward planning of national resources. All this is sensible enough, but in taking what may be called the narrow view, the commission has overlooked considerations such as the importance of siting airports in such a way that air traffic considered as a method of linking one place with another is most efficient. It has forgotten what it is fashionable to call the systems approach. If the summary now issued is not a travesty of the full report, there is no mention in the detailed discussion of where to put the airport, of the kinds of aircraft that will be using it and of the extent to which the journeys they are likely to be making will be affected. Instead, the commission seems to have fallen into the familiar trap of giving most attention to that part of the entire air journey which can be most closely analysed and which is of most immediate local concern—the journey from the airport to its parish. By doing so, it may have chosen a site but it has missed an opportunity.

The choice between Foulness and Cublington is Occam's Razor stuff. In the end, the Roskill Commission seems to have been frightened off by some of the drum-beating with which several development companies and hastily formed consortia threw themselves into the business of planning industrial developments on the north side of the Thames to surround and no doubt stifle the putative airport at Foulness. With justice, the commission is correct in rejecting the view of some capital developers (the Thames Estuary Development Company in particular) that there would be advantages in financing the whole operation with private capital—with a project of this size, no government could let its *rentiers* go bankrupt. But there are great benefits in any scheme that proposes to turn mud into dry land, and those who complain that Britain is already overcrowded must surely now be disappointed that several square miles of Buckinghamshire are to be covered with concrete if the Roskill Commission has its way. As well as such intangible benefits, however, Foulness is also admitted by the commission to be the least affected of any site at sea level by cloud, the least offensive to those with good planning at heart, the least likely to disturb dwelling-places and amenities, although it must, to be fair, be acknowledged that coastline of a kind would be consumed. The commission even says that an airport at Foulness would cause less indirect disturbance by requiring the construction of roads in inconvenient places, that it would fit in well with the pattern of minor airports around London

(while Cublington would put an end to flying from Luton), that it will cause less noise and that there is no technical barrier to its construction.

So why then settle for Cublington? The Roskill Commission says not merely that Foulness is comparatively inaccessible but that inaccessibility will breed disuse so that twenty years from now air passenger traffic through Foulness would be 12 million a year less than through one of the inland sites. It will be interesting to see, when the full report is published, how the commission arrives at this conclusion. It might have been thought that the usability of Foulness and therefore the traffic through it, like the usability of an airport elsewhere, would be determined by the ease of access. In the event the commission rejects the notion that there should be a high speed link by rail between London and Foulness on the grounds that a comparatively high fare could not be justified commercially, but this is yet another point on which only the full report will be able to throw light. Did the commission adequately discount the fact that an air traveller who has paid tens of hundreds of pounds for a ticket may be willing to stomach a few extra shillings for ground transport if the service is better? This is one crude representation of the systems approach. It is a pity that the Roskill Commission had not been indoctrinated by the cyberneticists. In the event it argues as if it has been living in the shadow of the economists—it claims that over the life of the airport, Foulness would consume national resources worth more than £100 million. This sum of money may be large compared with the cost of laying down a single runway, but in these sophisticated days everybody knows that this is not the critical parameter. What matters is the properly discounted and aggregated amount of money spent on construction, operation, maintenance and access. But this sum for any airport is likely to be merely peanuts compared with the equivalent economic value of operating aircraft likely to use the third London airport. In circumstances like these, the difference of £100 million which Judge Roskill has identified is not seriously to be counted an impediment to building at Foulness. Mr John Davies should feel quite free to say that the nuisance value of the noise that an airport built at Cublington would cause, when properly discounted and aggregated over thirty years, would be much greater than this figure. The hope then is that the Roskill report will be valued as a sensitive analysis of an important problem and that its recommendation will be politely discarded in favour of an airport in the Thames estuary with a fast rail link on the ground and hardly any noise.

In passing, the Roskill report has quite discredited the British Airports Authority, which is something to be grateful for. Over the years, Mr Peter Masefield and his colleagues have been representing that the existing airports would be choked solid with aluminium alloy and people unless work had begun on the third London airport a year ago. What the commission asks for is that the first runway of the third large London airport should be functioning in 1980 or soon afterwards. If Mr Masefield had been taken seriously, in his protests at the abandonment of the foolish site at Stansted, the United Kingdom would have ground to a halt long before then. Sensibly, Judge Roskill, who earns his living in the judicial system and not the management of airports, has nevertheless the acumen to see that there is plenty of scope for the further development of airports such as Luton and Gatwick, and for the imaginative exploitation of computer sequencing as well. The implication, then, is that there is plenty of time for careful study and reappraisal of the commission's work, and every opportunity for the present government or even its successor to agree with Professor Buchanan that Foulness is by far the best choice for a third airport. After all, nobody can tell just what the pattern of traffic within the United Kingdom will be in 1980, or whether the distribution of population will be radically different from what it is at present. The Roskill report, in other words, promises to become as much a conversation piece as a turning point in the history of air transport in Britain. It is therefore important to remember that it is also a monument to the efficacy with which common sense and public protest were able to overturn the previous government's reckless acceptance of its officials' devotion to the site at Stansted.

Chemists are Like Dodos

THE Royal Institute of Chemistry's committee under Professor Colin Eaborn (see page 1244) has produced one of the most profoundly conservative documents to have been published in the modern history of higher education. With touching devotion to the belief that chemistry is not so much a discipline as a virtue, the committee proclaims the advantages of the present system for training chemists at British universities, deplores the way in which students appear to be increasingly unwilling to exploit these advantages, bemoans the difficulties which beset chemistry graduates seeking jobs and then hopes—its recommendations are hardly better—that by some magic everything will come right. In much the same spirit, no doubt, the last of the now vanished quill pen manufacturers must have wrung their hands in bewilderment over the falling away in trade. Are not our quill pens as good or even better than ever? Is it not mere fickleness and even fecklessness that has driven the customers away? And will not everything be right again if we hang on (with government subsidies to help) waiting for other people to change? This, at least, is what the least adventurous among the quill makers would have said—their more farsighted colleagues would have been investing heavily in the manufacture of steel nibs. The danger now, in British chemistry, is that Professor

Eaborn's report will serve only to undermine the flickering resolution of those who may have considered that the time is right for change.

The object of the exercise was meant to be a study of the relationship between university chemistry and the needs of British industry. Fair play, Miss Elizabeth Paul, who was charged with making the survey of opinion among chemists, teachers and industrialists, has done a splendid job which throws a good deal of light on the sociology of industrial chemistry in Britain and which at the same time provides much evidence to contradict some of the committee's generalizations. The attitudes of chemistry students and recent graduates to their undergraduate education should have been a warning, for example. In the survey which Miss Paul has carried out, 72 per cent of undergraduates and 64 per cent of postgraduates considered that undergraduate courses required too much learning of factual material and 59 per cent of undergraduates and 57 per cent of postgraduates considered that ancillary courses should have been better linked to undergraduate chemistry. Most students expressed a need for more tutorials and seminars, more theory, less rote learning and less work. Miss Paul says that "the complaint most relevant to our inquiry relates to the breadth of the subject matter covered . . .". The same survey has revealed that most of those who graduate as chemists are dedicated to Professor Eaborn's monasteries before leaving school, thus confirming the common belief that chemistry departments at British universities are more exacting in their requirements of intending students than most other kinds of science departments. In circumstances like these, nobody will be surprised that "students, university staff and industrial employers and employees would welcome some broadening of university courses (in chemistry)". Indeed, the only puzzle is that the Eaborn committee should dismiss the case for a broader education without much concern for argument one way or the other.

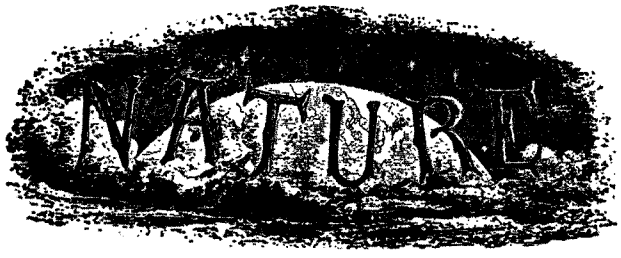
On the internal organization of chemistry courses, for example, the committee argues quite sensibly for a balance between basic and vocational training (and in particular between theoretical chemistry and practical chemistry), but then goes on to dismiss at least some of the complaints of those who consider that chemistry teaching should be more modern and lively with the cheap canard that "many of the students who do not do well in examinations are unlikely, on looking back, to express enthusiasm for their course". Is this belief compatible with the conviction among undergraduates and postgraduates alike that chemistry entails too much rote learning, for example? On the more general issues of whether undergraduate courses for chemists and other scientists at British universities should be leavened with other elements, the committee is at its most iconoclast, specifically taking issue with the Swann committee which two years ago put forward the belief that more general education would imply that graduates would be more adaptable to the needs of industry, chiefly on the grounds that the industrialists responding to questionnaires did not rank this high among their complaints. It may of course be true that "employers selecting chemists much prefer to recruit special rather than general degree graduates", but is it really in the best interests of the students that the opinions of potential employers on these questions, often based on misconceptions, should determine the character of the undergraduate curriculum? And is it right that the committee should respond to the general feeling among chemists and their employers that a capacity to write literate reports would be an asset by, first, woodenly proclaiming that "we cannot recommend that a formal course in report writing and the use of English should form part of all chemistry degree courses", in the process passing the buck downwards to the secondary schools where "basic command of correct English must be acquired" and upwards to induction training on the grounds that "it is at this stage that the student will recognise the importance of report writing to his career and so be influenced to give his mind to it"? The issue is not whether chemistry graduates should be taught to write after having been taught chemistry, but whether they should be educated in such a fashion as to be literate. On the similar complaint from industry that chemistry education should deal more explicitly with commercial applications of research and with techniques such as those of operational research, the committee takes the line that there is no time for luxuries like that, and that, in any case, undergraduates might be taught things not strictly relevant to their later life. In this spirit were those apprenticed to quill makers no doubt discouraged from worrying about metalworking.

The truth is, of course, that undergraduate education at British universities in chemistry is—with few exceptions—notoriously narrow and narrowing. The fact that the committee has discovered with understandable alarm that students drift away from chemistry after one or two years at those universities where they have the chance is one practical sign of trouble. Another is the way in which chemistry departments are hard pressed to fill their vacant places with suitable candidates. In circumstances like these, the committee's complacency about the present state of affairs is in the interests neither of chemistry nor of those students who are recruited to the cause. At the very least, the Eaborn committee should have considered in some detail the issue which it has studiously ignored that there should be a radical reconstruction of the pattern of undergraduate teaching in Britain, possibly with a period of more general education leading into one more concerned with specialized and vocational matters. But even within its chosen terms of reference, the committee should have paid serious attention to the problem of how to impart vitality and relevance to the teaching of chemistry at British universities.

Because of its internal defects, the committee's report is less convincing than it might have been in its largely sensible account of how relationships between chemistry departments and industry might be more efficiently regulated. One conclusion that deserves some publicity is, however, that the belief among

students of chemistry that industry is less likely to provide challenging opportunities for advancement than popular legend would suggest is exaggerated. To be sure, those already at work find their will to contribute to the commercial success of their enterprises frustrated, but it is not entirely clear whether these complaints arise from the unfamiliarity of work to those recently accustomed to academic life or from defects in the way in which industry itself is organized. In reality, the committee recognizes, the chemical industry is unusual among British industry in its willingness to support teaching programmes at universities, schemes for the encouragement of research and even relationships between undergraduates and potential employers. Whether it is sensible that there should be a standing committee of chemistry professors and representatives of the Chemical Industry's Association is another matter—this sounds too much like too much bureaucracy. But there is something to be said for some means by which university chemistry departments and the potential users of their product could work out the reform of chemistry teaching at British universities which the Eaborn committee has avoided.

100 Years Ago



NATURAL SCIENCE AT OXFORD

THE progress which Natural Science has made at Oxford within the last few years has far exceeded the anticipations of even the most sanguine of its promoters. It is but ten years ago that the New Museum was opened, and not much longer since the School of Natural Science was founded. Since then, year by year, the interest shown in these studies has steadily augmented, the number of undergraduates attending the University College Science Lectures has augmented in proportion as the number of these lectures has increased, and the School of Natural Science has become recognised as on a par with the other three great schools of Philosophy, Mathematics, and Law and Modern History. This has been chiefly brought about by the high standard of excellence required by the examiners in this school. When the position taken by Natural Science at a university which has commonly been condemned for neglecting this very subject, is fully recognised outside its own walls, there can be no doubt but that a far greater number than at present will come up to Oxford to pursue their science studies there. Hence it may not be here out of place to give as briefly as possible a short *résumé* of the opportunities held out to Natural Science students at Oxford, in the way of university and college lectures and the various scientific museums and libraries, as well as to notice the numerous rewards and honours which are open to all such students. To do this completely would far exceed the limits of this article, so that what follows must only be taken as a sort of index, as accurate as possible, to a subject, the details of which can be obtained by writing to the tutors of the various colleges mentioned.

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RESEARCH POLICY

Another Fresh Look

THE Council for Scientific Policy has embarked on a study of the pattern of expenditure in civil research, supported by the Department of Education and Science, and of the arrangements which at present exist for carrying out this work. For this purpose, the council has set up a working group under its chairman, Professor F. S. Dainton. The group is to examine the structure of the research councils and the relationship between them as well as the functions of the University Grants Committee in relation to research and the work of other public bodies and government departments with interests in civilian research and development.

The working group has been set up by the CSP on its own initiative and will provide advice to the Secretary of State for Education and Science, Mrs Margaret Thatcher. Typical of, although not prominent among, the questions to be considered is whether the Agricultural Research Council should continue in its present relationship with the Department of Education and Science or whether, alternatively, some of its functions and interests should be transferred to the Ministry of Agriculture, Fisheries and Food. (The notion that something along these lines should be done has for a long time been cherished in some Whitehall offices.) In this sense, the Council for Scientific Policy's working group will be complementary to the review of government policy on all kinds of research and development entrusted to Lord Jellicoe. Although the starting point for much of the CSP's work will be an attempt to establish criteria for assessing the social value of particular activities in research and development, so that the report may deal with matters such as research policy in fields such as telecommunications and transport, Lord Jellicoe's study will cover defence research as well. By all accounts, the CSP study may be complete within three months, in which case it should be well timed to play a part in the larger review of research and development policy to be expected later in 1971.

CHEMISTRY

No Formula for Change

by our Education Correspondent

A SORRY tale of a declining proportion of the most able students being attracted to university chemistry courses and of poor prospects for chemists in the job market is told in a report published this week by the Royal Institute of Chemistry. Based on a four-year study conducted by a committee under the chairmanship of Professor Colin Eaborn, professor of chemistry at the University of Sussex (report of the *Committee of Enquiry into the Relationship between University Courses in Chemistry and the Needs of Industry*, Royal Institute of Chemistry, £2), the report shows that chemistry departments are accepting about 80 per cent of the candidates who apply. Bearing in mind that about 20 per cent of all candidates who apply to universities through the Universities Central Council on Admissions fail to achieve the minimum entrance qualification of two A-level passes, this finding suggests

that chemistry departments are scraping the barrel to fill available places. Nevertheless, in all the four years which came under the scrutiny of the committee, university chemistry places were left unfilled. This state of affairs, the committee suggests, "is a matter of concern for the country in general and for the chemical profession in particular".

But the number of chemistry graduates likely to be produced by the universities during the next few years will not fall much short of Britain's requirements for specialist chemists. Chemistry graduates have consistently formed the largest proportion of unemployed scientists six months after they have graduated, and this factor has led to suggestions that output from university chemistry departments is already too great to be absorbed by industry. But the committee takes a different view. It suggests that one of the chief troubles is that too few chemistry graduates are accepted for jobs in management, administration, government and politics, and it points out that the situation in Britain is very different from that in the United States, where only half the chemistry graduates take up jobs specifically requiring chemical knowledge.

It is therefore surprising to find that the report pours cold water on the idea that chemistry courses should be despecialized—the committee has some sharp words to say about the suggestion canvassed in, for example, the Swann report, that graduates would be more useful to industry if they had studied a wider range of subjects to a less advanced level than is now customary in special degrees. "We do not accept that any possible gains would outweigh the loss of professional competence in the subject of specialization," the committee maintains, and moreover "we encountered no clear opinion in the chemistry industry as a whole that the present degree courses in chemistry are too specialized."

What, then, is to be done? The committee suggests that, for one thing, chemistry courses tend to involve "too much learning of facts", and it suggests that chemistry teachers should consider ways to get round this. There is also a suggestion that chemistry students should be given the option of studying courses such as economics, sociology and computer programming. But such courses should be available only as subsidiaries and should not dilute the specialist nature of the major chemistry course.

The committee is satisfied with the proportion of undergraduates in chemistry departments who intend to take up careers in industry, and it also suggests that employers are satisfied with the graduates they get. But the proportion of graduates entering teaching is another matter. The committee believes that it is "unfortunate" that the teachers must now have teacher training qualifications before they can take up school teaching, because this restriction is likely to deter the more able graduates away from the schools. A means of offsetting this deterrent should be found, the committee urges, and as a minimum, grants for the compulsory training year should be equivalent to those for PhD students.

As far as PhD courses in chemistry are concerned, the committee is generally satisfied: "We believe that existing PhD courses provide a good preparation for chemists who will take up research careers, including those who will move to non-research posts after a few years in industry". Nevertheless, the committee recommends that some variants of the normal PhD course should be introduced. One suggestion which is canvassed in the report is that PhD courses could combine the equivalent of two years' chemical research with one year of formal instruction in, for example, technological economics, business management, operational research or marketing.

The committee therefore sees room at postgraduate level for undoing some of the effects of specialization at earlier levels, but it has few formulae to offer for changing university chemistry education. The report is, on the whole, a depressingly conservative document.

ATOMIC ENERGY

Turning Point for Dragon

WITH the first commercial application of the high temperature reactor (HTR) in the offing, the emphasis of research in the OECD Dragon reactor project has shifted steadily away from reactor development to problems of high temperature fuels and of control systems. The annual report of the project up to April 1970 shows that expenditure on reactor construction continues to diminish in an overall budget which rose by more than 12 per cent last year compared with 1968/69. Growth is unlikely to continue beyond 1971, however, and spending should fall below the present level by the time the agreement between the twelve countries involved expires in 1973. The expenditure in 1969/70 of £2.3 million brings the total cost of the project since its inception eleven years ago to just over £31 million.

A chief part of the Dragon programme is now centred on the use of the 20 MW experimental reactor at Winfrith as a test facility for industrially developed high temperature fuels. Previous work on the properties of coated fuel particles compacted in graphite matrices has been extended to include the testing of complete fuel assemblies suitable for use in future power stations. Among the recent innovations is the introduction of fuel elements in the form of large blocks of graphite with machined holes to carry tubular pins.

During the period covered by the report, the reactor was shut down for the fitting of new heat exchangers and secondary circuit pumps to increase the heat removal capacity. Although most of the work in the Dragon project is now centred on the testing of fuel and core materials, the approach of the first contract for an HTR for a power station—probably for Oldbury B—has focused attention on a host of important secondary problems affecting the HTR. The design of heat exchangers, gas circulation pumps and control systems are prominent among these. The chemical interactions between the graphite and the helium coolant are being studied as well as systems for monitoring and maintaining the purity of the coolant.

Various safety tests have also been carried out to ensure that the leak detection system for the pressure vessel is adequate for commercial usage. New information on the fracture mechanics for partially penetrating cracks has led to fresh studies of the detection system but the equipment was found to be quite efficient enough to reveal a crack before it reached anywhere near its critical length under the operating conditions of the vessel.

GENERATORS

Some Light in the Gloom

THE Central Electricity Generating Board can take a crumb of comfort from the fact that the troubles with its 500 MW generating sets, which last December caused power reductions of about 6 per cent, are being resolved. And prospects for the smooth installation and commissioning of some of the 660 MW sets in the next few years are good. But by no means is everything in the garden now rosy. Other breakdowns and troubles last winter pushed the proportion of plant which was out of operation at critical times to over 20 per cent (*Nature*, **228**, 1125; 1970) and the shortage of smokeless fuel will not make things any easier for the rest of this winter.

The whole matter of the 500 MW sets was reviewed by the Select Committee on Science and Technology earlier this year (HMSO; 4*s*) and the conclusions were that the design work was insufficiently detailed, that there were welding defects and hairline cracks in the boilers and that some malicious damage had been done.

There are now twenty-three 500 MW sets at various stages of commission and some are producing over 95 per cent of their maximum power—the outputs vary from about 280 MW to 480 MW. Three of the four sets at Eggborough, Yorkshire, which contributed much to the trouble last winter have now been modified and are generating between 380 and 430 MW each; the fourth is being reconstructed. There has been no recurrence of the damage at Aberthaw B power station which was considered malicious by the Select Committee because a number of bolts were found inside the generators.

Manufacturers seem to be taking to heart the remarks about design contained in the Select Committee Report and the CEGB is confident that the 660 MW sets to be incorporated into the Hinckley Point B, Hartlepool and Dungeness B nuclear power stations will not show the type of fault which beleaguered the 500 MW sets. The board points out, however, that final tests can never be carried out until the sets are in position.

VENUS-7

Mystery and Speculation

from our Soviet Correspondent

THE degree of success of the Soviet Venus-7 probe would seem to have added a further, if minor, mystery to that already mysterious planet. There has been considerable speculation about whether the probe was intended to reach the surface of the planet in a viable condition and, if so, whether it succeeded. The relatively minor announcement for Venus-7 in *Pravda* (December 16, 1970)—a small article half-way down the front page, with no supporting articles in the inside pages—suggests, perhaps, that Venus-7 cannot yet be described as a major Soviet success in space research. The facts of the probe's mission, however, seem to be as follows.

Venus-7, launched on August 17, 1970, completed its 320 million km journey in 120 days, during which it had broadcast to Earth 124 sessions of telemetered information. Preparations for the descent began on December 12 at a distance of 1,300,000 km from the planet. At this point, the batteries for the descent stage were switched into a charging circuit from the solar cells. At the same time, cooling of the descent stage to a temperature of $-8°$ C began.

Immediately before entry into the Venusian atmosphere, the descent craft was separated from what is described as the "orbital section". The descent craft entered the atmosphere at 10·3 km/sec and this velocity was reduced by aerodynamical braking to 250 m/sec when the parachute system came into operation. The antennae were deployed and radio transmission of information began.

The transmission continued for 35 minutes. Until some preliminary results are released, whether the descent stage actually reached the surface of the planet, or whether it, or its transmitting system, was destroyed by heat while still parachute-borne is open to speculation. It is perhaps significant that, in the preliminary news releases from the TASS agency, the concluding stage of the experiment is described as "entry into the atmosphere", and no mention is made of any possible contacting of the surface.

CANCER THERAPY

Insufficient Evidence

THE publication last week by Dr Joseph Issels of a preliminary report of his unconventional method for treating cancer has done little to remove the controversy surrounding his techniques. The Medical Research Council's Joint Coordinating Committee on Cancer Research was quick to publish a statement pointing out that the evidence so far available is "insufficient to enable a scientific assessment to be made of Dr Issels's methods of treatment or of his results", and the committee added that such evidence as there is does not encourage the belief that any advance in the treatment of cancer has been achieved. The committee, nevertheless, intends to send a few experts to have a look at Dr Issels's clinic at Ringberg to see whether further study of his methods is necessary.

In several respects, Dr Issels's report of his work, published in the latest issue of *Clinical Trials Journal*, leaves unsaid more than it reveals. To be fair, Dr Issels himself claims that his results are not conclusive, and that a more detailed statistical investigation of the results of his technique should be made. He describes in his article a study of a sample of 242 from a total of 750 patients who attended his clinic. It turns out that 42 from the sample were alive and "fully fit for work" five years after treatment, and, of these 42, 39 still showed no signs of cancer 15 years after treatment. A preliminary study of a further 370 patients who were treated by Dr Issels shortly after surgery or irradiation shows that 87 per cent were still alive after a period of five years, with no detectable relapse. Dr Issels therefore claims that his treatment reduces the danger of relapse during the first five years to 13 per cent, compared with the world average of 50 per cent.

Whether or not these claims are justified depends to a great extent on the criteria by which Dr Issels selects his patients, and his report does not shed much light on this factor. The report also gives a very sketchy account of his method of treatment, and does not include analysis of control trials. Indeed, Dr Issels has consistently refused, on ethical grounds, to carry out control trials, without which an accurate assessment

of the efficiency of his method for various types of cancer at different stages of development cannot be made.

The treatment given at Dr Issels's Ringberg clinic is a mixture of immunotherapy, chemotherapy and psychotherapy, based on the concept that cancer is a chronic systemic disease. Elimination of the original tumour is carried out by surgery, irradiation, chemotherapy, enzyme therapy and "immunotherapy by both active immunization with mycoplasma vaccine (developed by Dr Gerlach) and passive immunization with antibodies prepared from cancer cells". The most interesting part of the immunotherapy is the use of the Gerlach vaccine, but, unfortunately, few details are given in Dr Issels's article. He says that the vaccine is a mixture of pure cultures of tumour mycoplasmas obtained after ultracentrifugation from tumour tissues of heterogeneous malignant growths. The vaccine is injected intramuscularly or intravenously "for weeks or months in doses depending on the patient's condition". With this sort of vague information to go on, the MRC was right to emphasize that no conclusive evaluation of Dr Issels's methods can yet be made.

POLLUTION

Dangers in Tinned Tuna

AFTER the finding of dangerous concentrations of mercury in tinned tuna fish in the United States, it is not surprising that the laboratory of the Government Chemist has reported similar concentrations in British tins. Although the Ministry of Agriculture has not released the precise results of the preliminary emergency survey of the tinned fish, the nature of the reports suggests that concentrations between 0·3 and 1·0 parts per million—similar to those found in the American fish—have been found. The Government Chemistry Laboratory itself, in its annual report last September, generally allayed the concern about mercury contamination and failed to detect mercury in the total British diet.

In the laboratory's preliminary survey, "a range of mercury levels" was found in samples of tuna, bonito and Ship Jack fish from Japan, Peru, Russia and Yugoslavia. Accordingly, the Ministry of Agriculture and Fisheries warned the public that no assurance could be given that all tinned tuna fish is safe for consumption in quantity over a long period. But "because the consumption a head in this country is only 10 per cent of that in the United States, and in the light of the figures revealed from the small sample examined so far, the problem does not require additional action before more complete information and expert advice has been obtained". This advice will come from the government laboratory after a more extensive examination of fifty species of fish.

This recent wave of concern comes at the end of a year during which the inadequacy of restrictions on the disposal of mercurial residues in industrial wastes have been made painfully clear. In the United States, the widespread contamination of lakes and the high concentrations found in some bird species are causing considerable concern; in Canada, 0·3 p.p.m. have been found in chicken livers and 0·2 p.p.m. have been found in bread. What has also been made clear is that nobody knows what the effects of smaller, sublethal concentrations of mercury are. Whilst it is well known that the metal persists and accumulates in the body, with a multitude of effects ranging from skin rashes to insomnia and loss of memory, very little is known about the long-term effects of concentrations well below the generally accepted safety limit of 0·5 p.p.m. on, for example, phytoplankton, the first link in the food chain. As long as this ignorance persists, safeguards to protect water quality standards will remain inadequate.

NEWCASTLE DISEASE

Live Vaccine Approved

ALMOST nineteen million birds in forty-three counties in Britain are now suffering from fowl pest, and there is little sign that the epidemic has spent its force. Some weeks ago it seemed that the worst was over, but the dismal daily tally of fresh outbreaks has, at last, forced the hand of the Minister of Agriculture—poultry farmers will in future be permitted to use the hitherto illegal live vaccine.

This last ditch attempt to turn the tide of infection follows six weeks of intensive field trials in which the live vaccine was tested for efficacy and safety under close Ministry supervision. The results of these trials indicated that live vaccine—only the strain Hitchener B1 has so far been permitted—may give somewhat better protection to very young birds, and it will have few adverse side effects. No significant side effects were detected when the vaccine was used on 10–14 day old chicks. The chief advantage of the live vaccine lies in its cheapness of manufacture, and the ease with which it can be used to treat poultry *en masse*. Whereas inactivated vaccine has to be administered to each bird individually, the live equivalent can be applied as an aerosol to many birds simultaneously.

It has been suggested that the virulence of the present epidemic, and the necessity to use live vaccine are evidence that Newcastle disease is now endemic in Britain, but this the Ministry have denied. However difficult it may prove to break the hold the disease has, there can be no doubt that the use of live vaccine will have economic repercussions. The veterinary controls which have previously invalidated the use of live vaccine have similarly excluded the import of cheap overseas poultry. When the use of live vaccine becomes general, this ban will no longer apply. The Minister of Agriculture said last week that when the ban is lifted, arrangements would have to be made to safeguard the home market from being undermined by unduly low priced imports.

COUNTRYSIDE COMMISSION

Causes for Dissatisfaction

THE Countryside Commission has good cause for dissatisfaction. It has a staff complement of little more than two-thirds that originally envisaged in the Countryside Act, 1968, and a budget substantially less than the £2 million considered necessary in 1967 for its new powers and responsibilities. And it is also unhappy about the low priority given by the government to landscape values and to conservation in general and in particular, in the national parks and areas of outstanding natural beauty. In its latest annual report (HMSO, 13*s*.) the commission criticizes government

approval of the potash mining activities in the North Yorks Moors National Park, of the scheme for a reservoir at Meldon in the Dartmoor National Park, and of the route of the M40 motorway (London to Oxford) through the Chiltern Hills.

Referring to the potash workings, the commission repeats its concern about the way in which public money could be used to aid private enterprise developments which conflict with the purposes of a national park, and it also questions the national need for three separate companies to exploit so rapidly the potash deposits. The commission's next important battle will be to oppose the building by the Central Electricity Generating Board of a new 1,300 MW power station at Insworke Point near Plymouth, a site which the commission will say at the enquiry next February is a "beautiful and sensitive area of countryside and coast which ought to be conserved and protected from large scale industrial intrusions".

The report also mentions the Arnside–Barrow link road in the north-west of England. This route, chosen by the Ministry of Transport, would run through an area of coast and countryside in the Arnside/Silverdale area which the commission decided in 1967 should be designated an area of outstanding natural beauty. After considering an independent consultant's report on the proposed new road, the commission made history by objecting to the proposal at the public enquiry held earlier this month. It condemned the proposal as misconceived, and claimed that the road would be substandard, create noise and pollution in the countryside and effect only an "absurdly small" saving in time for traffic travelling to Furness from the A6 and M6.

The commission also reports that more than 100,000 visitors called at the Lake District National Park Centre near Windermere during its first full season. And in the Peak District National Park, the traffic management scheme, which was started last July in the Goyt Valley, received almost unanimous approval. Visitors to the valley were persuaded to leave their cars, and were taken into the valley area in minibuses. The success of this scheme may pave the way for similar experiments in other national parks.

CONSERVATION

Balancing the Water Budget

from our Soviet Correspondent

THE Supreme Soviet of the USSR has adopted new legislation governing water usage and water conservation to take effect from September 1, 1971. The law covers all water resources in the Soviet Union—rivers, lakes, reservoirs, inland seas and territorial waters for industrial, agricultural, fishing, transport, power production, fur-producing, cooling, effluent removal, recreational and other purposes. Although the preliminary paragraphs of the law note that state ownership of the water resources of the USSR creates favourable conditions for optimum planning and use, a considerable number of the 46 articles of the law seem to be aimed at resolving the conflicting demands of different sectors of the community. Under the new legislation, all water resources are to be the subject of measures against pollution, blockage and depletion. Although penalties for infringement have not yet been published, special attention is drawn to the possible pollution of reservoirs and lakes by the fouling of their winter ice cover. All existing sources of industrial pollution are to be "liquidated", and no new projects —factories and so on—can be commissioned unless they are equipped with anti-pollution devices.

In addition to pollution, the law also forbids waste and uneconomic use of water, because for some years the availability of the water resources of the USSR have been causing concern. The new law discusses means of government assessment of total and accessible water resources, and emphasizes the need for proper maintenance of a balanced water economy for the basins, economic regions and Union Republics of the USSR. Anti-erosion measures for preserving this balance are especially emphasized and the law also provides for the rapid introduction into the water-budget of any underground reserves which may be brought to light as a result of mining or oil and gas drilling operations.

SEA BED

Exploitation Rights

ALTHOUGH oceans and seas cover some 360 thousand km² of the Earth's surface, with an average depth of about 3,700 m, exploitation of their vast potential is only just beginning. A symposium on the exploration and exploitation of the sea bed and its subsoil, held in Strasbourg earlier this month, was organized by the Council of Europe as a first step towards a possible unified European effort to tap this reservoir of potential wealth.

Although the symposium came to few conclusions, there was valuable discussion of the legal position regarding ownership of the sea bed. The present position is confused, despite the Geneva Convention of 1958 (now ratified by more than 40 countries) covering ownership of the continental shelf. Many countries have already granted licences for oil exploration in areas well out to sea in the oceans bordering their coastlines—this is taken as making *a priori* claims to the ownership of mineral rights in these areas. In Europe, with a large number of countries bordering a small amount of sea, the legal problems are of very great importance, as has been shown by the recent dispute over just which parts of the North Sea should be allocated to West Germany.

But the legal problems still remain largely theoretical for the greater part of the Earth's oceans, and there is urgent need of formal international agreement. Reporting to the symposium, Professor W. H. van Eek suggests that within a few years divers will be able to work usefully down to at least 200 metres, the depth below which even major surface storms have no influence on the local environment.

Discussions are now taking place at the UN about this problem, but national governments in general are very ill informed. A full report of the Council of Europe's symposium will be put before the council's Parliamentary Assembly in January 1971, after which precise recommendations for the establishment and terms of reference of a European Ocean Space Commission and an Institute for Ocean Studies could be made. If this results in a wider understanding of the

problems involved, and produces a common European attitude on how they should be solved, the Council of Europe will have made a major contribution to the future development of world resources.

JOURNALS

Filling a Gap

In line with increasing acceptance of the need for a scientific approach to the management of research and development, the only British journal devoted entirely to the field has recently been published (*R & D Management*, Basil Blackwell). Dr E. A. Davies, formerly Joint Parliamentary Secretary to the Ministry of Technology, says in the foreword that its principal aim should be to promote the " . . . efficient and purposeful management of our R & D whether in government, industry or universities. . . ."

The new journal is edited by Mr Alan Pearson, director of the R & D Research Unit of the Manchester Business School where contacts with industrialists have emphasized the demand for such a journal. He sees it as combining some of the best features of the two principal research management journals in the US. These are *IEEE Transactions on Engineering Management*, which tends to be rather theoretical, and *Research Management*, which has an editorial board of industrialists and errs rather on the side of publishing subjective accounts of individual R & D policies. Mr Pearson says that his journal will attempt to adopt a disciplined academic approach to a topic which must at all times remain both practical and applicable.

These views are also borne out by the activities of the Science Policy Foundation whose aim of promoting the scientific investigation of science itself includes studies of the economics of research and development. The need to ensure that discussion and exchange of information are on an academically sound basis and as free as possible from subjective bias is reflected in the nature of the seminars organized by the SPF. A measure of the interest among middle and top management can be gauged from the attendance of 70 people at the second of a series of five such seminars on the theme "Industrial R & D—sound business or act of faith ?".

The editorial board of *R & D Management* hopes that the circulation of the new journal, published three times per year at a subscription rate of £5, will soon pass the 1,000 mark and that it will be appropriate reading for a wide spectrum of people from directors of research to practising scientists. Its role in the realms of formal education is stressed by Professor F. R. Jevons, head of the Department of Liberal Studies in Science at the University of Manchester and a member of the journal's advisory board. He emphasizes the possible uses of the journal both in joint courses such as those run by his own department and the Manchester Business School and in those optional courses in management economics offered by many engineering schools.

The first issue contains papers on topics such as innovation and the small firm, communication networks in research laboratories and methods for research project selection. It is, however, rather disappointing in its layout, but a rather uninspiring front cover and some blank spaces inside will no doubt disappear as the journal finds its feet.

Parliament in Britain

Open University

The government's plans for the Open University were the subject of several questions last week. But Mr William van Straubenzee, Under Secretary of State for Education and Science, refused to be drawn into making definite commitments. The university's grant for 1971–73 has been assessed for an intake of 25,000 students in the first year, and between 36,000 and 42,000 a year for the next two years, but annual intakes for the following years have not been determined. Asked by Mr Peter Hardy for an assurance that the university will be allowed to make unimpeded progress and will not be used as a cheap form of education for those leaving school at the age of 18, Mr van Straubenzee said that the government's decisions have been announced and will be adhered to. Nevertheless, the government has asked the Open University to look into the possibility of taking students at the age of 18. The government also has no plans for increasing the educational facilities of the Open University. (Oral answers, December 17.)

Post Office Equipment

Asked by Dr Maurice Miller whether the government intends to change the right of the post office to manufacture its own equipment, Mr Christopher Chataway, Minister of Post and Telecommunications, said that he has no proposals to amend present legislation. There has been some speculation recently that the Post Office is considering acquiring some manufacturing facilities of its own. (Written answers, December 14.)

Royal Commission on Environment

Mr Peter Walker, Secretary of State for the Environment, said that he has no intention of reviewing the terms of reference of the Royal Commission for the Environment that was set up by the previous government. Mrs Peggy Fenner said that she was sceptical, however, about delaying tactics like the setting up of Royal Commissions. She asked the minister to bear in mind that what the public wants is not advice, consultation, or coordination, but action. Mr Walker replied that the commission should have freedom to choose which aspects of pollution should be given priority. (Oral answers, December 16.)

Museums Charges

During a debate in the House of Lords on the government's proposal to institute charges for admission to museums and galleries, Lord Eccles, Paymaster General, announced that the grant to the Arts Council will be increased next year by £2·6 million to £11·9 million. Lord Eccles also made it clear that he is entirely in favour of the proposed charges, and that this was not a measure foisted on him by his colleagues. I thought the choice was right, he said, and I asked my colleagues to endorse it. Lord Eccles offered the increase in the grant to the Arts Council as proof of the government's intention of giving more aid to the arts, and he was also considering ways of releasing the publications department of the British Museum from having to return its profits to the government. Despite strong criticism of the government's proposals, members of the House of Lords endorsed them by a vote of 121 to 84. (Debate, Lords, December 16.)

Competition for University Places

A-LEVEL results, viewed as the chief criterion for selecting students for university places, are singularly inefficient. That is one conclusion to be drawn from a report published by the Universities Central Council on Admissions (UCCA, 15*s*). Although that may not be too surprising a conclusion, another fact to emerge from the statistics in the report—that British universities attract a disproportionate number of applicants from the middle classes—is even less of a shock. But the report is, nevertheless, a valuable document. It is the first attempt by the UCCA to analyse in depth the social and academic backgrounds of the students who pass through university selection procedures and the wealth of information which the report provides may serve as useful indicators for higher education planning.

Based on a ten per cent sample of the applicants to British universities for October 1969, the report gives detailed information on the academic qualifications (A-level results, or their equivalent), the social backgrounds, the regional distribution and the subject preferences of aspiring university students. It paints a picture of the many hurdles which lie in the path of such students on their way to a university place, and it gives some indication of the intensity of the competition for university places.

The first fact to emerge from the report is that 64 per cent of the sample of candidates from the UK who achieved at least the minimum entrance qualifications of two A-levels were accepted by the universities in 1969. There were, however, 2,721 applicants in the sample with such qualifications who were rejected. If the sample is representative—and there is little reason to suspect that it is not—some 27,000 people were unsuccessful in the competition for places. Bearing in mind that many of these students eventually found their way into colleges of education and the polytechnics, the so-called pool of untapped ability shrinks to slightly more acceptable proportions, but the output of students from schools with at least two A-levels is expected to more than double during the next ten years. Unless extra places are found, the competition will therefore get even more intense. The report also shows that 46 per cent of the accepted students were given places at their first choice university.

Are the universities, in any case, taking the students with the highest qualifications, in terms of A-level passes? The UCCA report shows that there is a very high acceptance rate for candidates with the best A-level results, but those with average or relatively poor results more or less take pot luck. By scoring A-level results as 5 for a grade *A* down to 1 for a grade *E*, it is clear that those who achieved a score of 9 or more (equivalent to an *A* and a *B* or three grade *C*s) stand a chance of better than 80 per cent of getting into university (see Table 1), but those with lower grades stand an almost even chance of gaining a place. A more revealing analysis, however, is that which shows the ranges of ability of the students accepted for various subjects. There is strong evidence to show that students intending to take arts or social science subjects must achieve higher A-level grades than those intending to take science or technology courses. Of the arts students accepted with three or more A-levels, 85 per cent had scores of 9 or more and 80 per cent of the social science students had similar scores, but of those accepted for science courses, 67 per cent scored 9 or more for three A-levels, and 65 per cent of the technology students had similar scores. But for medicine, only 59 per cent of the students accepted had achieved these results.

Table 1. A-level achievement and university acceptances, 1969

Score on 3 or more A-levels	Accepted	Not accepted	Per cent accepted
15	266	12	93
14	359	26	93
13	406	22	95
12	453	39	92
11	492	49	91
10	488	65	88
9	462	118	80
8	395	150	73
7	267	222	55
6	164	230	42
5	125	199	32
4	90	128	43
3	58	80	42

Evidence of the overwhelmingly middle class backgrounds of university students is given by the finding set out in the report that 31 per cent of the accepted candidates in the sample came from families which belong to the upper economic groups, and only 27 per cent to the lower groups. The percentages of economically active males in the total population in these two groups, however, are 8 per cent and 64 per cent respectively. But the report shows that the bias is not introduced during the university selection procedure, for the number of applications correlate closely with the number of acceptances in each economic group. The universities therefore reflect the middle class bias in the secondary schools.

The suggestion that cost savings in the universities may be made by regionalizing intake—insisting that universities take a large proportion of their students from surrounding areas—is dealt something of a blow by the findings revealed in the report. In regions such as the North-West, London and the West Midlands, which have urban populations large enough for regionalization of intake to be a real possibility, less than half the students accepted in 1969 came from the region in which the universities are situated. Universities in the North-West, for example, accepted only 28 per cent of their students from that region, and similarly West Midlands universities accepted only 20 per cent of their students from surrounding areas. And the situation is even more difficult for universities in East Anglia and the East Midlands—these universities accepted 3·6 per cent and 10 per cent respectively of their students from surrounding areas. More than a third of the sample of students who applied to universities in 1969 came from the South-East, and is seems that the North, East Anglia and the Midlands as a whole supplied less than their fair share of candidates.

NEW WORLD

Tolbutamide Study Raises Blood Pressures

by our Washington Correspondent

DR CHARLES EDWARDS, the new head of the Food and Drug Administration, has stumbled into a controversy that could prove nearly as troublesome to the agency's reputation as was the handling of the cyclamates incident. Two months ago the FDA acted on the basis of a single epidemiological study, still not published in full, to urge that tolbutamide and other antidiabetic agents taken by mouth be used by doctors only on a last resort basis. The sharp dispute that has now erupted within the medical community over the methodology and interpretations of the study has made the action of the FDA look awkwardly premature.

As a result of the FDA's hasty ruling, an atmosphere of suspicion and resentment has been created among diabetologists in addition to their doubts about the validity of the study. Matters came to a head at a recent clinical convention of the American Medical Association in Boston when forty specialists signed a statement on November 30 deploring the FDA's course of action as uncritical, premature and scientifically unacceptable.

The source of the controversy is an ambitious prospective study of tolbutamide that started in 1961 and eventually included some 800 diabetic patients in 12 university centres. The conclusion of the study, reached after 8½ years, is that the tolbutamide therapy, combined with a controlled, low sugar, diet, is no more effective than diet alone in prolonging life. The investigators went on to conclude that tolbutamide and diet may even be less effective than diet alone, or diet and insulin, with respect to the risk of cardiovascular mortality.

Tolbutamide is a chemical that transiently lowers the quantities of sugar in the blood, thus mimicking one of the functions of the natural hormone insulin. It is valued by doctors because unlike insulin it does not have to be injected and can be taken in pill form. Of the 4½ million Americans who suffer from diabetes, about 1½ million have a milder form of the disease which can be treated by oral antidiabetic drugs such as tolbutamide, and about 800,000 use tolbutamide itself (which is marketed as Orinase by the Upjohn Company). The tolbutamide study, a $7 million dollar project financed by the National Institutes of Health and known as the University Group Diabetes Program (UGDP), first came to public attention through press articles in May. The Food and Drug Administration convened an advisory committee on May 20 to advise on its reactions to the UGDP report. The recollection of one of the members is that the committee heard conflicting opinions about the report from three biostatisticians and concluded by saying that the significance of the results needed further discussion. In any event the FDA issued a statement the following day saying that it accepted the UGDP study and would issue a bulletin to doctors later, and order appropriate labelling changes. The bulletin sent out at the beginning of November went rather further than just telling doctors of the UGDP results; it also told them how to treat their diabetic patients. First the patient should be treated by diet and weight control alone, with no other therapy if this is adequate. As a next step insulin should be given, and only as a last resort should the oral hypoglycaemic agents be used.

FDA spokesmen point out that this action has been supported by the American Diabetes Association and the American Medical Association's Council on Drugs. These organizations have indicated their agreement with the stated conclusions of the UGDP study and the AMA group has confirmed the FDA's recommendations on the primacy of diet alone but gives rather more discretion to the doctor in deciding whether to prescribe insulin or an oral diabetic agent as the next step if diet fails. Unfortunately, the sanction of these authoritative bodies has not defused criticism to anything like the same extent as full publication of the UGDP report might have done.

In the first place there have been many criticisms of the methodology of the UGDP study. Although these criticisms may turn out to be groundless or overstated when the full data are published, they are sufficiently important to justify serious reservations in the meantime about the implications of the study and the wisdom of the FDA's action. Defenders of the UGDP survey point out that the final paper has been vetted by no less than six referees and the project as a whole was intensively reviewed at each stage of its eight year existence —but this is not the same as open discussion throughout the usual scientific channels. Perhaps as a result of the relevant facts being unavailable, the air is thick with accusations of bias, chicanery and character assassination.

On top of the reservations about scientific method, there is resentment among the medical community at what is regarded as the FDA's "practising medicine" by specifying how diabetics should be treated, instead of merely passing on to the doctors information about the new results. The statement prepared by doctors at the Boston meeting of the American Medical Association declared the FDA's action an "unprecedented interference with the practice of medicine in a controversial area (which) is not only outside the province of a Government regulatory agency but . . . has also damaged the welfare of a million diabetic patients".

Reception of the FDA's decision has also been made more difficult by the existence of long-standing differences of medical opinion on the management of diabetes. One school of thought, represented by the Joslin Clinic in Boston, where the AMA convention took place, believes in the importance of controlling the level of blood sugar in diabetes. Another view, held by some of those involved in the UGDP survey, is that control of blood sugar level does not have a significant effect on longevity. The results of the UGDP study inci-

dentally tend to favour the latter view, with the result that in the process of confirming the UGDP results on tolbutamide the FDA has seemed to its critics to be cutting across a vexed and as yet unresolved academic controversy.

Dr Robert Bradley of the Joslin Clinic, who helped draft the statement criticizing the FDA, believes along with other doctors that the agency has gone too far in extrapolating the results of the UGDP survey from patients not dependent on insulin, who were the subjects of the survey, to all kinds of diabetics. The agency also seems to have taken a more definite view of the toxicity of tolbutamide than the AMA Council on Drugs felt was justifiable. The council in its statement said only that tolbutamide "*may* have played a 'toxic' role with respect to cardiovascular mortality" but that this interpretation "is quite difficult and may or may not be valid".

Adding to the bad feeling between the agency and the medical community is the series of press leaks, both in this and previous instances, that have alarmed the public and made quick decisions necessary. The AMA at its annual convention in June this year went out of its way to reprove the FDA for its news policies which had "created undue anxiety" among diabetics. According to the November 2 issue of the AMA's newsletter, *American Medical News*, the agency also failed to prepare its bulletin on tolbutamide by the deadline agreed with the AMA and the American Diabetes Association, according to which all three organizations would release statements at the same time. The plan also called for the publication of the full article in October, now not expected until the end of this month.

The fact that the UGDP report is still unpublished seems much to blame for the bickering and backbiting that now surround discussion of the survey and may cling to it for some time to come. Why could the FDA not have waited until the full report was out? "The political pressures are too great to withstand," an FDA official explained last week—"the medical profession always chides the agency as premature when it acts promptly and it is pilloried by Congress and in the press if it bides its time." But if the Food and Drug Administration does not stand up to political pressures in order to allow scientific procedures due process, how is a rational decision to be arrived at? "You must ask Congress that," the official said.

COOPERATION

Brighter Outlook for Astronauts

by our Astronomy Correspondent

ONE of the ways in which 1970 has been a remarkable year is that cooperation between the United States and the Soviet Union in space exploration has at last seemed a possibility, although a remote one. *Nature* has already chronicled the long history of attempts at collaboration (**225**, 1094; 1970) and, whatever the reasons, the tale is depressing. But this autumn, almost out of the blue, moves towards cooperation took place whose outcome so far has, for once, not been disheartening.

Nobody knows what part the near-disaster of Apollo 13 played in the prehistory of the present negotiations with the Soviet Union. But the notion that there could be some kind of standardization of systems allowing Soviet spacecraft to dock with those of NASA and vice versa surfaced at the autumn congress of the International Astronautical Federation (see *Nature*, **228**, 198; 1970). After that the talks progressed remarkably quickly, and the fact that an agreement had been reached between NASA and the Academy of Sciences of the USSR was announced on October 29. On the Soviet side of the talks held in Moscow were B. N. Petrov, K. P. Feoktistov, V. S. Syromyatnikov, V. V. Suslennikov and V. A. Lavrov, and on the American side Robert R. Gilruth, Arnold W. Frutkin, George B. Hardy, Caldwell C. Johnson, and Glynn S. Lunney. Now that the agreement has been confirmed by an exchange of notes between the acting administrator of NASA, Dr George Low, and the president of the Soviet Academy, Academician M. V. Keldysh, the text has been made available.

During the talks, which appear to have been limited to the question of compatibility of rendezvous and docking systems, the two sides exchanged information about their existing methods, and decided on twelve topics which merit further study:

(1) Passive reflectors of the radio guidance systems, their location and characteristics.

(2) Passive reflectors of the optical guidance system, their location and characteristics.

Apollo 14 Timetable

NASA has now published the preliminary timetable for the Apollo 14 mission, which will leave on January 31 for the Fra Mauro formation. All times are in BST.

Event	Date/time (BST)
Launch from Cape Kennedy	Jan. 31, 9.23 p.m.
Leave Earth orbit	Jan. 31, 11.54 p.m.
Leave free-return trajectory	Feb. 2, 3.59 a.m.
Lunar orbit insertion (57 × 170 nautical miles)	Feb. 4, 7.58 a.m.
Descent orbit insertion of docked spacecraft (10 × 58 nautical miles)	Feb. 4, 12.17 p.m.
Lunar module undocks and separates from spacecraft	Feb. 5, 5.47 a.m.
Command/service module moves into circular orbit (56 × 63 nautical miles)	Feb. 5, 7.07 a.m.
Lunar module powered descent to lunar surface	Feb. 5, 10.02 a.m.
Landing at lat. 3° 40′ 19″ S., long. 17° 29′ 46″ W.	Feb. 5, 10.14 a.m.
The two astronauts will stay about 34 hours on the surface.	
Begin 4 to 5 hours of lunar surface activity (EVA)	Feb. 5, 2.50 p.m.
Begin second 4 to 5 hours of lunar surface activity	Feb. 6, 11.30 a.m.
Liftoff of lunar module from Moon	Feb. 6, 7.44 p.m.
Docking with command/service module	Feb. 6, 9.30 p.m.
Lunar module jettisoned on to lunar surface	Feb. 6, 11.43 p.m.
Apollo 14 leaves lunar orbit	Feb. 7, 2.35 a.m.
Splashdown in Pacific Ocean for recovery by USS New Orleans	Feb. 9, 10.01 p.m.

(3) The radio-technical guidance equipment, utilizing active radio signal transmission and return, including its elements, location and characteristics.

(4) Lighting equipment for rendezvous and docking, its elements, relative location and characteristics.

(5) Benchmarks and reference marks for orientation during rendezvous and docking, their location and characteristics.

(6) Coordinate systems for reference in developing rendezvous and docking techniques for spacecraft and space stations.

(7) The docking assembly, inner tunnel and electrical, pneumatic and hydraulic couplings.

(8) Pneumatic, hydraulic and electrical couplings and connectors between space suits and on-board equipment of the spacecraft or stations and their characteristics, the dimensions of hatches and the means of opening them from the outside (in the event that assistance needs to be rendered from the outside).

(9) Composition and characteristics of the cabin atmosphere.

(10) Voice and code communications between spacecraft.

(11) Dynamics of docking and the stabilization of system after docking.

(12) Constraints on the location of thrusters, solar batteries and other design elements which should be borne in mind to assure the possibility of docking.

The agreement goes on to list the next steps to be taken. During November of this year there was to be an exchange of technical details on radio guidance and rendezvous systems, on the composition and characteristics of the spacecraft atmospheres, and the voice communication systems. Then each side is to prepare its own draft of the technical requirements for the systems for which it considers it advisable to ensure compatibility, to be exchanged in January or February next year. This will be followed by a meeting in March or April to discuss and refine the technical requirements that have been put forward, and the agreement allows for an earlier preliminary meeting to prepare the ground. Once agreement has been reached each side, independently, will work out preliminary designs which will then be put to the representatives of each side who will decide what further work is to be done.

Does this mean then that the future Apollo astronauts will enjoy an extra lifeline not available to their predecessors? Unfortunately not. The Apollo programme is almost certainly too unmalleable to make the changes now. The same goes, of course, for the next step by the Americans, the Skylab programme which is still based on Apollo technology. What was being talked about at Moscow then was the compatibility of docking systems in programmes which in the United States have not yet received the go-ahead, the shuttle and the space station. A start has been made nevertheless.

LEAD POISONING

Children at Risk

from our New York Correspondent

LEAD poisoning is one of the most important paediatric problems in New York City according to Dr Vincent F. Guinee, director of the City Health Department's Bureau of Lead Poisoning Control. Speaking at the New York Academy of Sciences last week, Dr Guinee estimated that between 6,000 and 8,000 children in the city have significant levels of lead in their blood. The number of deaths due to lead poisoning has dropped sharply because of improved testing, and is down to two so far this year, but for the same reason the number of known non-fatal cases has risen from 151 in 1959 to nearly 2,500 in 1970.

The chipping paint in older apartments is the primary source of lead poisoning. Paint with a high content of lead was banned in 1959, but Dr Guinee estimated that there are 450,000 apartment units in New York in such bad repair that children could easily eat leaded paint chips. Twelve per cent of the ingested lead is absorbed into the blood, with high enough levels causing irreparable brain damage.

The Bureau of Lead Poisoning has for the past year been seeking out cases, screening more children and attempting to provide preventive care. Dr Guinee hopes that each hospital and clinic will soon automatically include lead-poison screening in its testing facilities. The bureau itself screened more than 79,000 children last year to find the 2,500 with at least 60 micrograms of lead in their blood, the definition of a positive case. The apartments of all positive cases are tested by the bureau; if any painted wall contains more than one per cent lead, the area must be repaired and repainted, either by the landlord or by the bureau at the landlord's expense. Unfortunately, with only 200 workers and a budget of $2·4 million, the bureau cannot begin to test the apartments of those 31,000 children who, when tested, had positive amounts of lead in their blood, not to mention the other 400,000-odd apartments that are probably contaminated.

According to Dr Guinee, the bureau's work during the past year has taught the researchers that (i) results vary greatly according to the depth of the sample; the more children who are screened, the more cases of lead poisoning that will be discovered. (ii) Study in this field has been hampered by semantics—what determines a "case"; where should the cut-off point be? And (iii) there is still a great lack of knowledge—how does a child react to his condition? Is there any psychological damage? Which children can receive treatment and what is a satisfactory or realistic end-point? Also, how is a health hazard measured and how efficiently can the law be enforced?

Lead poisoning is obviously an environmental problem, directly related to deteriorating housing. The obvious answer is to improve the housing, but this takes time and money. Workers in the field see the necessity for enacting stiffer laws and for educating the federal government, but fear that the problem will continue to grow not only as investigation improves, but while more housing is allowed to deteriorate.

But it seems possible that in this field, too, those involved are no longer going to sit back and wait for a change. One of the doctors at the meeting, Dr Rothschild of the Sloan-Kettering Institute for Cancer Research, stated: "What is lacking is that having identified the vector, we throw up our hands and go on treating the symptoms without trying to do anything about the environmental problem that causes it". Dr Rothschild went on to raise the possibility of becoming directly involved in social action. The meeting was receptive to the idea, if not prepared to act on it immediately, a hopeful sign at least for change in the future.

NEWS AND VIEWS

A Prediction Fulfilled

If the only thing more gratifying than having one's predictions confirmed by somebody else's experiments is being able to confirm experimentally one's own predictions, Dr Bernard Davis must be feeling very satisfied. In 1968 he and two colleagues predicted the existence of a factor in *Escherichia coli*, the specific function of which is to cause the two subunits of a ribosome to dissociate. And on pages 1273 and 1269 of this issue of *Nature* he and Dr A. R. Subramanian and Dr S. Ochoa and three colleagues report experiments which prove that dissociating ribosomes of *E. coli* is the function of an initiation factor F_3.

In *E. coli*, the initiation of the translation of a messenger RNA molecule depends on the formation of a complex between the smaller of the two ribosomal subunits, the 30S subunit, a molecule of messenger RNA, and the initiating transfer RNA carrying a formyl-methionine residue (fMet-tRNA$_f$). Only after this initiation complex, as it is called, has been formed can the larger of the two ribosomal subunits, the 50S subunit, associate to yield a 70S ribosome which can then catalyse the formation of peptide bonds and the translation of the entire messenger RNA. Obviously this stepwise initiation process depends on a supply of free 30S and 50S ribosomal subunits, but in 1968 Davis and two colleagues reported that the ribosomes which run off a messenger RNA after translating it completely remain intact as 70S particles. Although there has been something of a dispute about whether or not ribosomes dissociate into their two subunits at the end of each round of protein synthesis, Davis and his colleagues stuck to their guns, maintaining that the dissociation does not occur automatically at the termination of protein synthesis. They therefore postulated that some dissociation factor must exist which functions by complexing with the 70S ribosome, probably to its 30S moiety, and causing it to fall apart into its subunits. And by remaining associated with the 30S subunit the factor would block the spontaneous binding of free 30S and 50S subunits. In other words, the factor would produce and maintain the supply of 30S subunits required for initiation complexes.

Those workers who postulate the existence of factors are behoven to search for and identify the putative molecules, and in 1968 Davis and his colleagues reported that a dissociation factor is indeed solubilized by washing ribosomes with ammonium chloride solutions. But the washings they obtained in this way clearly contained several factors including some which catalyse the steps in the initiation of protein synthesis. Since 1968, several groups have pursued the question of the part various factors play in initiation and three distinct initiation factors, F_1, F_2 and F_3, have been defined and purified.

Davis and his colleagues repeatedly observed that their dissociation factor purified along with the F_3 initiation factor, and now with pure F_3, supplied by Dr Ochoa, they have confirmed that whatever other functions F_3 may have in initiating protein synthesis it is able to dissociate 70S ribosomes into their two subunits. Apparently one molecule of F_3 is able to dissociate one 70S ribosome. Purified initiation factors F_1 and F_2 by contrast have no ribosome dissociation activity and the ability of only partially purified preparations of F_1 to effect dissociation is attributed by Subramanian and Davis to contamination with F_3. They claim that F_3 is the sole dissociation factor, and suggest that each molecule of F_3 remains associated with a 30S ribosomal subunit until it is released during the formation of an initiation complex, whereupon the molecule of F_3 is available to recycle and dissociate another 70S ribosome.

Ochoa and his colleagues, Sabol, Sillero and Iwasaki, have also reached the conclusion that F_3 dissociates ribosomes, but they suggest that this factor may as well function in the formation of the initiation complex. The F_3 which they prepared and supplied to Subramanian and Davis is a basic protein weighing 21,200 Daltons, which can bind to both synthetic and natural messenger RNAs. This binding does not result simply from the basic nature of F_3 because factor F_1 is even more basic, but it fails to bind to the messengers tested. This result led Ochoa and his colleagues to investigate the role F_3 might have in promoting the binding of a 30S ribosome to messenger RNA. They find that both F_3 and F_1 must be present for the formation of a complex between a 30S ribosomal subunit and a messenger RNA. The third initiation factor F_2 is then required for the binding of the initiating fMet-tRNA$_f$ to the ribosome/messenger complex, and after this step has been achieved the 50S subunit can associate and the first peptide bond can be formed. According to Ochoa's group therefore F_3 has a dual function, dissociating 70S ribosomes and promoting the binding of messenger to 30S ribosomes.

Less committed than Davis to the notion that ribosomes running off a messenger remain as intact 70S particles until they meet with a molecule of F_3, Ochoa and his colleagues say that the precise way in which the F_3 factor functions remains an open question. Either as Davis suggests F_3 dissociates from the 30S

ribosome during initiation and then is free to bind to and dissociate a 70S ribosome leaving a messenger, or the F_3 molecules may remain bound, one per 30S subunit, throughout the ribosome cycle. If the latter is the case, ribosomes should dissociate at the termination of a round of protein synthesis; furthermore, the dissociating activity of the factor must be blocked by an aminoacyl or peptidyl-tRNA, otherwise 70S ribosomes would never form. Deciding between these alternatives (and the odds are on the former) will complete the picture of the ribosome cycle in bacterial cells.

REVERSE TRANSCRIPTASES

Roundabouts and Swings

from our Cell Biology Correspondent

On Monday, December 14, Dr Sol Spiegelman, lecturing at the Royal College of Surgeons at the invitation of the Imperial Cancer Research Fund, treated his audience to an adroit review of the prodigious amount of research which he and his colleagues have done on reverse transcriptases in animal cancer viruses and human cancer cells. After briefly discussing the three possible models which account for the replication of RNA viruses in a cellular environment dominated by DNA, and outlining his work on the replication of the phages, Dr Spiegelman reached the meat of his lecture—the significance of the discovery of reverse transcriptases to cancer research and the search for a human RNA leukaemia virus.

The presence of reverse transcriptases in animal tumour viruses makes possible the synthesis of a highly radioactive labelled DNA with a base sequence complementary to that of a tumour virus RNA. If human tumour viruses exist, and if they are related in their base sequences to the animal tumour viruses, such DNA might be an effective probe, in hybridization experiments, for a human tumour virus nucleic acid in a cancer cell. But unfortunately, Spiegelman and his colleagues found in hybridization tests that the base sequences of the various animal tumour viruses are not closely related. This slightly disappointing result means, of course, that there is no good reason to anticipate a close relationship between the base sequences of a putative human tumour virus and any of the animal tumour viruses. DNA complementary to tumour virus RNA is therefore a much blunter probe than everybody hoped it might prove to be.

Synthetic double stranded RNAs and DNAs and RNA/DNA hybrid molecules, on the other hand, have been shown to be more useful than anybody had a right to expect. Certain synthetic nucleic acids are by about two orders of magnitude better templates than any natural nucleic acids so far tested for reverse transcriptases in animal tumour viruses. Armed with this information, Spiegelman *et al.* began to search for polymerase activities, first in the plasma of human leukaemia patients and then in extracts of leukaemia cells. In the cells of more than forty leukaemics, they have now found a polymerase activity which uses poly dT·poly rA as a template, as well as an activity which replicates poly dC·poly dG. This latter activity seems to be present in all proliferating cells irrespective of whether or not they are malignant, but the former activity, which utilizes an RNA/DNA hybrid as a template, seems to be restricted to cancer cells. In addition to being present in leukaemia cells, it has been detected in the cells of two osteosarcomas and one chondrosarcoma. It is interesting, however, that only one of seven Burkitt's lymphoma biopsies, supplied by Dr G. Klein, contained detectable amounts of the poly dT·poly rA polymerase, whereas all contained the activity which replicates poly dC·poly dG.

So far, as Dr Spiegelman took pains to stress, neither his nor anybody else's work on the nucleic acid polymerases in human cancer cells proves the existence of human leukaemia or sarcoma viruses. The polymerase activity which distinguishes human cancer cells from other non-malignant, but proliferating cells, differs markedly from the reverse transcriptase in animal tumour viruses; the two activities can, for example, be differentiated on the basis of their preferences for synthetic templates of various base compositions. But irrespective of any question of the viral aetiology of human cancers, the presence in cancer cells of a polymerase activity which can use a hybrid nucleic acid as a template provides a new tool for diagnosis of cancer and for following the course of remissions and relapses during therapy.

PROTEIN SYNTHESIS

Inside and Outside the Ribosome

from our Nucleic Acids Correspondent

Studies of the structure and assembly of the ribosomal particles from *Escherichia coli* continue to burgeon, and a rich crop is to be found in the November issue of the *Proceedings of the National Academy of Sciences*. Among these articles are the striking results of Chang and Flaks (*Proc. US Nat. Acad. Sci.*, **67**, 1321; 1970) and Craven and Gupta (**67**, 1329; 1970), who have attempted to examine the topography of 30S ribosomal particles by partially digesting them with low concentrations of trypsin or treating them with protein reagents. Chang and Flaks reasoned that if some of the ribosomal proteins are more accessible to the enzyme than others, these will be most rapidly digested. Hence it might be possible to deduce the relative accessibility of the proteins (those which are external and those which are not) in the particle. They therefore analysed the proteins remaining in variously digested ribosomal particles by polyacrylamide gel electrophoresis and compared them with the proteins from undigested controls run in parallel. They report the remarkable finding that those proteins which become associated early with the RNA in the course of the reconstitution scheme for the 30S particles described by Nomura are usually the least sensitive to digestion. Those proteins added at intermediate or late times in the reconstitution are, in contrast, digested more easily. In other words, there is an inverse relationship between the sensibility to digestion of the ribosomal proteins and the order in which they map in the reconstitution of the 30S ribosomal particles. Although there were a few discrepancies and ambiguities, there is, nevertheless, a close agreement to this model considering the relatively unsophisticated method used.

Craven and Gupta report analogous results when the 30S ribosomes are treated with iodoacetate or 2-methoxy-5-nitrotropone (derivatizing —SH and free $-NH_2$ functions respectively). They have also used trypsin insolubilized by attachment to a cellulose matrix, as an agent exclusively restricted to acting at the surface of the particles. This method showed that certain proteins are not attacked by any of these agents, whereas others are consistently accessible to the action of all three. A further class displays intermediate reactivity towards these agents. Once again the accessibility of these proteins is inversely related to their positions in the assembly map.

It is concluded therefore that the proteins which bind to the 16S RNA early in the reconstitution are sheltered within the interior of the 30S ribosomal particle, with the late-binding proteins predominantly towards the outside. This result is a further contradiction of the models of ribosomal structure previously proposed, in which the proteins would be more or less equally accessible to the action of enzymes or chemical reagents, and it reaffirms that the order of assemblage is in part determined by protein–protein interactions.

NUCLEOLI

rRNA Synthesis in *Urechis*

from a Correspondent

THE activities of the fibriller core (which presumably contains the DNA coding for rRNA) and granular cortex of the nucleolus have been extensively and successfully pursued in a wide range of organisms. Cytological studies suggest that RNA is first synthesized in the core and it then moves to the cortex. Biochemical analysis shows that this RNA is the precursor to ribosomal RNA and that this is cleaved and processed to the mature ribosomal particles in the nucleolus. The use of biochemical and cytological techniques in one system has enabled Das *et al.* (*Proc. US Nat. Acad. Sci.*, **67**, 968; 1970) to correlate the labelling of the pre-rRNA components in eggs of *Urechis caupa* with autoradiographs of the nucleoli. The eggs of this marine worm are easily obtained and are free of the follicle cells which interfere with biochemical studies in amphibian eggs. Their slow rate of processing of precursor rRNA makes it easier to follow the fates of all the components. The one very large nucleolus gives good autoradiographs and the rDNA is easily detected in the cores by Feulgen staining. And most important, clean nucleoli can be isolated from the eggs without degradation of the pre-rRNA.

After labelling for 1 to 2 h with ^{3}H-uridine, Das *et al.* found radioactivity in the 38S precursor rRNA (by sucrose gradient centrifugation); silver grains were confined to the nucleolar core. After several hours, the cleaved products of the 38S precursor, the 30S and perhaps 20S precursors to mature rRNA, were labelled, and radioactivity had spread to the cortex. Das *et al.* used actinomycin D to inhibit further RNA synthesis after 2 h labelling and incubation continued for a further 6 h. Then only the 30S and 20S components were labelled and silver grains were confined to the granular cortex. The 38S, 30S and 20S RNA thus methylated after incubation in methyl-labelled methionine. This work probably confirms that the components are ribosomal, but it might be useful to take the experiments further to investigate the processing of the pre-rRNA.

What Das *et al.* did not mention is that the *Urechis* oocyte represents a special case of ribosome synthesis, in which the large size of the nucleolar core seems to be caused by an increased number of ribosomal genes. Dawid and Brown (*Dev. Biol.*, **22**, 1; 1970) have examined the amplification of mitochondrial DNA and of rDNA and have shown that the nucleus of *Urechis* oocytes contains about six times the normal complement of ribosomal genes. Their conclusion is based on hybridization of *Urechis* DNA with *Xenopus* rRNA in non-saturating amounts—not the best of conditions, but their argument seems reasonable. They have shown that *Xenopus* rRNA is about 60 per cent homologous with the rDNA of *Urechis*, fractionated on a caesium chloride gradient, and that the amount of RNA hybridized is proportional to the amount of DNA immobilized on the filters. *Urechis* oocyte DNA hybridized with about three times as much RNA as sperm DNA. Because the oocyte also contains a large excess of mitochondrial DNA, about 60 per cent of the total per cell, it seems that the nuclear DNA contains rDNA amplified at least six-fold.

These results may be compared with those in Amphibia and water beetles, as studied in Birnstiel's and Gall's laboratories. The mature *Urechis* egg contains, according to Dawid and Brown, 12 ng of RNA which is about 3×10^9 ribosomes, some 1,000 times more than in somatic cells. But the rDNA is amplified only six-fold and in one nucleolus, which may be contrasted with the 1,000 or more extra-chromosomal nucleoli, in *Xenopus* oocytes. In both cases sufficient maternal ribosomes are made for early embryonic development, and rRNA synthesis does not need to start again until later in embryogenesis. Thus, amplification of rDNA seems to be associated with extensive synthesis and storage of ribosomes for later use, whether this amplification is in one nucleolus or many. This correlation suggests that the extra chromosomal rDNA may be under different control from the rDNA integrated in the chromosomes of somatic cells. Such a difference would permit oocytes to uncouple the synthesis of ribosomes from protein synthesis, in distinction to somatic cells where these are tightly coupled.

ENZYMES

Spectroscopist's Guide

from our Molecular Biology Correspondent

MANY a sanguine hope has been expressed about the promise of various spectroscopic techniques for determining stereochemical details of interactions at active sites of enzymes, and observing catalytic events. As long as X-ray crystallography remains a static method, they are obviously worth nurturing, even if so far few very memorable results have accrued. Some interesting explorations, using fluorescence, electron spin resonance and nuclear magnetic resonance are to be found in the November issue of the *Proceedings of the National Academy of Sciences.*

Latt, Auld and Vallee (**67**, 1383; 1970) have constructed an excitation transfer system to observe the interaction between carboxypeptidase and its substrates. Three chromophores are involved: tryptophan residues and the cobalt atom (replacing zinc) in the protein, and a dansyl group, attached to a peptide substrate at its N-terminus. The conditions for energy transfer between two chromophores—that the absorption spectrum of the acceptor must overlap with the fluorescence spectrum of the donor—is doubly fulfilled, in that the tryptophan fluorescence overlaps with the dansyl absorption, and the dansyl emission with the cobalt absorption. The enzyme–substrate interaction can then be observed during turnover in a stopped-flow machine by looking at the quenching of the protein tryptophan fluorescence by the bound substrate. In the zinc enzyme this is accompanied by the growth of dansyl emission, which in the cobalt enzyme is wholly or partly extinguished by transfer to the quencher, cobalt. The extent of the latter effect depends on the distance between the dansylated N-terminus of the peptide, and the cobalt, which is close to the C-terminus, where hydrolysis occurs. The extent of transfer depends on the sixth-power of the separation, and from the overlap, and other known parameters, the distance between the cobalt and the dansyl can be calculated, and agrees well with values from model-building for a series of peptides, assuming extended chains. Many such systems could no doubt be devised, and somewhere, some time, if there is any justice, there will be a problem ripe for the plucking.

Another method, which has enormous steric sensitivity, and can be applied to metallo-enzymes, is electron spin resonance. Taylor, Mushak and Coleman (**67**, 1410) have examined the copper and cobalt forms of carbonic anhydrase, also in nature a zinc enzyme. The hyperfine structure of the spectrum reflects the coordination state of the metal, which in the inactive copper enzyme can be deduced to be in the square planar form. The interaction with nitrogenous ligands engenders large changes in the spectrum, from which the degree of covalent character in the binding can be deduced. In the active cobalt enzyme, interpretation of the spectra is more hazardous. The authors suggest that the zinc and cobalt complexes admit of more distortion than the copper, and that this is favourable for ligand exchange. They suggest an equilibrium between square planar and approximately tetrahedral configurations, the latter being involved in the transition states.

Sarma and Kaplan (**67**, 1375) have used nuclear magnetic resonance at 220 MHz to study the interaction of NAD with lactate dehydrogenase. In free solution the co-factor is in the folded, stacked state, as shown by the shifts due to interaction of protons with the currents in the aromatic rings. The barrier for interconversion between the stacked and open forms is low. From the binding constant of NAD with the protein, the proportions of free and bound NAD can be calculated. The spectrum of firmly bound NAD would undergo line-broadening to the point of vanishing, whereas the observed appreciable but not inordinate broadening of all lines can be explained in terms of rapid exchange of bound and unbound co-factor. The ring-current shift is retained on binding, and it is deduced from this and other evidence that the NAD is bound in the folded form. Now this is at odds with the unequivocal crystallographic evidence of Rossmann and his group, as is the statement that nicotine mononucleotide in the presence of AMP does not bind to the enzyme. It seems then that Sarma and Kaplan's hare will not run, in spite of a careful and seemingly convincing analysis of the NMR spectra. One must suppose that they are the victims of an unlucky circumstance (or Murphy's law, as it used to be called), whereby an interaction with an aromatic ring in the protein generates a ring current equal to that arising in the stacked form of the NAD alone. The situation might be further complicated by rapid exchange between the two conformational forms of the enzyme, which, as Rossmann and his colleagues found, interconvert in response to the binding of the adenine ring. The failure of this change in Sarma and Kaplan's conditions would explain both the absence of interaction with nicotinamide mononucleotide and the retention of the stacked conformation in the co-factor. It is even possible that their enzyme (from chicken heart) behaves in a different way from the dogfish enzyme used by Rossmann's group.

ICHTHYOLOGY

Brotulid from the Bahamas

from our Marine Vertebrate Correspondent

THE brotulid fishes present a diversity surprising even to the ichthyologist. They range in size from more than 1 m long to dwarf forms of 50 mm when adult. Most of the 150 or so species that have been described are marine fish, many of them inhabitants of the deep sea; indeed, the deepest known fish from the oceans is a brotulid *Bassogigas* from the Puerto Rico Trench at a depth of nearly 8,000 m (*Nature*, **226**, 501; 1970). It is surprising therefore that other brotulids live in freshwater in the Caribbean region. An interesting new freshwater species, *Lucifuga spelaeotes*, has now been described by Daniel M. Cohen and C. Richard Robins (*Proc. Biol. Soc. Wash.*, **83**, 133; 1970) from a habitat near Nassau in the Bahamas.

Two freshwater species have long been known from caves in the island of Cuba, and Cohen and Robins regard *L. spelaeotes* as congeneric with the two Cuban fishes. Strangely, this group of three species does not seem to be closely related to the Mexican freshwater cave brotulid *Typhliasina*. Cohen and Robins consider that the three species are instead most closely related to the known deep water genus *Cataetyx*, although a moderately close relative, *Calamopteryx*, a small secretive coral reef fish, lives in the tropical West Atlantic. The common ancestor of both *Lucifuga* and *Calamopteryx*, Cohen and Robins conclude, "may await discovery, hidden in the crevices of a tropical Atlantic reef".

The Bahaman species was discovered by Cohen and



Lucifuga spelaeotes, 109 mm long.

Robins in the Mermaid's Pool, a little known limestone sink near Nassau on New Providence Island. This pool is about 75 feet across, with several small openings nearby; the water level is that of the exposed water table. Its salinity is low, about 4·6 parts per thousand. Only two specimens were collected by a diver at a depth of 5 m. The species grows to at least a length of 10·9 cm. Its body is covered with small scales, but the head region is naked. Very large pores on the head open on to a cavernous sensory canal system, and there are well-developed sensory papillae along the sides of the body. The eye is small but relatively well developed and has a lens protruding through an elongate clear window of the outer skin. The fish is thus obviously able to see (in contrast to some of its other cave-dwelling and deep-sea relatives, which are blind), as well as to take full sensory advantage of its near stygian habitat. The colour of the only known specimens is a rich walnut brown, with rather lighter edges to the fins, although Cohen and Robins describe the naked skin in the mucous chambers of the head as an intense white.

The outlook for the continued survival of this fascinating fish can only be described as bleak. New Providence Island is the scene of a booming real estate development, and much of the area surrounding Mermaid's Pool has already been cleared for property development. Even if the pool survives the immediate programme of building (and inland waters have a knack of bringing out the worst in building man), can it survive the almost inevitable pollution which water extraction, sewage effluents, accidental kerosene leakage, and garbage will produce?

MEMBRANES

Transport Systems

from a Correspondent

How cell membranes are organized to perform various specialized functions has been and will continue to be an intriguing problem. That this problem is capable of being solved, however, seems no longer in doubt. This was the generally optimistic view which emerged from a meeting held at the Royal Society on December 10 and 11 to discuss the mechanisms for the active transport of salts and water in living tissues. In the past, progress in this field has depended on the representation of transport processes as simple model systems. An early warning of the dangers of implicit belief in such models was provided by Professor H. H. Ussing (Institute of Biological Chemistry, University of Copenhagen). The critical approach should show that simple models will have only a limited use in explaining how complex tissues operate. Future developments are more likely to arise from an appreciation of how tissues work as a whole, coupled with a detailed knowledge of how transport processes operate at a molecular level.

Nevertheless, the selection for study of epithelia peculiarly suited to perform single functions can still be expected to show how more general processes might work. That such epithelia exist was reinforced through contributions by Professor J. A. Ramsay (Zoology Department, University of Cambridge), on the extraordinary ability of insects to recover water from relatively dry vapour held within the rectum; by Dr K. Zerahn (Institute of Biological Chemistry A, University of Copenhagen), on the peculiarities of potassium transport by the midgut of the American silkworm and by Professor E. J. Denton (Laboratory of the Marine Biological Association, Plymouth) on the possibilities of water and salt transport by epithelia subjected naturally to high hydrostatic pressures.

The association of many epithelia with layers of non-transporting cells has in the past added considerably to the difficulties inherent in studying epithelial transport, but the use of new techniques should lessen these difficulties. Dr D. Erlij (Centro de Investigacion y Estudios Avanzados, Instituto Politecnico Nacional, Mexico DF) described how epithelia could be separated from underlying tissues in the frog skin through the combined use of collagenase and hydrostatic pressure, and Dr M. W. Smith (ARC Institute of Animal Physiology, Cambridge) showed how transport into the intact intestinal epithelium could be studied for periods of time independently from the underlying tissues. In other cases, where there is little opportunity of simplifying the tissue studied, adaptational changes can be used to investigate transport mechanisms. Dr J. Maetz (Groupe de Biologie Marine, Station Zoologique, Villefranche-sur-Mer), for example, spoke about the reversal of sodium transport which takes place in fish gills as they adapt to environments of different salinity. The initiation of membrane changes seems to be induced by changes in blood sodium. Kinetic studies of sodium fluxes in whole fish indicate that new transport sites are being created during adaptation.

A feature of the meeting was the continual reference to the work of Dr J. M. Diamond (Physiology Department, Center for Health Sciences, Los Angeles, California). His original suggestion that intercellular spaces can function as standing-gradient flow systems and that this largely explains the coupling of water to active solute movements is now widely accepted. Clearly, many workers are now re-evaluating their own findings in the light of this still relatively new concept.

STELLAR CLUSTERS

Disruption by Interstellar Clouds

by our Cosmology Correspondent

THE importance of interstellar clouds as disruptive influences on open star clusters has only recently been studied in detail, because of the lack of sophisticated high-speed computers. P. Bouvier and G. Janin have now tackled this problem by numerical experiments, using the CDC 3800 of Geneva University (*Astron. Astrophys.*, **9**, 461; 1970).

Earlier rough analytical work has shown that for interactions occurring at distances comparable with the cloud radii repeated disturbance of a cluster by passing clouds produces a dispersion of its component stars, resulting in the dissolution of the cluster after some 2×10^9 years. Not surprisingly, distant encounters have little effect on the star cluster (compare the results of L. Spitzer, *Astrophys. J.*, **127**, 17; 1970). Like earlier workers, Bouvier and Janin have used the impulsive approximation for the collisions, assuming that the duration of an encounter is much less than the typical periods of stellar motions within the cluster. They have also neglected the tidal influence of the galaxy, so their results can be taken as an upper

limit for the lifetime of these clusters, but their numerical calculations implicitly include the effects of all encounters, ranging from the very distant to close near collisions.

For a cluster containing an initial population of twenty-five stars, the total energy was dissipated over a period of $4{\cdot}5 \times 10^8$ years, as a result of interactions with clouds distributed randomly with a number density of 4×10^{-5} pc^{-3}. In the same period of time, a similar model cluster of twenty-five stars, left to evolve without any interference from interstellar clouds, maintained its original total energy to within 0·1 per cent. The lower value for the cluster lifetime found by this technique shows, when compared with Spitzer's work, that close encounters with interstellar clouds are of great importance in the disruption of open clusters. The process seems therefore to be similar to the evaporation process, where a large number of distant interactions cannot give an individual star sufficient energy to escape from a cluster, but close encounters with other stars can give it a net positive energy.

METEOROLOGY

Hot Line to Weather

from our Geomagnetism Correspondent

HOT lines, hitherto restricted to politicians anxious to avoid the hazards of instant war, are now to be used to combat another potential source of catastrophe —the weather. In any period of 24 hours, more than 100,000 observations of weather conditions at the Earth's surface and more than 11,000 observations of the upper atmosphere are recorded at about 8,000 land stations scattered throughout the world, aboard 4,000 merchant ships and on 3,000 transport and reconnaissance aircraft. The transmission of this growing quantity of data to a central point for the preparation of six-hourly weather charts presents a formidable problem of information exchange. Present communications systems are in danger of being swamped.

To come to terms with this problem, the World Meteorological Organization, an agency of the United Nations, set about planning the World Weather Watch in 1967. The first phase of the new global telecommunications system, a hot line between Washington and Tokyo which transmits information at the rate of 3,000 five-character words a minute, came into use last week. If all goes according to plan, the high-speed network will be extended to other major world cities during the next five years, though in most cases this will just mean speeding up existing links. Among the expected benefits of the new system is longer range forecasting.

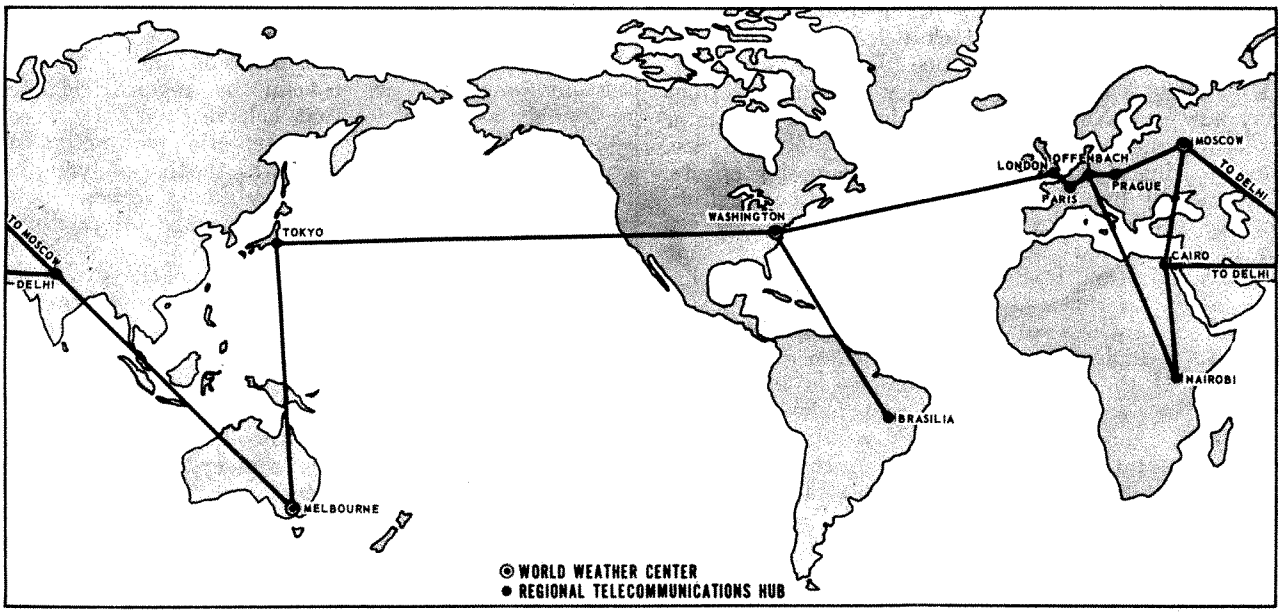
TEKTITES

Australite Analyses

from our Geomagnetism Correspondent

THE origin of tektites is as obscure as ever. So, in the meantime, work continues on individual samples and groups of samples in an attempt to determine how many different tektite falls there have been and how many different mineralogical types exist. The ultimate hope is that from detailed chemical comparisons some unifying property will emerge to permit an unambiguous interpretation of tektite origin.

That many more hard data are required before even simple relationships can be defined properly is evident from, for example, the problems which have arisen in connexion with the Australian tektite field. The microtektites found in deep sea sediments close to the tektite strewn fields of Australasia and the Ivory Coast apparently fall into two distinct chemical groups—the so-called "bottle green microtektites" which have low silica and high magnesium contents, and the so-called "normal microtektites", the chemistry of which is very similar to that of the Australasian tektites, especially in that the silica contents are high. It is debatable, however, whether there really are two distinct types of microtektite. It is sufficient to say that the range of microtektite compositions is apparently wider than the range of tektite compositions.



Proposed high-speed global telecommunications system forming the World Weather Watch.

In particular, there is a lack of reports of high magnesium tektites, the continental equivalent of the deep sea bottle green microtektites.

This lack of symmetry has led some people to question the identification of the Australasian microtektites. Other workers, on the other hand, believe that the absence of high magnesium tektites is more apparent than real, the problem being merely one of unrepresentative sampling. Moreover, and for a similar reason, the compositional range of even the normal tektites may not be as restricted as it has hitherto appeared. Glass, for example, has been carrying out detailed electron microprobe analyses of a single flanged australite (Australian tektite) and has found a remarkably wide range of composition within the one sample (*Earth Planet. Sci. Lett.*, **9**, 240; 1970). In particular, the silica content varies from 59 per cent to 85 per cent, which easily encompasses the 59 per cent to 76 per cent range of previously analysed normal microtektites. Furthermore, it compares with the previously reported range of 69 per cent to 79 per cent silica for twenty-three australite analyses. The point is that if a single australite can vary to this extent, similar analyses of other australites could easily result in a wider silica range which would encompass all microtektites whether normal or bottle green.

Thus, contrary to the impression given by previous limited data, Australasian tektites probably have a wide range of compositions. If this is the case, there is then no reason why the tektites and adjacent microtektites should not have had a common origin. Such a conclusion has always had strong circumstantial support but it has, until now, seemed to contradict the evidence from detailed analyses.

LIQUIDS

New Light on Solutions

from our Statistical Mechanics Correspondent

WHAT exactly happens when two liquids "mix" or when a solid is "soluble" in a liquid? This may be one of the oldest problems of physical chemistry but perhaps it is beginning to crack at last; some preliminary studies by Weres and Rice (*J. Chem. Phys.*, **52**, 4475; 1970) suggest that a theory of liquid solutions may now be possible. In solid solutions the atoms or molecules involved are usually about the same size, thus creating a balance between two opposing effects. Energetically like atoms usually prefer to be together. On the other hand, "mixed-up" configurations are far more numerous than "segregated" ones, so that thermal agitation tends to cause mixing of unlike molecules. In thermodynamic language, "there is a gain of entropy as a result of mixing" and, if the temperature is high enough, this can balance a loss of energy. If there were no difference in interaction energies between pairs of like and pairs of unlike molecules, "good" solubility could result (miscibility in all proportions), for the same reason that a mixture of red and blue grains of sand assumes a uniform colour when well stirred up.

This problem, of following qualitatively the balance between energy and entropy in a solid solution, has been studied, with various degrees of mathematical subtlety, since about 1930 a very important landmark being Onsager's famous *Physical Review* paper of 1944. Paradoxically therefore solid solutions are better understood than liquid solutions. One solid can be dispersed in another without, in many cases, any significant change of lattice structure, but this is probably never true of liquid solutions.

In a liquid, the interchange, by thermal agitation, of a pair of unlike molecules changes not only the energy and entropy, but also the immediate surroundings of both of these molecules. A liquid does not possess an actual lattice structure, but a "distribution function" can be defined that describes the average surroundings of a typical molecule. This function can be determined by X-ray and neutron scattering and, since about 1960, there have been theories for calculating it with reasonable accuracy. What still has to be allowed for, in discussing the solution process, is that the immediate surroundings of a large molecule may be very different from those of a small one.

Much theoretical work has been done on mixtures of non-attracting rigid spheres of different sizes, partly analytical, partly based on the use of large computers. Not surprisingly, theory predicts that, without interactions, there should be miscibility in all proportions. It should now be possible to put together a theory that balances the rather subtle geometric effects against the direct effects of intermolecular interaction and thermal agitation.

In some well known work in 1967, Barker and Henderson (*J. Chem. Phys.*, **47**, 2856 and 4714; 1967) showed that, if the properties of a (fictitious) assembly of hard non-interacting spheres are regarded as known, it is possible to devise a perturbation procedure that allows for the "softness" of real molecules and for the fact that, at large distances, they attract one another. This approach has been followed up by various other workers with very promising results. (It is obviously better to take the "rigid sphere fluid" as a starting point than to begin, as older theories did, with a solid or gas-like structure.) Many details remain to be worked out, but it is clear that this approach has come to stay. More recently, Weres and Rice have reported work on a perturbation treatment of liquid mixtures, starting from the now fairly well known properties of mixtures of non-attracting rigid spheres of two sizes. They studied the mixtures hydrogen–argon and methane–tetrafluoromethane which can be regarded fairly reasonably as molecules that behave as if they were spherical. After adjusting the interaction parameters to give the best possible agreement with the thermodynamic properties of the substances, they used a rather rough and ready procedure for estimating the interactions between unlike molecules and then attempted to calculate the thermodynamic properties of the mixtures. The agreement with experiment is reasonable for hydrogen–argon, less good for methane–tetrafluoromethane.

As always in this type of work, it is difficult to separate out the effects of the approximations used from the effects of our imperfect knowledge of the interactions. Nevertheless, the preliminary results of Weres and Rice suggest that recent work on mixtures of rigid spheres may be a satisfactory starting point for a theory of liquid solutions. As always, there are nagging doubts about the best way to set up a perturbation treatment, the rapidity of its convergence and the like, but these will not deter the enthusiast.

Wordsworth and Hamilton

by
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The 200th anniversary of Wordsworth's birth seems to be a good time to recall one of the most interesting and best documented associations between an outstanding poet and a major scientist. The Irish mathematician Hamilton and the English poet Wordsworth were friends and correspondents for more than twenty years. Each helped to mould the ideas of the other significantly.

It comes as a surprise to most scientists to think of Wordsworth—the leader of the romantic poets—as having an interest in and expressing opinions on scientific matters. That he did so was largely due to his friendship in his later years with the Irish mathematical-physicist William Rowan Hamilton. The bond which first brought them together and held their active interest in a correspondence lasting more than twenty years was Hamilton's poetry. From his early youth and concurrently with his mathematics, Hamilton had been a prolific poet. For several years (1822–1829) the young Hamilton could not decide whether to abandon mathematics for poetry and wondered whether he had a true instinct for poetry. What was more natural for the young mathematician, distinguished at so early an age, than to seek the advice of the current doyen of English poetry. Wordsworth in his later years spent considerable effort in advising would-be young poets. He took a particular interest in the young Hamilton and from this grew a deep mutual respect. Later Wordsworth was to say that the only two people in whose presence he felt inferior were Coleridge and Hamilton.

Hamilton was born in Dublin in 1805 (ref. 1). His father, a solicitor, had defended the outlawed Irish patriot Archibald Hamilton Rowan and had obtained a reversal of his sentence. From Rowan, who acted as sponsor at the baptism of the infant William, the boy received his second Christian name. His early development is well known. He was at three a superior reader of English and was considerably advanced in arithmetic. At five, besides being a good geographer, he was able to read and translate Latin, Greek and Hebrew and he loved to recite long passages from Dryden, Milton and Homer. And so he continued until at the age of thirteen he could speak a language for every year that he had lived. Mathematics was not neglected, however, and in his seventeenth year, while heavily engaged in classical studies for university entrance, he records that he was also reading the works of Newton, Lagrange and Laplace. He discovered a mistake in the reasoning in the *Mécanique Céleste* of Laplace and brought it to the attention of Brinkley, the Astronomer Royal of Ireland. The first sign of his creative ability came in a letter to his sister Eliza in the same year, in which he spoke of "some curious discoveries". These were the essentials of what he developed in the next five years into his *Theory of Systems of Rays*. In it he explicitly recognized that optics and dynamics are essentially a single mathematical subject. His plan was to characterize or describe any optical or dynamical system by means of a single mathematical function and has led to one of his enduring memories, the familiar Hamiltonian functions of classical and quantum mechanics[2,3].

The year of Hamilton's birth, 1805, was also a landmark in Wordsworth's life[4]. The poet was in his thirty-fifth year and had been married for three years. In this year his brother John was drowned. This family tragedy moved him deeply and turned his thoughts towards more spiritual things. There began a more mature and less hectic phase of his poetry. The earlier, bolder, Wordsworth who lived in revolutionary France during 1791–92 (and who returned to England in 1792 leaving behind a daughter who had been born out of wedlock) matured into a sober middle-aged Anglican poet. The synergistic creativity of Wordsworth and Coleridge which produced *The Lyrical Ballads* in 1798 and began the romantic movement in poetry was over by 1805 (ref. 5).

From 1802 to 1822 Wordsworth wrote most of his major mature works including "The Excursion", "The Immortality Ode", "The White Doe" and "The Prelude" (which was not published in his lifetime). During this period Hamilton had come of age, intellectually, and had entered Trinity College, Dublin, at the end of 1823 to begin a career that was brilliant in both classical studies and mathematical research. His letters show that he first began to write poetry at the age of fourteen. The visit of King George IV to Dublin in 1821 excited the young Hamilton and he began to produce his first mature verse. At Trinity College his prizes for science and classics were complemented by prizes for his verse. The last year of Hamilton's undergraduate studies, 1827, is an important landmark both for his mathematical research and for his relationship with Wordsworth. Newton had died exactly one hundred years earlier in 1727. In the ensuing century the initiative in mathematics was taken by the continental schools[6] and the imposing edifice of classical mechanics was erected, notably by Lagrange and Laplace. This phase ended with the death of Laplace in 1827. Hamilton, while still an undergraduate, consciously tried to continue and extend Lagrangian dynamics. In 1827, before he graduated, he had brought his youthful discoveries to fruition and in April of the same year he presented his *Theories of Systems of Rays* before the Royal Irish Academy[7]. Later in the same year, though still an undergraduate, he was elected as Professor of Astronomy and Astronomer Royal of Ireland, over the heads of established English astronomers.

Hamilton was always supremely confident of his mathematical ability. He was, however, unsure of his poetic ability and took steps to gain a critical appraisal in 1827. Following the excitement of his election as professor of astronomy in June, he went on an extensive tour of Ireland and England culminating in a visit to the Lakes and Wordsworth in September[8]. A deep rapport was instantly established between the two men. In after years Wordsworth referred with pleasure to their first meeting. Hamilton and his friends had tea and spent the evening with the Wordsworths at Rydal. Wordsworth walked back with his guests to their lodgings about a mile away near Ambleside. Here, such an interesting conversation had developed that Hamilton decided to accompany Wordsworth back to Rydal. On arrival there Wordsworth decided they could not break off the conversation and so

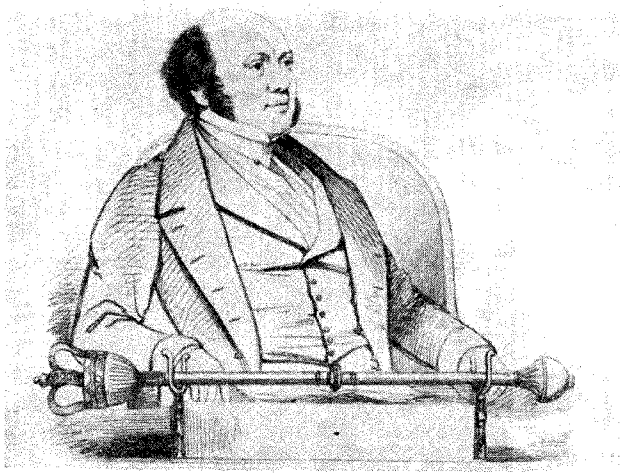


Fig. 1. W. R. Hamilton.

they turned around and walked back to Hamilton's lodgings, where once again they turned and walked back to Rydal, Hamilton returning at a very late hour. His meeting with the poet inspired him to compose a poem "It Haunts me Yet" which he sent to Wordsworth for approval[9]. In reply Wordsworth wrote "You will have no pain to suffer from my sincerity. With a safe conscience I can assure you that, in my judgement, your verses are animated with true poetic spirit, as they are evidently the product of strong feeling,...you will not, I am sure, be hurt, when I tell you that the workmanship (what else could be expected from so young a writer ?) is not what it ought to be.

> "Some Touch of human sympathy find way
> And whisper that while Truth's and Science's Ray
> With such serene effulgence o'er thee shone"

Sympathy might whisper, but a Touch of sympathy could not. "Truth's and Science's Ray for the ray of Truth and Science is not only extremely harsh, but a ray shone is, if not absolutely a pleonasm, a great awkwardness; a ray fell or shot may be said, and a sun, or a moon or a candle shone, but not a ray."

Similar comments were made on some other parts of the poem. Hamilton made a spirited reply justifying his choice of certain terms but still felt unsure of himself as a poet. He concluded: "I am, I assure you, sincerely conscious of the general defects of my poetry and deeply feel the little likelihood that there is of one so devoted to Science as myself ever attaining a high place in the ranks of poetical composition".

In 1829, at Hamilton's suggestion, Wordsworth fulfilled a long-cherished desire by visiting Ireland[10]. Unhappily Ireland was then, as now, in a "rioting time" and Wordsworth wondered if it was safe to come but was reassured by Hamilton. In spite of home sickness and anxiety for his family, the tour was one of the most energetic made by Wordsworth. Of his tour in the county Kerry he wrote: "We left Kenmare each on a vile Irish hack horse at five in the morning...Rode 11 Irish miles (about 15 English). Took to our feet, clomb and clomb till we reached the summit of a ridge, descended,...Took the mountain of Carranthouel, the highest in Ireland,—having taken nothing all day but a bad breakfast—one crust of bread and two basins of milk and a glass of whiskey"[4].

The tour began with Wordsworth visiting Hamilton and his family. Hamilton's sister Eliza has left a detailed account of the visit[10]. When the ladies entered the drawing room before dinner they found Wordsworth reading some of "The Excursion" aloud to his host. "The poet hastily turned round, with a gesture of politeness, moving his face, and indeed his whole body, in the direction to which we passed." The passage which he had been reading had displeased Hamilton by what appeared to be its "slight reverence for science". It was evidently those lines in the fourth book[11] in which the wanderer criticizes the "presumption" of men of science who "pry" and "pore".

> "Viewing all objects unremittingly
> In disconnection dead and spiritless;
> And still dividing, and dividing still,
> Break down all grandeur, still unsatisfied
> With the perverse attempt, while littleness
> May yet become more little; waging thus
> An impious warfare with the very life
> Of our own souls! . . . .
> Philosophers, who, though the human soul
> Be of a thousand faculties composed,
> And twice ten thousand interests, do yet prize
> This soul, and the transcendent universe
> No more than as a mirror that reflects
> To proud self-love her own intelligence;
> That one poor finite object, in the abyss
> Of infinite Being, twinkling restlessly!"

This attitude which Wordsworth had maintained ever since these lines were written in 1798 he now had to defend in the presence of a scientist.

"Wordsworth", says Miss Hamilton, "first finished the passage, in a very low impressive tone, moving his finger under every line as he went along, and seeming as he read to be quite rapt out of this world. He then defended himself, with a beautiful mixture of warmth and temperateness, from the accusation of any want of reverence for science, in the proper sense of the word—on the contrary he venerated it 'when legitimately pursued for the elevation of the mind to God'. But science as the mere accumulation of facts, and above all science which waged war with and wished to extinguish imagination in the mind of man was worse than useless. He would rather be a superstitious old woman than have his mind engrossed with knowledge that was merely material in its aims and uses. As for Newton, and men of science such as he, to whom he paid no tribute in "The Excursion"—" 'What', he said, 'would have been the use of my praising such men as Newton ? They do not need my insignificant praise, and therefore I do not allude to such sons of science'." Yet not very long after his discussion with Hamilton, he made to Newton the most famous allusion ever made to a scientist. In the third book of "The Prelude" he had spoken of the statue of Newton in the chapel of Trinity College, Cambridge

> " . . . Newton, with his prism and silent Face"

When, early in 1832, he began his great revision and correction of "The Prelude" texts, he added to this line two more

> "The marble index of a mind for ever
> Voyaging through strange seas of thought, alone."

Whatever had been lacking before in tribute to Newton was now supplied in marvellous perfection. It is likely that the memory of that evening in Dublin with Hamilton played its part in his decision to make the change.

An immediate effect of the visit was that Wordsworth managed to persuade Hamilton that poetry absorbed the whole man and that he must renounce the hope of habitually cultivating both poetry and science. Characteristically, Hamilton's decision was made concrete in a poem "To Poetry" which concludes

> "Spirit of Beauty, though my life be now
> Bound to thy sister Truth by solemn vow;
> Though I must seem to leave thy sacred hill,
> Yet be thine inward influence with me still;
> And with a constant hope enquire,
> And with a never quenched desire
> To see the glory of your joint abode,
> The home and birthplace, by the throne of God."

In spite of his good intentions, Hamilton continued to produce poetry, some of which was submitted for criticism to Wordsworth. From time to time Wordsworth further urged him not to get engrossed in poetry to the detriment of his mathematics. Two years later Wordsworth writes "You send me showers of verse, which I receive with much pleasure, as do we all yet have we fears that this employment may seduce you from the path of Science which you seem destined to tread with so much honor to yourself and profit to others".

In the early 1830s Hamilton made important advances in his optical and dynamical researches. Although the two basic principles of least time in optics and least action in dynamics had been known for many years, he was the first to appreciate that there is a close mathematical analogy between the path described by a ray of light in a medium and the path described by a material particle in a field of force[12]. He quickly realized that the methods which he had discovered earlier in optics could be extended to dynamics[3,13]. This application of his methods culminated in his famous paper in the *Philosophical Transactions of the Royal Society* in 1834—"Essays On A General Method in Dynamics"[14]—in which the methods of what is now called Hamiltonian mechanics were outlined. This work attracted a good deal of attention and in 1835, on the occasion of the meeting of the British Association for the Advancement of Science in Dublin, he was knighted at the age of thirty. For Wordsworth it was a case of "Whether Sir William or plain Mr. Hamilton be you assured of my affectionate admiration". The friendship between the Hamilton and Wordsworth families was further cemented by Hamilton becoming, at Wordsworth's request, a godfather to one of his grandchildren.

In their correspondence, Wordsworth was interested, as a layman, in scientific matters but regretted his lack of knowledge. In a letter thanking Hamilton for a gft of his "Introductory Lectures on Astronomy" he said: "Your lectures I have read with much pleasure. It is philosophical and eloquent and instructive and makes me regret, as I have had a thousand occasions of doing, that I did not apply to Mathematics in my youth. It is now, and has long been, too late to make up for the deficiency". Wordsworth was critical about the behaviour of some of his contemporary scientists. Just before the meeting of the British Association in 1833 he wrote to Hamilton: "I hope that in the meeting about to take place in Cambridge there will be less of mutual flattery among the men of Science than appeared in that of the last year in Oxford. Men of Science in England seem inclined to copy their fellows in France, by stopping too much out of their way for titles and baubles of that kind, and for offices of state and political struggles which they would do better to keep out of".

When Hamilton became a friend of Wordsworth, it was inevitable that in time he would meet Coleridge. Coleridge's poetry had flourished in the six years when he was in constant contact with Wordsworth (1797–1803). When Hamilton came to know him in 1832, two years before the poet's death, it was in the time of Coleridge the philosopher and theologian rather than the poet. Hamilton, like Coleridge, was fond of metaphysical speculations. His metaphysical thinking and reading provided the intellectual framework which led to his conceptual breakthroughs in dynamics and algebra[15]. Wordsworth provided Hamilton with an introduction to Coleridge and he paid a visit to the ailing philosopher, in London in 1832. This led to an interesting correspondence on the philosophical notions of matter and atoms. From it we learn that Hamilton, like his contemporary Faraday, was influenced by Boscovichean atomism[16]. Wordsworth was delighted that his two great friends had met each other and had philosophical ideas in common.

For both Hamilton and Wordsworth 1843 was a particularly important year. Wordsworth was appointed poet laureate in April. In a letter to Hamilton he relates how initially he refused the flattering invitation on the grounds of his advanced age but that eventually, as a result of the urgings of the Lord Chamberlain and Sir Robert Peel, he accepted. For Hamilton it was the year in which he discovered quaternions[17,18]. During the previous fifteen years he had been studying problems in the algebra of imaginary numbers. How, he wondered, can imaginary numbers, which can be represented in the plane by the Argand diagram, be generalized and represented in space[19]. The answer, which came to him literally in a blinding flash, was that although a two term expression sufficed for the Argand diagram, a four term expression—a quaternion, and not a three term expression as had been commonly supposed—was necessary for the three dimensional situation. It was also necessary to do what was then virtually unthinkable, to violate a familiar algebraic operation and introduce the notion of non-commutative multiplication[20]. These novel concepts were seminal for the development of linear transformations, matrices, and vector analysis by the British mathematical schools of the nineteenth century.

From this year to Wordsworth's death in 1850 there was less contact but no diminution in affection between the two. Hamilton threw his immense energy into the exploitation of quaternions and still found time to write poetry. The ageing Wordsworth, who found it increasingly difficult to read and write, spent his time agitating about the incursion of the railroad into the lakes, besides his official duties of laureateship and the more pleasant duty of playing the grand host at Rydal[4]. Hamilton paid his last visit to Wordsworth in 1844 (ref. 4). On a hot July day, they walked, with mutual friends, to Loughrigg Tarn. The familiar scene stimulated Wordsworth to write a happy little poem in a new form of verse—triple measure.

Wordsworth's death in 1850 deeply grieved Hamilton. He thought of those "personal visits which are among my brightest and fondest recollections". Fittingly, some time afterwards, he commemorated the passing of his friend and mentor in a religious poem "To An Afflicted Friend".

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Transfer RNA Methylases and Cancer

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There is much evidence to suggest that tRNA methylases in tumours differ from the enzymes in normal tissues. The experimental basis and possible significance of the changes in activity and in specificity of these enzymes are discussed in this article.

ABOUT ten years ago the rather unexpected discovery was made of a group of enzymes which methylate nucleic acids at the polynucleotide level. It had been known for some time that small amounts of methylated bases were present in RNA from various sources[1,2], but it had been tentatively assumed that this could be explained by incorporation of the bases during nucleic acid synthesis, rather than by modification at the macromolecular level. This assumption was proved wrong during work on a methionine auxotroph of *Escherichia coli*.

Most amino-acid auxotrophs synthesize RNA only when the amino-acid is supplied in the medium; if it is not, synthesis of both protein and RNA is inhibited. But although the *E. coli* in question was unable to synthesize methionine and so required an external supply for growth, it continued to synthesize RNA in the absence of the amino-acid. The RNA formed in these conditions was deficient in methylated bases[3] but became methylated when methionine was added to the incubation medium[4]. The enzymes responsible, the nucleic acid methylases, have since been found in almost every type of cell system investigated. The function of this universal methylation of tRNA remains obscure, however. Because current concepts had no obvious need for tRNA to be methylated in order to carry out its functions in protein synthesis, it was suggested that methylation was involved in the control of protein synthesis at the level of translation. In determining what type of protein is made it would thus determine the type of cell formed and so control differentiation. Thus, as cancer can be considered to be a disease of differentiation, involving essentially a change in cell type, it was conceivable that changes in tRNA might be responsible for carcinogenesis. The alterations in tRNA which have been studied most in relation to carcinogenesis have been changes in methylation.

Simultaneous with the proposal that changes in tRNA methylases might be involved in carcinogenesis, a different line of work led to the suggestion that methylation of nucleic acids was significant in the action of chemical carcinogens. This work began when it was found that administration of the carcinogen dimethylnitrosamine to the intact animal led to the methylation of liver and kidney DNA and RNA[5]. Methylation occurred chiefly on the N^7-position of guanine. Dimethylnitrosamine is metabolized in liver and kidney to form an alkylating intermediate. Other nitrosamines, nitrosamides, and N-methyl-N-nitroso-N′-nitro-guanidine act as carcinogens for different organs, and in each case where alkylation of nucleic acids has been studied, with a few possible exceptions, it has been shown to occur in organs which would have developed a tumour if the animal had been kept alive[6]. Thus there was debate as to whether cancer could be due to alkylation of RNA or of DNA.

When the nucleic acid methylases were discovered it seemed possible that certain carcinogens, such as nitrosamines and nitrosamides, might act by causing direct alkylation of nucleic acids, while others, which could not act as alkylating agents themselves, might bring about a similar end result by causing an increase in activity or an aberrant activity of the methylase enzymes. For these reasons, investigations of nucleic acid methylases in relation to cancer began in several laboratories. Evidence was found for an increase in activity and a change in specificity of the enzymes in various tumours. The sequence of events which brings about these changes is not known, and the exact nature of the changes is not understood. But, perhaps most important in the present context, it is not known whether the changes observed are an essential part of carcinogenesis, or whether they are coincidental to the malignant process.

Properties of tRNA Methylases

tRNA methylases are probably located in the nucleus of the cell[7], the tRNA being methylated as it is synthesized and before it passes into the cytoplasm. The tRNA methylases alkylate each of the four major bases and also the 2′ position of the ribose moiety[8]. Although the enzymes responsible for methylation of the sugar have not yet been studied in relation to carcinogenesis, base methylation has been studied in some detail. Only certain methylated bases are formed and these differ according to the source of the tRNA. For example, the major methylated bases in rat liver tRNA are 5-methyluracil, 5-methylcytosine, 7-methylguanine, 1-methylguanine, N^2-methylguanine, N^2-dimethylguanine and 1-methyladenine[9]. tRNAs of HeLa and L cells contain the same methylated bases but in slightly different proportions[10], while tRNA of *E. coli* contains 2-methyladenine and N^6-methyladenine also[11].

Sequence studies have shown that methylation occurs only at specific sites in the tRNA of each amino-acid and for each cell type studied. Two examples are given in Fig. 1. As a result of this specificity, methylases from one species can methylate tRNA from that species to a certain level only, and a saturation point is reached. A methylase preparation from another species can bring about further methylation until a new saturation level is attained[12]. Thus it was clear why such highly active enzymes had escaped detection. In normal conditions, tRNA as isolated as fully methylated by its homologous enzymes as is compatible with their specificities. Incubation with enzymes from the same source would not methylate tRNA, as the sites for methylation would have been alkylated *in vivo*. But because the enzymes are species specific, incubation of tRNA from one species with enzymes from another species can lead to "unnatural" hypermethylation. Thus it is possible

to assay mammalian enzymes by incubation with tRNA from bacteria, and this is what has been done in most studies on tumour tissue. It is important to remember in assessing the results of this work that the enzymes are not acting on their normal substrates.

Altered Methylation and Cancer

If tRNA is normally fully methylated by its homologous enzymes as it is synthesized, it is difficult to see how an increase in activity of the methylase enzymes could have any effect on the level of methylation reached in the intact animal. There are, however, two ways in which the pattern of methylation could be changed. There could be a change in specificity of some or all of the methylase enzymes, so that their action on normal pre-methylated tRNA would bring about methylation of bases at sites not normally methylated. Alternatively, there could be a change at the level of transcription of tRNA, resulting in an altered base sequence. Then normal methylase enzymes acting on an altered substrate would bring about a different pattern of methylation. The cell is known to contain a multiplicity of tRNAs accepting the same amino-acid—isoaccepting tRNAs. Thus a change could be due either to a change in the relative proportions of the different isoaccepting tRNAs, or to the synthesis of new species of tRNA. While the relative proportions may be similar in different tissues[13], there is evidence for a shift in the relative proportions[13,14], and also for a new species of tRNA[15] during carcinogenesis. The complex relationship between synthesis and methylation has been studied in the intact animal, while isolated enzyme systems have been used to study the methylases without the complication of concomitant synthesis.

Evidence for aberrant methylation of tRNA during carcinogenesis comes from four sources: (1) direct analysis of RNA; studies of methylation in (2) the intact animal and (3) isolated systems, and (4) studies of the action of the carcinogen ethionine which is an analogue of methionine.

The obvious direct approach to the analysis of RNA would be to determine the amount of each methylated base in each tRNA of each amino-acid, and to compare the results obtained for tRNA isolated from normal tissue, precancerous tissue and from tumours. Because so little tRNA is present in the cell, and because of the multiplicity of tRNAs, only mixed tRNA has been studied in this way. There is evidence that tRNA isolated from mammary adenocarcinoma and S180 ascites tumours in mice contains more methylated purines than does tRNA from normal mouse tissues[16]. Accurate quantitative estimation of minor bases in small amounts of tRNA is difficult, however. Apart from work on surgically removed gliomas[17] this approach has not been followed up.

As far as the intact animal is concerned, measurement of methylated bases in urine from patients with leukaemia gave some of the first evidence for an increased level of methylation of tRNA in tumours[18]. During catabolism of tRNA, most of the methylated bases are probably deaminated or otherwise degraded. 7-Methylguanine, however, is excreted unchanged from the rat, and is partly converted into 8-hydroxy-7-methylguanine in man. Most, possibly all, of the 7-methylguanine originates in tRNA, and an increased excretion therefore implies that tRNA is being degraded more quickly than normal, or that hypermethylated RNA is being degraded at the normal rate, or that both events occur. Greater than normal excretion was found in rats and mice carrying thymic lymphomas and mammary carcinomas[19] and in hamsters with tumours induced by adenovirus-12 (ref. 20). A recent survey has shown, however, that this is not general among cancer patients with solid-tumour cancer (personal communication from S. Mirvish). It is possible that, in some of the cases cited, the response is due to the viral nature of the disease, rather than to its being associated with malignancy. It is known that, either directly or indirectly, viral infection can cause a change in methylase activity[21,22]. The cell death which is often concomitant with carcinogenesis may also lead to an

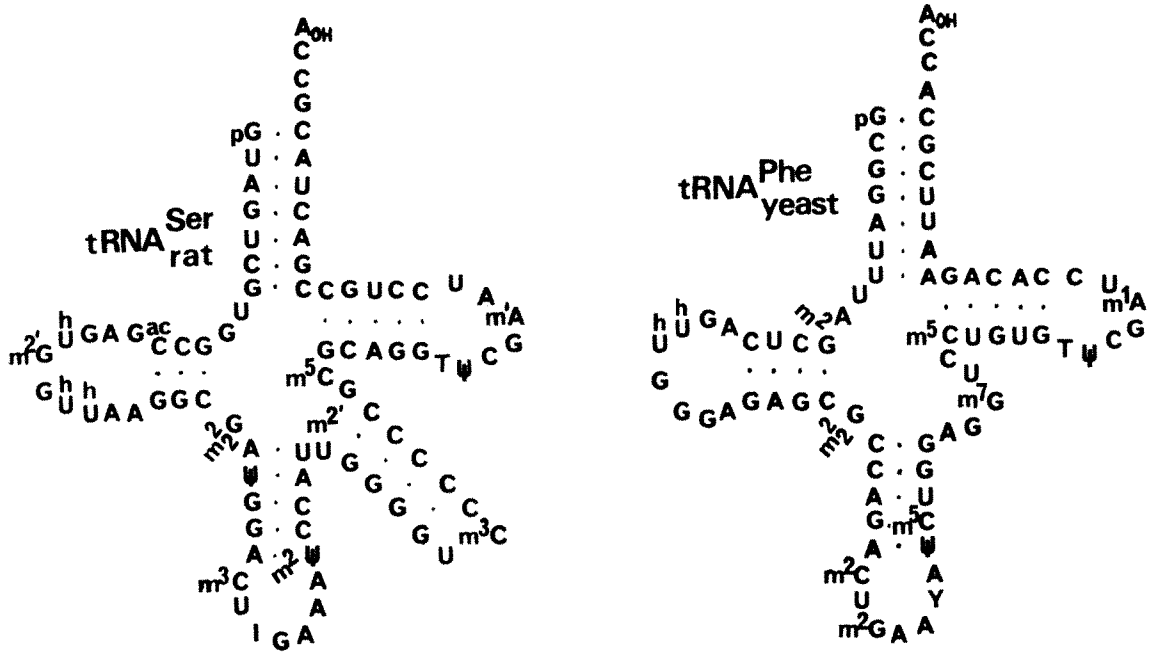


Fig. 1 Sequences of two tRNAs (from ref. 58).

increased excretion of methylated bases. Studies of urine therefore cannot give reliable information about the state of methylation of tRNA.

To study methylation of nucleic acids in the intact animal it is possible to give an injection of (^{14}C-methyl)-methionine, and later to kill the animal, isolate the tRNA, and after hydrolysis to analyse it for methylated bases. Normal rats and animals treated with several hepatocarcinogens have been studied in this way[9]. The methylated bases of normal liver become labelled within 2 h (Fig. 2*a*) after which the level of radioactivity slowly decreases, due to the slow turnover of tRNA. Liver tRNA from animals fed a diet containing dimethylnitrosamine for 23 weeks shows a much higher level of labelling of the methylated bases (Fig. 2*b*). This increase could possibly be due in part to an increased rate of total synthesis of tRNA, although this seems unlikely as there was no correspondingly large increase in synthesis of rRNA, DNA or protein. In addition, the different methylated bases increase in labelling to different extents, so that there is a change in their relative proportions. Similarly, in a rat which had been fed a diet containing aflatoxin, tRNA isolated from the liver tumour had a higher level of labelling of each of the methylated bases than had tRNA isolated from the liver lobes which had remained comparatively normal in appearance. Again the methylated base ratios from both samples differed from normal. Simultaneous measurement of synthesis and methylation by double-isotope techniques should help to elucidate these changes.

Isolated Systems

Crude extracts from various normal tissues and from cancerous tissue have been studied for their methylase activity, using *E. coli* tRNA as substrate and methyl-labelled S-adenosylmethionine as methyl donor. The level of activity has been studied by determining the rate of methylation of the substrate. The specificity of the enzymes has been investigated by measuring the maximum level of methylation which can be obtained when all susceptible sites have been methylated, that is, at the saturation level. A change in specificity might also be revealed by the fact that whereas normal methylases do not methylate their homologous tRNA when incubated *in vitro*, enzymes prepared from, for example, a liver tumour, might methylate normal liver tRNA.

Experiments of this type have given evidence for an increased activity and in some cases for an aberrant activity of methylases in various tumours, for example, in leukaemic leucocytes[23], Novikoff hepatoma[24], SV40-induced hamster tumours[25], mouse hepatoma[26], tumours induced by adenovirus-12 (ref. 27), tumours induced by viruses in chick liver[28], and neoplastic cell lines *in vitro*[29]. Methylases extracted from kidney tumours produced by injection of dimethylnitrosamine have also shown different properties from enzymes of normal kidney (my unpublished results). The kidney tumours were composed of a mass of light coloured cellular tumour, partly mixed with dark necrotic tissue. As far as possible, these tissues were homogenized separately, and their ability to methylate *E. coli* tRNA was studied (Fig. 3). A higher level of methylation was reached using an enzyme extract from the cellular tumour than from normal kidney or from the necrotic tissue. This suggests that specificity of the enzymes changes, but several complicating factors made interpretation of such results difficult.

First, there is the problem of the type of normal tissue to use as a control. In the experiments I have described, kidney tumours induced by dimethylnitrosamine were derived from mesenchymal cells (personal communication from G. C. Hard and W. H. Butler), and therefore connective tissue cells of the renal cortex would be a more appropriate control than normal kidney. Second, cellular changes in the tumour may alter the state of extractability of the methylase enzymes from the nucleus, and because it is often only the high-speed supernatant fractions from

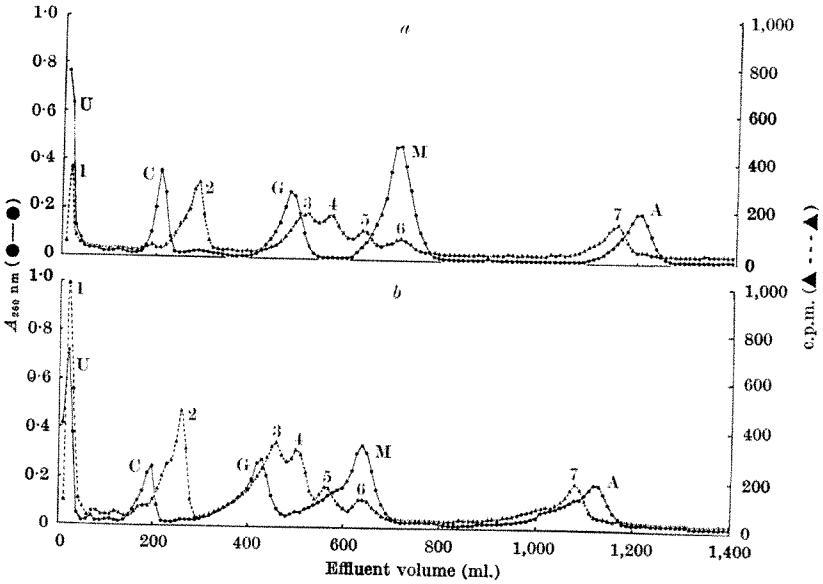


Fig. 2. Column chromatography on Dowex-50 of an $HClO_4$ hydrolysate of rat liver tRNA isolated after treatment with (^{14}C) methionine. *a*, Normal; *b*, after feeding the carcinogen, dimethylnitrosamine, in the diet for 23 weeks. A_{260} nm profile (●—●): U, uracil; C, cytosine; G, guanine; M, marker 7-methylguanine; A, adenine. Radioactivity profile (▲---▲): peak 1, methyluracil; peak 2, methylcytosine; peak 3, N^2-dimethylguanine; peak 4, N^2-methylguanine; peak 5, 1-methylguanine; peak 6, 7-methylguanine; peak 7, 1-methyladenine (*a* is taken from ref. 9).

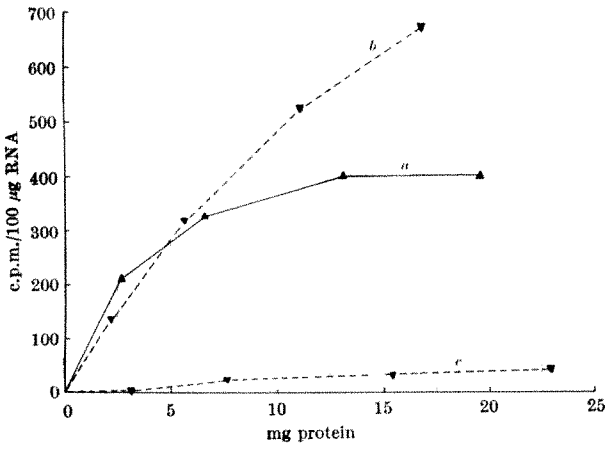


Fig. 3. Methylation of tRNA of *E. coli* K-12 during incubation with (^{14}C) adenosylmethionine and methylase preparations from (*a*) normal kidney; (*b*) cellular region of kidney tumour; (*c*) necrotic region of kidney tumour.

tissue homogenates which are studied, the results could be misleading. Furthermore, the presence of varying amounts of inhibitors[30] and activators[31] in tumours may alter the apparent enzyme activity. Ribonucleases present in crude methylase preparations may alter the tRNA during incubation in the assay procedure, making the substrate resistant to methylase activity, and thus causing a premature termination of methylation and a false plateau value.

It is therefore necessary, when studying methylase activity and specificity, to use enzyme preparations which have been at least partly purified. This has been done in the case of 1-methyladenine methylase from normal and leukaemic rat spleen[32,33]. The results supported the idea that there is a genuine increase in the amount of enzyme in leukaemic spleen, but no saturation level was reached when the purified enzyme was used in conditions which gave a plateau when crude extracts were used. Determinations of apparent saturation levels should be regarded as a preliminary stage in studies of the specificity of methylases.

More illuminating information concerning a possible change in specificity is given by determination of the sequence of bases around the methylated base. It was found that 1-methyladenine methylase from normal and from leukaemic spleen methylated adenine in the same tetranucleotide sequence[32]. Possibly analysis of a sequence longer than a tetranucleotide would have revealed a difference in specificities. More experiments of this type should show whether enzymes from normal and from tumour tissues have different actions on *E. coli* tRNA. The results would be more meaningful if tRNA from the same animal species could be used as substrate. If unmethylated precursor tRNA can be isolated from mammalian cells[34–36], this may be possible.

Ethionine Carcinogenesis

Ethionine is a potent hepatocarcinogen when given in appropriate doses in the diet. The mechanism of carcinogenesis of this analogue of methionine is of special interest in assessing the role of the nucleic acid methylases in carcinogenesis. After injection of ethionine, the ethyl group reacts with tRNA[37], but possibly not with rRNA or DNA[38]. Analysis of the tRNA shows that the ethyl group is probably attached to the base residues. One possible explanation is that ethionine is activated to form S-adenosylethionine (SAE), and that the methylase enzymes then use SAE in place of S-adenosylmethionine, so that tRNA is ethylated where it would normally have been methylated. There is also an increase in the level of methylase activity in the supernatant fraction of liver of mice fed ethionine[39] and of tumours induced by ethionine in rats[40]. Whether either this increase in methylase activity or the ethylation of tRNA is involved in carcinogenesis caused by ethionine is impossible to say at present.

In studies with most carcinogens which react with nucleic acids, reaction is found to occur with tRNA, rRNA and DNA, and the formidable problem is to determine which, if any, of these reactions is responsible for cancer. Only in the case of ethionine has reaction found to be almost entirely with tRNA. This is true even when the liver is rapidly synthesizing DNA and the nascent DNA is being methylated, as when ethionine is given to partially hepatectomized rats in a dose which does not inhibit regeneration (my unpublished results). Unfortunately, however, even in the case of ethionine, the carcinogen ethylates preformed protein[41] and is also incorporated as ethionine into protein[42]; possibly these reactions rather than those with tRNA are the carcinogenic event.

Biochemical Consequences of Altered Methylation

To assess the relevance of methylation of tRNA in carcinogenesis, and to decide whether a change in methylation is likely to explain the properties of cancer cells, it is necessary to understand the detailed functioning of tRNA. Conceivably, methylation of tRNA could affect any of the stages at which tRNA functions in protein synthesis, that is, in the acceptance of the appropriate activated amino-acid, in binding to the ribosome–mRNA complex, and in codon recognition. Methylation of the bases may alter their hydrogen bonding and stacking properties, and so alter the secondary and tertiary structure of the molecule, thus affecting each of these reactions. But it has been a working hypothesis that each activity of tRNA is associated with a definite region of the molecule. Then whether or not an activity is affected by hyper or hypomethylation will depend on whether or not the appropriate region contains a methylated base.

With reference first to the acceptor function, the sites on the clover leaf recognized by the amino-acyl tRNA synthetases have not yet been located. These sites could contain methylated bases, in which case methylation could affect charging of tRNA. There was evidence that methyl-deficient phenylalanyl tRNA had a lower rate of acceptance of phenylalanine than had the normal tRNA[43]. This result was not confirmed[44], however, when rigorous methods were used to avoid degradation of tRNA by nuclease activity during the separation of methyl-deficient from normally methylated phenylalanyl tRNA. In the case of ribosome dependent binding to mRNA, it has been suggested that, because the sequence TψCGp in the ψ loop is common to many tRNAs, this may be the ribosome binding site. Thus methylation of uracil to form thymine in this position might be essential for binding to occur. There is evidence that binding of leucine tRNA[45] and phenylalanyl tRNA[46] is reduced if the nucleic acid is deficient in methyl groups. Of most interest in relation to carcinogenesis is the possible effect of methylation on codon recognition. Sequence analysis has shown that the base next to the anticodon is often a methylated or otherwise modified rare base. Whether or not this

base is modified may determine the nature of the codon recognition, and thus determine which amino-acid is inserted into the protein chain, and thus the type of protein synthesized. Experimentally, however, the effect of methylation at this site has yet to be demonstrated.

Possible Significance for Carcinogenesis

There is good evidence that certain tumours have a higher level of methylase activity than do related normal tissues, although the increase may not occur in the early stages of the disease. Many enzymes are known to alter in activity during carcinogenesis[47] and so the problem is to determine whether any particular change is relevant to cancer or not. With reference to the nucleic acid methylases, the increase in activity is possibly specific for methylases of tRNA. There is evidence that the activity of rRNA methylases[9] and DNA methylases[48] does not change during carcinogenesis, although these enzymes have not been studied in detail. If tRNA is normally fully methylated soon after synthesis, it is difficult to assess the effect of an increase in methylase activity, unless there is at the same time a change in specificity of the enzymes. As I have discussed, the evidence for such a change is far from conclusive.

When the methylase enzymes were discovered it was suggested that they may play a part in differentiation. Some evidence for this has come from experiments on systems in which cell differentiation is taking place, for example, during insect metamorphosis[49], during morphogenesis of the slime mould[50], and in developing foetal rat brain[51]. The amount of work done so far is too limited for the suggestion to be more than an attractive hypothesis. Another idea is that methylase activity is related more to growth and protein synthesis. Thus there is a high concentration of methylases not only in tumours but also in embryonic liver[52] and in chick oviduct[53] and mouse mammary gland[54] after stimulation by hormones. On this basis one might expect to find high levels of activity in regenerating liver. Determination of methylase activity in samples of regenerating liver gave no evidence for this[55], although *in vivo* experiments showed that the ratio of synthesis to methylation of tRNA decreased at that time after partial hepatectomy when protein synthesis increases[56]. The relation between rate of protein synthesis, tRNA synthesis and methylation of tRNA is not clear, and the sites at which control mechanisms might act are unknown. At present there is no compelling evidence for either nuclear or cytoplasmic control of differentiation or of protein synthesis in eukaryotic cells. There is simply a good case for not continuing to look "too hard at the nucleus and not hard enough at the cytoplasm"[57].

In view of the lack of information concerning the control of differentiation and of protein synthesis, including the uncertainty of the part played by tRNA, it is only worthwhile to speculate very briefly on the significance of changes in tRNA methylases during carcinogenesis. There are two possibilities. One is that a change in methylase activity affects the synthesis of a specific regulator protein. Loss of such a protein may result in uncontrolled cell division, and thus start the carcinogenic process. Alternatively, changes in methylases may not be involved in the primary initiation of cancer but may be an essential part of the subsequent stages of the process. It now seems certain that carcinogenesis is not a one or two stage event, but that a multi-stage process causes the gradual evolution of cancer cells. The changes in protein synthesis which must occur during this process may be mediated by the changes in methylase activity. All that can be said with certainty is that there are alterations in methylase activity and possibly in specificity; the fact that these enzymes act on molecules concerned in what could be a key stage of gene translation is a compelling reason for finding out how these changes are brought about, and what their biological consequences may be.

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Purification and Properties of Initiation Factor F_3

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Homogeneous preparations of initiation factor F_3 have both RNA binding and ribosome dissociation activities. But although DF activity could entirely account for the role of F_3 in the ribosome cycle this factor has an additional function in formation of the initiation complex that may be related to its RNA binding properties.

INITIATION factors of *Escherichia coli* (F_1, F_2, F_3) are loosely associated with 30S ribosomal subunits[1,2] and can be extracted by washing the ribosomes with concentrated salt solutions, such as 1·0 M NH_4Cl (refs. 3 and 4). F_2 has essential SH groups[5] and mediates the binding of the initiator, fMet-$tRNA_f$, to a messenger–30S ribosome subunit complex[3,6]. This reaction requires Mg^{2+} and GTP and is accompanied by hydrolysis of GTP[6–8]. When the trinucleoside diphosphate ApUpG (AUG) is used as messenger in conditions where F_3 is not required, the AUG–30S–fMet-$tRNA_f$ complex can be formed at 0° C with F_2 as the only initiation factor, but at 25° C both F_1 and F_2 are essential[6]. This suggests that the complex formed in the absence of F_1 is very unstable and is stabilized when F_1 is present. A requirement for F_3, both for translation and formation of the initiation complex[3], is found when natural mRNA (such as phage RNA) is used as messenger. Our results[3] suggest that formation of the initiation complex involves two steps: (*a*) the F_1 and F_3-dependent binding of mRNA to the ribosomes, and (*b*) the F_2-dependent binding of fMet-$tRNA_f$ to the mRNA–ribosome complex.

All three initiation factors are now available in virtually homogeneous condition. The purification and properties of F_2 have been described[9] and the preparation of homogeneous F_1 has been reported[10]. We describe here the preparation of homogeneous, or nearly homogeneous, F_3 (see Fig. 1) and further studies on the properties and function of this factor. F_3 has an RNA binding activity, as judged by 'Millipore' filtration assays, and promotes dissociation of 70S ribosomes to 30S and 50S ribosome subunits[11]. Insofar as initiation requires the availability of 30S subunits[12] the dissociation factor activity of F_3 could account entirely for its role in the ribosome cycle[13,14]. But we show also that F_3 is directly involved in formation of the chain initiation complex.

The purification procedure is summarized in Table 1. Step 6 F_3 has an A_{280}/A_{260} ratio of 1·8, indicating that it is free of nucleic acid. Fig. 2 shows that it is an essentially homogeneous, basic protein as judged by disc electrophoresis at two *p*H values in 8·0 M urea and by SDS-gel electrophoresis. It is better than 90 per cent pure. When estimated by ultraviolet absorption the values are less than one-third, according to the Lowry method. Because of this exceptional discrepancy the protein concentration of step 6 F_3 solutions was also determined refractometrically[17] and gravimetrically. For the latter measurement the protein, extensively dialysed against NH_4HCO_3 solutions, was lyophilized, then repeatedly dissolved in water and relyophilized to constant weight. The relative values given by the four methods were: Lowry, 1·0; gravimetric, 0·93; refractometric, 0·87; ultraviolet absorption, 0·32. Lowry values will be used throughout unless otherwise stated.

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Properties of F_3

A molecular weight for F_3 of 21,000 was determined by SDS-gel electrophoresis[18]. Free SH groups were titrated with 5,5′-dithio-*bis*-(2-nitrobenzoic acid) (DTNB) by the

Table 1. PURIFICATION OF F_3

Step	Volume ml.	Protein mg	Units† $\times 10^{-3}$	Specific activity‡	Yield per cent
NH_4Cl wash*	1,600	22,900	52	2·3	100
$(NH_4)_2SO_4$ fractionation	85	3,850	38	9·9	73
DEAE-cellulose chromatography	109	914	34	37·2	65
Phosphocellulose chromatography	28	107	34	318	65
'Sephadex G100' chromatography	40	44	23	523	44
'Sephadex G75' chromatography in 6 M urea§	2	15	10	670	10

* From 3·4 kg of frozen cells.
† 1 unit = 1·0 nmole of ^{14}C-lysine incorporated under standard assay conditions (20 min, 37°).
‡ Units/mg protein.
§ Peak fractions.

The standard assay of F_3 is described in the legend to Fig. 1. In the conditions of the assay, the incorporation of ^{14}C-lysine into acid-insoluble material was proportional to the concentration of F_3 up to about 2·0 μg/0·125 ml. Protein was determined by the method of Lowry *et al.*[16] using bovine serum albumin as a standard. Blanks in the absence of F_3 averaged 0·07 nmole and in the absence of MS2 RNA (with 2·5 μg F_3) 0·02 nmole. Omission of F_1 and F_2 still permitted significant incorporation (about 0·26 nmole with 2·5 μg F_3). We isolate the three initiation factors from the 1·0 M NH_4Cl wash of unfractionated *E. coli* Q13 ribosomes. All operations are carried out at about 4° C. The wash, obtained as previously described[3], was fractionated with ammonium sulphate. The precipitate between 55 and 70 per cent saturation (at 4° C), containing the bulk of F_1 and F_3, was dialysed against 0·005 M phosphate-Tris-glycerol (PTG) buffer (0·005 M phosphoric acid with Tris base added to *p*H 7·5, 5 per cent glycerol). Phosphate was used in all buffers because it has a stabilizing effect on F_3. The dialysed solution was diluted with the same buffer to 10 mg protein/ml. and applied to a column (3 × 70 cm) of DEAE-SH cellulose (Serva, 0·8 mequiv/g) equilibrated with the same buffer. F_1 is not retained by DEAE-cellulose. The column was washed with this buffer until the A_{280} of the effluent was near zero and F_3 was eluted with 0·15 M PTG buffer (0·15 M phosphate). F_3 activity was found in the initial fractions. These were pooled and dialysed against 0·1 M phosphate-Tris-sucrose (PTS) buffer (0·1 M phosphoric acid with Tris base added to *p*H 7·5, 10 per cent sucrose). The dialysed solution was applied to a column (2·2 × 48 cm) of phosphocellulose (Serva, 0·68 mequiv/g), previously washed with 25 per cent NaCl and equilibrated with 0·1 M PTS buffer. Washing with the same buffer until the A_{280} of the effluent was near zero removed large amounts of inactive protein. F_3 was then eluted with 0·5 M PTS buffer (0·5 M phosphate). The active fractions were pooled, the protein concentrated by precipitation with ammonium sulphate at 80 per cent saturation, and the precipitate dissolved in a minimal volume of 0·5 M PTS buffer (0·5 M phosphate). This solution was chromatographed on a column (2·5 × 88 cm) of 'Sephadex G100' equilibrated with 0·5 M PTS buffer. F_3 activity coincided with a protein peak eluting about 100 ml. after the void volume. The pooled active fractions were concentrated either by dialysis against saturated ammonium sulphate or by chromatography on a small phosphocellulose column. At this stage (step 5) polyacrylamide gel electrophoresis revealed a broad, fuzzy band in the absence of urea and two sharp bands in 8·0 M urea. Further purification (step 6) was carried out by 'Sephadex' chromatography in 6·0 M urea; brief exposure to this concentration of urea does not inactivate F_3. The concentrated solution from step 5 was dialysed against a buffer (PTU-SH buffer) containing 0·1 M phosphoric acid, with Tris base to *p*H 7·5, 6·0 M deionized urea, and 0·002 M dithiothreitol, and the dialysed solution applied to a column (1·5 × 88 cm) of 'Sephadex G75' equilibrated with the same buffer. On elution with this buffer the F_3 activity coincided with the principal A_{280} peak which emerged from the column at a volume slightly greater than the void volume. Appropriate peak fractions were pooled and dialysed at once against 0·5 M PTS buffer. When kept in this buffer at 4° C, F_3 is stable for several months. Two preparations of F_3 by the method described gave essentially identical results. F_3 can be lyophilized salt-free after dialysis against NH_4HCO_3 solutions without substantial loss of activity. The lyophilized material is stable at refrigerator temperatures and can be redissolved with retention of activity for one week in a phosphate-free buffer containing 0·25 M Tris-HCl (*p*H 7·8)–10 per cent sucrose.

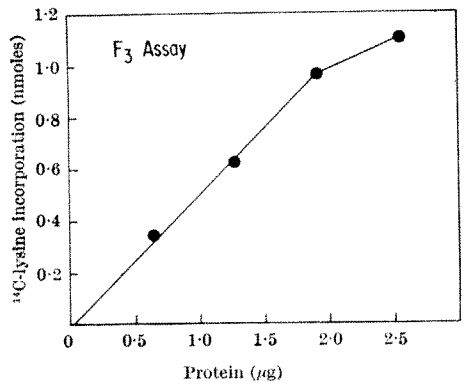


Fig. 1. F_3 assay. Assay is based on F_3 dependence for translation of mRNA by 1·0 M NH_4Cl-washed *E. coli* Q13 ribosomes in the presence of excess F_1 and F_2. The samples contained, in a volume of 0·125 ml., Tris-HCl buffer (*p*H 7·8), 60 mM; phosphate buffer (*p*H 7·8), 4 mM; NH_4Cl, 70 mM; magnesium acetate, 14 mM; 2-mercaptoethanol, 16 mM; ATP, 1·3 mM; GTP, 0·3 mM; phosphocreatine, 17 mM; creatine kinase, 3 μg; tRNA (*E. coli* W), 55 μg; MS2 RNA (as messenger), 1 A_{260} unit (about 40 μg); purified ribosomes, 5 A_{260} units (about 200 μg RNA); *E. coli* Q13 s150 supernatant (as a source of amino-acyl-tRNA synthetases, chain elongation and chain termination factors) with about 0·3 mg protein; F_1 (about 50 per cent purity), 2 μg; F_2 (about 10 per cent purity), 40 μg; F_3 (about 75–80 per cent purity), from 0·64 to 2·56 μg; ^{14}C-lysine (specific radioactivity 10 μCi/μmole), 0·1 mM; and the remaining (unlabelled) 19 amino-acids, each 0·1 mM. After incubation (20 min, 37° C) acid-insoluble radioactivity was determined as previously described[3]. The blank in the absence of F_3 (about 0·07 nmole) was subtracted. Lysine incorporation in the presence of F_3 2·5 μg) but without F_1 and F_2 averaged 0·26 nmole; with all three factors (2·5 μg F_3) but without MS2 RNA it averaged 0·02 nmole.

procedure of Ellman[19]. The results were the same with native F_3 or with F_3 exposed either to 8·0 M urea for 1 h at 30° C or to 1 per cent SDS for 2 h at 37° C. The molar ratio bound DTNB/F_3 was about 0·6 on the basis of Lowry protein (about 0·7 on a refractometric protein basis). Thus F_3 seems to contain one reactive SH group. Preincubation of the factor with 10–20 mM N-ethylmaleimide resulted in moderate inhibition (~40–50 per cent) of its activity. The degree of inhibition, however, was about the same in the standard MS2 RNA translation assay and in the RNA binding and DF assays described below. This suggests that these activities reside in the same protein.

When labelled RNA or synthetic polynucleotides are incubated with F_3, as described in Fig. 3, the retention of label on 'Millipore' filters is proportional, if within narrow limits, to the concentration of F_3. Fig. 3 shows that peaks of RNA binding and amino-acid incorporation activity overlap the principal protein peak on chromatography of step 5 F_3 on 'Sephadex G75' in 6·0 M urea. The same is true of DF activity although, in the case of Fig. 3, this was ascertained for only three of the fractions. F_3 is not the only protein in the ribosomal wash that has RNA binding activity. We have seen other fractions with similar properties although without F_1, F_2 or F_3 activity. Fig. 3 also shows that the small protein peak between fractions 45 and 60, which is devoid of F_3 activity, has some RNA binding activity. A protein with similar RNA binding properties has been isolated by Brown and Doty[20] from the 1·0 M NH_4Cl wash of *E. coli* ribosomes. We have tested this protein (kindly provided by Dr Brown) for activity in fMet-tRNA$_f$ binding and mRNA translation, but with negative results. The RNA binding activity of F_3 cannot be solely ascribed to its basic nature because F_1, a more basic protein, lacks this property. F_2 is also inactive in the RNA binding assay.

Davis[21] predicted the occurrence of a factor causing dissociation of 70S ribosomes (ribosome dissociation factor, DF) and suggested that it would function in chain initiation by providing a supply of 30S subunits. DF activity is found in crude preparations of initiation factors[22,24,25]. Fractionation of the factors by DEAE-cellulose chromatography[3] showed that DF is associated with F_3 (ref. 11), a finding that we have confirmed with highly purified F_3 preparations[11,23]. The DF activity of step 6 F_3 is show in Fig. 4. In this experiment, 2·6 μg F_3 promoted ov 75 per cent dissociation of 40 μg ribosomes. By contra (Fig. 4) 14 μg of crystalline F_1 had no activity[12]. In oth experiments, F_2 was found to be free of DF. The results are incompatible with a recent suggestion b Miall *et al.*[26] that DF activity is associated with t initiation factor F_1. The reported DF activity of this preparation, however (judged to be about 95 per ce pure), is probably the result of contamination with for it amounts on a molar basis to only 5–10 per cent t DF activity of our step 6 F_3. According to so workers[11,24] DF activity is stimulated by GTP. In o assay conditions, however, we found no effect of GT on the dissociation reaction[23]. Similar results we reported from two other laboratories[25,26].

Chain Initiation

Stimulation by F_3 of mRNA translation and formatic of the initiation complex in experiments with unfra tionated ribosomes[3], containing a fairly large proportic of 70S particles, could be indirect and caused entire by its DF activity. But, using 30S subunits, or a mixtu of 30S and 50S subunits that do not reassociate spo taneously, shows that this factor is also directly involv in chain initiation. We have analysed the factor requi ments for initiation complex formation with ^{3}H-M RNA, ^{14}C-methionine labelled fMet-tRNA$_f$ and no spontaneously reassociating 30S and 50S ribosomes zonal centrifugation in sucrose gradients with use homogeneous or nearly homogeneous F_1, F_2 and F_3.

As shown in Fig. 5, a 70S complex was formed only the presence of the three factors. Panel *D* shows t appearance of an absorbance shoulder in the 70S regi of the gradient with overlapping peaks of ^{3}H and radioactivity. Similar results are obtained if F_1 omitted[3] but in this case the extent of complex formati is 50 per cent or less of that observed with the th factors. In particular, no complex was formed in t absence of F_3 (see panels *A* and *B*). With F_1 and

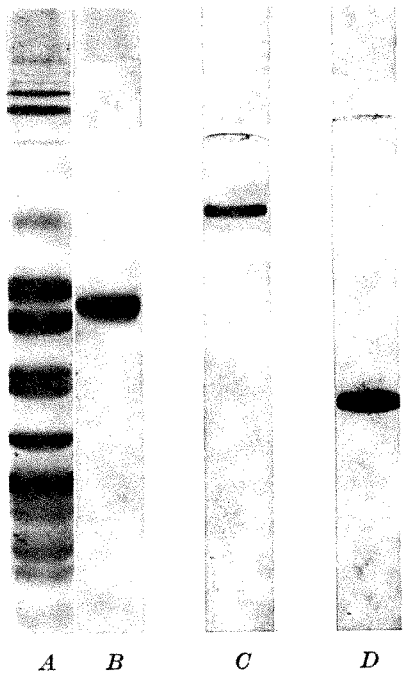


Fig. 2. Polyacrylamide gel electrophoresis of F_3 (step 6). *A* and 8·0 M urea (*p*H 4·5) (origin at top, cathode at bottom); *A*, protei (about 30 μg) from 1·0 M NH_4Cl-washed 30S ribosomes; *B*, F_3 (abo 8 μg). *C*, F_3 (about 7 μg), *p*H 12·7, 8·0 M urea (origin at top, anode bottom). *D*, F_3 (about 5 μg), SDS gel electrophoresis, *p*H 7·0 (orig at top, anode at bottom). Disc electrophoresis in 8·0 M urea was carri out as previously described[3] and SDS-gel electrophoresis as describ by Weber and Osborn[16].

alone (panel *C*) there was a small shoulder in the ^3H radioactivity curve in the region between 30S and 50S and the absorbance trough in this region was less deep than in the other panels. This may reflect formation of a 30S–MS2 RNA complex in the presence but not in the absence of F_3 (see panel *B*). Because the sedimentation coefficient of MS2 RNA is about 28S, such a complex might sediment somewhat faster than 30S. Little or no 70S complex was formed in control experiments without MS2 RNA, 30S subunits, or on substitution of uncharged tRNA for fMet-tRNA[28]. Thus, in the conditions of the present experiments, joining of 30S and 50S ribosomes requires formation of a complete initiation complex including both mRNA and fMet-RNA_f. This lends further support to the view[12–14,29] that joining of the ribosomal subunits to a 70S couple directly follows the formation of the initiation complex on the 30S ribosome. In the experiment of Fig. 5 one starts with fully dissociated ribosomes and that, despite its DF activity, F_3 promotes formation of 70S ribosomes (see panels *B* and *D*).

Direct involvement of F_3 in formation of the initiation complex can also be shown with 30S subunits. In the experiment of Table 2, ribosomal binding of ^{14}C-methionine-labelled fMet-$tRNA_f$, in the presence of unlabelled MS2 RNA, was measured by the 'Millipore' filter technique[30].

Initiation Factors and the Ribosome Cycle

Our results suggest a dual function for F_3 in the ribosome cycle shown schematically in Fig. 6: (*a*) to ensure a supply of 30S subunits at or after chain termination (step 5); and (*b*) direct involvement in initiation (step 1). Some workers believe that chain termination liberates 70S ribosomes[11,31] which dissociate subsequently[11] by interaction with F_3 released from 30S subunits when the 50S subunit joins the 30S initiation complex (step 3). This would imply that F_3 recycles between a ribosome-bound and a free form. Other workers hold the view that the ribosomes dissociate at chain termination[13,32]. If so, F_3 might remain ribosome-bound throughout the entire cycle so long as ribosomes bearing F_3 can associate and remain associated when they carry aminoacyl- or peptidyl-tRNA[25]. At present, the precise manner in which F_3 functions in the ribosome cycle remains an open question.

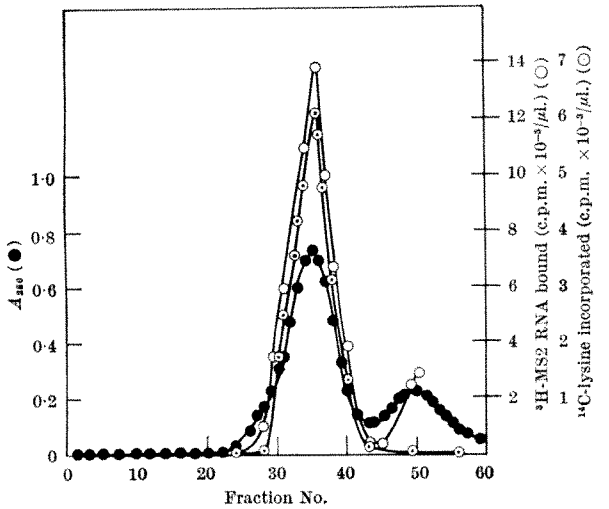


Fig. 3. Amino-acid incorporation and RNA binding activity of F_3. Chromatography of step 5 F_3 on 'Sephadex G75' in 6·0 M urea. Effluent fractions assayed for (*a*) translation of MS2 RNA in the standard F_3 assay (^{14}C-lysine incorporation) and (*b*) retention of ^3H-MS2 RNA on 'Millipore' filters (RNA binding). Some fractions were also assayed for DF activity (see Fig. 4). The percentage dissociation of 70S ribosomes was 38, 75 and 28 with aliquots (1 μl.) of fractions of No. 31, 35, and 40 respectively. For the RNA binding assay, the samples contained, in a final volume of 0·105 ml., phosphate buffer (*p*H 7·5), 20 mM; bovine serum albumin, 0·1 mg; ^3H-MS2 RNA, 2 μg (about 14,000 c.p.m.) and sample to be assayed (for step 6 F_3 with 0·2 to 0·9 μg protein). After incubation for 20 min at 0° C, 1 ml. of 0·1 M PTG buffer was added, the mixture was filtered through 'Millipore' membranes, at the rate of 1 ml./15 s, and the radioactivity retained on the filter measured.

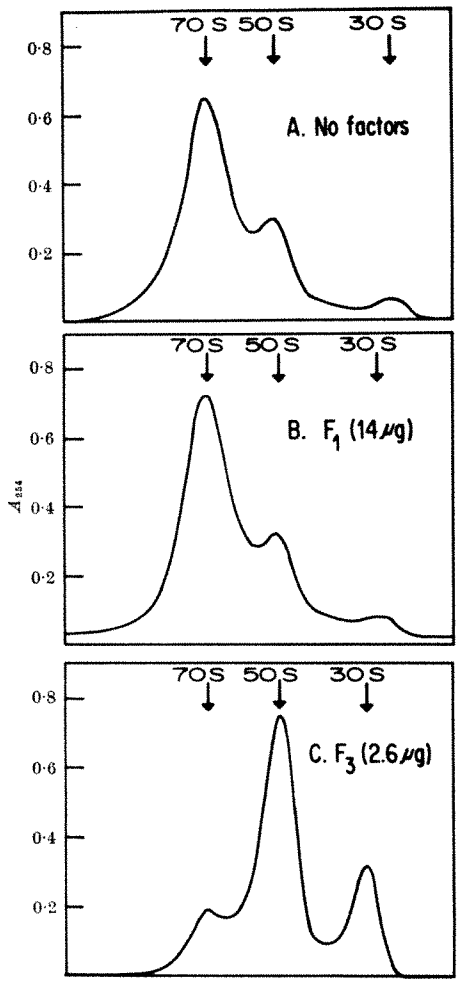


Fig. 4. Ribosome dissociation activity (DF activity) of F_3. The assay was carried out with 1·0 M NH_4Cl-washed *E. coli* MRE 600 ribosomes. The ribosomes were preincubated for 15 min at 37° C in a buffer containing 50 mM Tris-HCl (*p*H 7·8), 5 mM magnesium acetate and 1 mM dithiothreitol (DTT). The assay samples contained, in a final volume of 0·125 ml., Tris-HCl buffer (*p*H 7·8), 50 mM; phosphate buffer (*p*H 7·8), 4 mM; NH_4Cl, 40 mM; magnesium acetate, 6·6 mM; DTT, 1 mM, and preincubated ribosomes, 0·8 A_{260} unit (32 μg RNA), with or without factors as indicated. Homogeneous F_3 (step 6) and F_1 were used. After incubation at 37° C for 20 min, 0·1 ml. of the incubation mixture was layered on 5 ml. of a 5–20 per cent sucrose gradient containing 50 mM Tris-HCl buffer (*p*H 7·8), 60 mM NH_4Cl and 4 mM magnesium acetate, and centrifuged for 2 h at 4° C and 37,000 r.p.m. in the Spinco SW 39 rotor. The gradients were analysed in an Isco (model D) gradient analyser using a 10 mm light path flow cell.

At which step in initiation is F_3 directly involved? Our previous experiments with unfractionated ribosomes[3] were interpreted to mean that F_3 functions, along with F_1, in mRNA binding (Fig. 6, step 1). These experiments were similar to those of Fig. 6 except for the preparation of ribosomes and the use of ^{32}P–Qβ RNA as messenger. With F_1 and F_3 alone a peak of mRNA radioactivity appeared under the 70S ribosomal peak, but there was no

Table 2. EFFECT OF INITIATION FACTORS ON MS2 RNA-DEPENDENT RIBOSOMAL BINDING OF fMet-$tRNA_f$ WITH 30S RIBOSOMES

Factor additions (μg)	fMet-^{14}C-tRNA binding (pmoles) 30S	30S + 50S
None	0·09	0·05
F_3 (2·6)	0·15	0·08
F_1 (1·2), F_3 (2·6)	0·62	0·31
F_1 (1·2), F_3 (2·6), F_3 (1·1)	3·20	4·62

Samples contained, in a final volume of 0·125 ml., Tris-HCl buffer (*p*H 7·8), 50 mM; NH_4Cl, 60 mM; magnesium acetate, 5·4 mM; GTP, 0·16 mM; MS2 RNA, 1 A_{260} unit; fMet-^{14}C-tRNA, about 16 pmoles fMet-$tRNA_f$ (about 4,000 c.p.m.); and either 30S, 2 A_{260} units (left column), or 30S, 1 A_{260} unit, and 50S, 2 A_{260} units (right column), ribosomes. Incubation: 15 min at 37° C, 'Millipore' assay. The subunits were prepared as described for Fig. 5. F_1, F_3 and F_3 were homogeneous preparations.

significant binding of fMet ^{14}C-tRNA$_f$ unless F_2 was also present. The observed association of mRNA with 70S ribosomes, in the absence of substantial fMet-tRNA$_f$ binding, could result if non-specific binding of uncharged tRNA, present in the preparations of fMet-tRNA, permits joining of the 50S ribosome to the 30S–mRNA complex[13]. But, in any case, there is little doubt that F_3 promotes binding of mRNA to the ribosomes, an activity that may well be related to the RNA binding property of F_3 described here. This effect of F_3 is always markedly augmented by F_1. For this reason we believe that F_1 also functions at this stage of initiation (Fig. 6, step 1). Moreover, at 25° C, F_1 is essential for the AUG-dependent binding of fMet-tRNA$_f$ to 30S subunits[6]. In this system F_1 decreases the K_m for AUG (unpublished work of R. Mazumder), suggesting that this protein stabilizes mRNA binding to the ribosome. Thus, there seems to be little basis for the assumption[34] that F_1 is active in joining 50S subunits to the 30S initiation complex because of its apparent failure to stimulate AUG- and F_2-dependent binding of fMet-tRNA$_f$ to 30S subunits.

Finally, as regards F_2, work in this and other laboratories demonstrates that this factor functions specifically in the mRNA-dependent binding of the initiator fMet-tRNA$_f$ to the 30S subunit. We see no indications from our previous[3] or present work (Fig. 5*B*) that F_2 participates in the binding of mRNA to the ribosome. Thus, we believe that F_2 is involved at step 2 (Fig. 6) of the ribosome cycle. A reported stimulation by F_2 of mRNA binding to 30S subunits[34], if not arising from impurities, may reflect the occurrence of non-specific interactions in certain conditions.

We thank Mr Horace Lozina for growing bacterial cells, Mr Morton C. Schneider for preparation of unlabelled and ^{3}H-labelled MS2 RNA, Miss Maryanne Pendergast for the photograph used in Fig. 2, and Dr R. Mazumder and Dr W. Szer for discussions and Mr F. Zaboretsky for the refractometric protein determination. The work was aided by

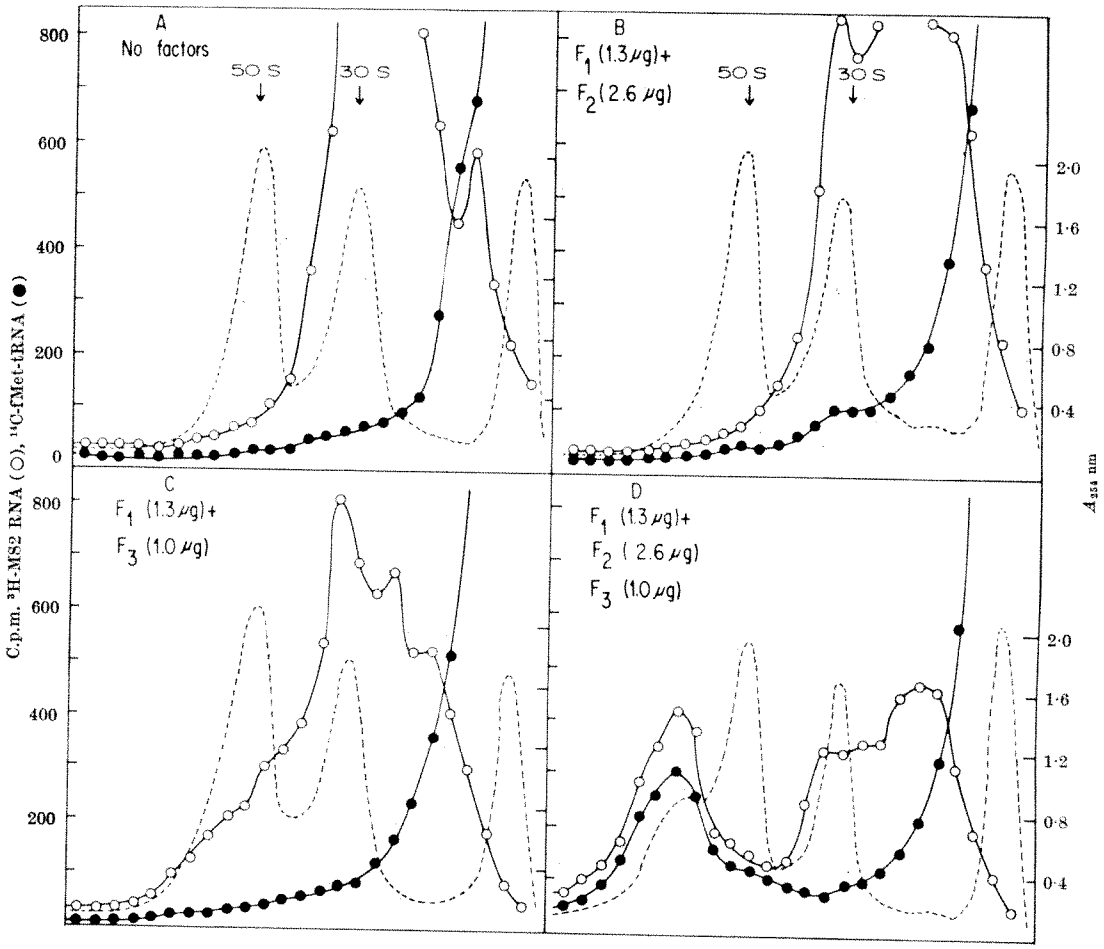


Fig. 5. Effect of initiation factors on formation of the chain initiation complex with non-spontaneously associating 30S+50S ribosomal subunits. Complex formation, using ^{3}H-MS2 RNA and ^{14}C-methionine-labelled fMet-tRNA, was analysed by zonal centrifugation in sucrose gradients. The subunits were prepared as follows: *E. coli* Q13 ribosomes were washed for 10 h at 4° C with a buffer containing 20 mM Tris-HCl (*p*H 8·1), 10 mM magnesium acetate and 1·0 M NH_4Cl. The ribosomes were sedimented by centrifugation at 4° C for 3 h at 60,000 r.p.m. in the Spinco 65 rotor, washed in the above buffer for a further 5 h, sedimented as before and suspended in 20 mM Tris-HCl (*p*H 7·8)—5 mM magnesium acetate—0·1 mM DTT—50 mM NH_4Cl (buffer A). The subunits were isolated by centrifugation through 50 ml. of a linear 10–30 per cent sucrose gradient in buffer A. The gradients were equilibrated for 8 h at 4° C, loaded with 1·5 ml. sample containing 300 A_{260} units of purified ribosomes (previously incubated for 5 min at 37° C in buffer A) and centrifuged for 11 h at 4° C and 24,000 r.p.m. in the SW 25·2 rotor of the Spinco L-2 centrifuge. The bands of 30S and 50S subunits could be easily seen through light scattering and the subunits recovered by suction. After adjusting the Mg^{2+} concentration of the ribosomal suspensions to 20 mM, the subunits were concentrated by centrifugation for 8 h at 4° C and 50,000 r.p.m. in the Spinco 65 rotor. The pellets were resuspended in a buffer containing 20 mM Tris-HCl (*p*H 7·8), 10 mM magnesium acetate and 0·5 M NH_4Cl and stored at 4° C. Before use the subunits were activated[27] by incubation for 2 min at 50° C in 10 mM Tris-HCl (*p*H 7·8)—250 mM NH_4Cl—30 mM magnesium acetate. The samples contained, in a final volume of 75 μl., Tris-HCl buffer (*p*H 7·8), 50 mM; NH_4Cl, 80 mM; magnesium acetate, 4·2 mM; GTP, 0·13 mM; DTT, 0·8 mM; ^{3}H-MS2 RNA, 0·58 A_{260} unit (about 8,500 c.p.m.); fMet ^{14}C-tRNA, about 20 pmoles fMet-tRNA$_f$ (about 5,000 c.p.m.); 30S ribosomes, 0·9 A_{260} units; 50S ribosomes, 1·9 A_{260} units, with or without homogeneous factors as indicated. After incubation for 15 min at 37° C, 60 μl. aliquots were layered on 5 ml. of a 5–20 per cent sucrose gradient, containing 50 mM Tris-HCl buffer (*p*H 7·8), 60 mM NH_4Cl, 5 mM magnesium acetate, and centrifuged for 55 min at 4° C and 63,000 r.p.m. in the Spinco SW 65 rotor. The gradients were analysed in an Isco gradient analyser (as in Fig. 4) attached to a fraction collector.

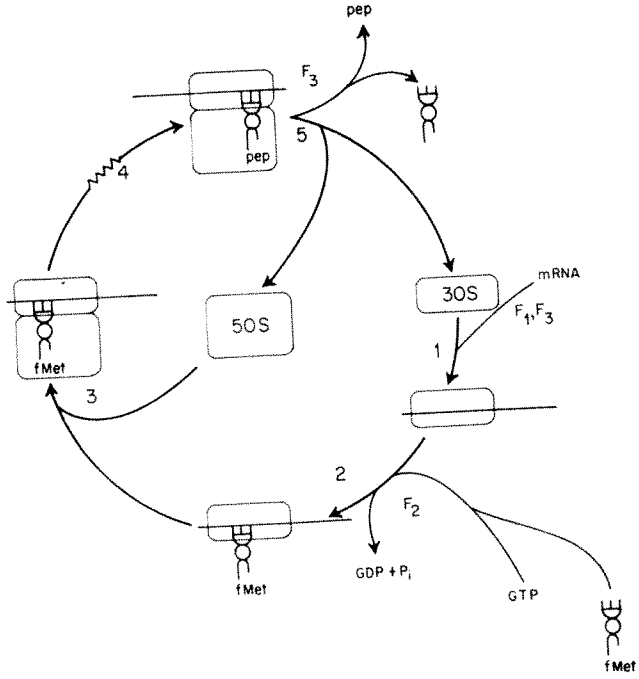


Fig. 6. Chain initiation and the ribosome cycle. (1) Binding of mRNA to 30 S ribosome subunits. (2) Binding of fMet-tRNA$_f$ to mRNA-30S ribosome complex. (3) Joining of 50S ribosome subunit. (4) Chain elongation. (5) Chain termination and dissociation of 70S ribosome.

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Activity of Initiation Factor F_3 in Dissociating *Escherichia coli* Ribosomes

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Pure initiation factor F_3 dissociates 70S ribosomes into 30S and 50S subunits in a way which suggests that *in vivo* interaction with runoff ribosomes is responsible for their dissociation into subunits.

When the products of polysome runoff are caused to accumulate in *Escherichia coli* cells or extracts they appear as 70S ribosomes[1]; and such "free" runoff ribosomes differ from complexed monosomes carrying messenger RNA and nascent polypeptide, in being more easily dissociated by low Mg^{2+} concentrations[2] or by Na^+ (ref. 3). Because runoff causes no parallel increment of ribosomal subunits, and because subunits are required for initiation of protein synthesis[4], this implies that the dissociation step in the ribosome cycle does not occur spontaneously. We have suggested that runoff ribosomes must complex with a dissociation factor, which would be present in the cell in limiting supply and would have to recycle, in the course of initiation, from a completed initiation complex to another free ribosome[1]. We have found this dissociation factor (DF)[5] in the mixture of initiation factors obtained by extracting the 30S ribosomal pellet with 1 M NH_4Cl; and as required for its proposed function, it dissociates free but not complexed (polysomal) ribosomes[5–8].

Because NH_4Cl-washed 70S ribosomes can perform all the steps in initiation with a mixture of three known initiation factors[9,10], dissociation factor must be one or more of these factors. Indeed, separation of the initiation factors on DEAE-cellulose regularly yields a good recovery of DF activity in the F_3 fraction, while lower activities appear variably in the F_1 fraction[6,11]. Identity of DF with F_3 is further strongly supported by our finding that with

purified preparations of the three initiation factors[12], kindly provided by Dr S. Ochoa, the F_3 preparation causes dissociation but F_1 and F_2 do not[6].

Dr Ochoa has now kindly provided us with an essentially pure preparation of F_3, described in the preceding article[13]; and we have confirmed the observation that this material is active in dissociating ribosomes. As a further test of the physiological significance of the reaction we have examined its stoichiometry at low Mg^{2+} concentrations when the response is maximized[5]. Our results support the conclusion that F_3 is the factor responsible for the dissociation step in the ribosome cycle.

Dissociation of Free 70S Ribosomes by Factor F_3

Fig. 1 shows the effect of pure F_3 on the sedimentation pattern of NH_4Cl-washed ribosomes in a buffer containing 2 mM Mg^{2+}. Incubation of 30 μg of ribosomes with 0·5 μg of F_3 raised the level of subunits from an initial value of 15 per cent to a final value of 60 per cent and 1·0 μg raised it to 90 per cent.

The number of 70S particles dissociated by a given amount of unpurified dissociation factor depends strongly on the concentration of Mg^{2+} (ref. 5). Because free *E. coli* ribosomes dissociate spontaneously at Mg^{2+} concentrations below 1·5 mM* (in the presence of 10 mM Tris and 50 mM KCl), our earlier standard assay used 5·0 mM Mg^{2+} (plus 1 mM GTP) to provide a safe margin against spontaneous dissociation. To measure the dissociating capacity of pure F_3 under more optimal conditions it was tested at various lower Mg^{2+} concentrations down to the level of incipient spontaneous dissociation. As Fig. 2 shows, while 2 μg of F_3 gave only about 15 per cent dissociation of 25 μg of 70S ribosomes at 5·0 mM Mg^{2+} it gave nearly 100 per cent at 2·0 mM. Smaller amounts of F_3 yielded parallel curves.

Unwashed runoff ribosomes responded to low Mg^{2+} or to F_3 very similarly to our ribosomes which had been washed with 1 M NH_4Cl after extraction from exponentially growing cells (Fig. 1)†. This shows that DF is not a ribosomal protein artificially extracted by 1 M NH_4Cl, but that it is physiologically detached from the ribosome at some stage before runoff. The distribution of DF among the various ribosomal particles[5] has suggested that the detachment occurs at some stage in the transition between an initiating complex and a functioning polysomal ribosome.

In Fig. 3 the data of Fig. 2 are redrawn to plot dissociation against amount of F_3. The curves obtained, at different Mg^{2+} concentrations, are each quite linear with F_3 up to at least 50 per cent dissociation. This result would be expected for a first-order reaction, in which one molecule of F_3 combines with a ribosome.

Because the molecular weight of F_3 is 21,200[13], and that of NH_4Cl-washed ribosomes is $2{\cdot}6 \times 10^6$ (ref. 15), a 1 : 1 stoichiometry would give 124 μg of ribosomes dissociated per μg of F_3. The ratios we observed at 2·0 and 1·5 mM Mg^{2+} are 28 and 53, respectively, which are equivalent to about 4 and 2 molecules of F_3 per ribosome. Moreover, from Fig. 2 it seems that even 1·5 mM Mg^{2+} is not low enough to permit a maximal response. These ratios, in a reaction that does not go to completion in the conditions of testing, also support the model in which one molecule of F_3 combines with the 30S moiety of a 70S ribosome and causes it to dissociate from the 50S moiety.

The dissociation reaction is strikingly inefficient at the Mg^{2+} concentrations optimal for *in vitro* protein synthesis with natural messenger (5·0 mM or above)—less than one tenth as efficient as at 1·5 mM. If the response is similar in the cell it would seem that a low affinity for DF is necessary in the ribosome cycle, perhaps to permit the binding of DF to be reversed on reassociation of the initiating 30S subunit with a 50S subunit.

The apparent affinity constant of F_3 for the ribosome at 4 mM Mg^{2+} can be calculated, from the 50 per cent dissociation of ribosomes in equilibrium with 2 μg/ml., as $(2 \times 10^{-3})/(22 \times 10^{3}) \approx 1 \times 10^{-7}$ M. This affinity, which seems very low in dilute solution, is still sufficient to cause most DF molecules to be bound in the cell. For in our assays the response in 4 mM Mg^{2+} on the linear part of the F_3 concentration curve corresponded to 1 DF molecule causing dissociation, and therefore bound, for about every twenty molecules not causing dissociation and hence presumably free. In these assays 25 μg of 70S ribosomes were incubated in 0·1 ml. of solution. Because the ribosome concentration in the cell is roughly 150 times as great, a similar equilibrium in the cell would yield a ratio of bound to free DF of about 8 : 1, which is consistent with our earlier failure[5] to detect any DF in the supernatant fraction of a lysate.

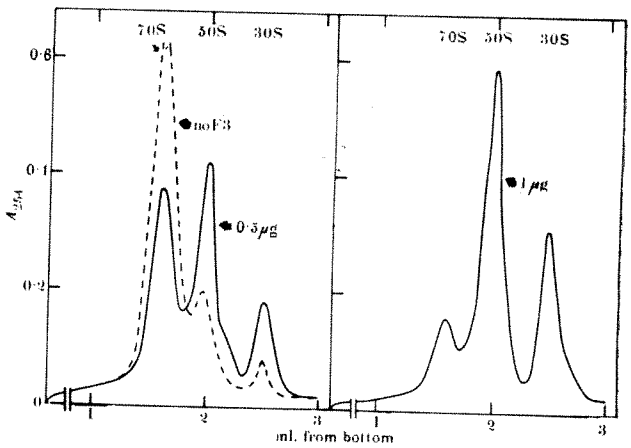


Fig. 1. Dissociation of 70S ribosomes by initiation factor F_3. Thirty μg of ribosomes (0·50 A_{260} unit), of which 25 μg was 70S particles, were incubated at 30° C with 0, 0·5, or 1 μg of F_3 in 0·1 ml. solution of 10 mM Tris-HCl (*p*H 7·8), 50 mM KCl, 2 mM magnesium acetate, and 2 mM dithiothreitol (DTT). After 15 min the mixture was chilled in ice and 0·08 ml. was layered on a 10–30 per cent sucrose gradient in TKM (10 mM Tris-HCl *p*H 7·8, 50 mM KCl, 5 mM magnesium acetate). The gradients were spun at 4° C for 1 h at 60,000 r.p.m. ($RCF_{max} = 321{,}000g$) in an International B-60 ultracentrifuge, and the sedimentation patterns were obtained on an Isco gradient analyser. The ribosomes, washed with 1·0 M NH_4Cl, had been obtained as a byproduct in recovering DF from cells of *E. coli* MRE-600. The ribosomal pellet from the 1 M NH_4Cl solution was dissolved in 10 mM Tris-HCl (*p*H 7·8), 50 mM KCl, 10 mM magnesium acetate, 5 mM DTT, and 20 per cent glycerol, to an A_{260} of 500 units/ml. These ribosomes were stored up to 4 weeks at 4° C; and with the RNA of phage MS2 as messenger and with initiation factors added they incorporated 14 moles of ^{14}C-valine (about 1 mole of coat protein) per mole. This activity compares well with that of freshly made extracts (S-30) from log phase cells[14]. The purified F_3, provided by S. Sabol[13] as a powder lyophilized from NH_4HCO_3, was dissolved in 0·25 M Tris-HCl (*p*H 7·6), 1·0 mM DTT, and 7·5 per cent sucrose to 1 μg/μl.; dilute solutions (0·1 μg/μl.) were made with the same buffer.

In the cell, of course, the response to DF may well be enhanced by other factors: for example, the inhibition by Mg^{2+} can be antagonized by increased concentrations of KCl or of Tris (unpublished observations). Moreover, although 5 mM Mg^{2+} or more is required for optimal protein synthesis in an *in vitro* system with natural messenger, the free Mg^{2+} concentration in this system (and hence presumably in the cell) may be lowered substantially by the nucleotides and nucleic acid present in this system but not in our DF assay system. The ratio of bound to free DF in the cell is therefore likely to be even higher than the 8 : 1 calculated above.

With unpurified preparations of DF we[6] and Gonzalez *et al.*[16] have observed that dissociation is stimulated

* In the experiments of Ron *et al.*[2], in which the effect of low Mg^{2+} in the gradient was tested, some dissociation was observed with Mg^{2+} as high as 2 mM. In the present experiments the ribosomes were incubated at low Mg^{2+} and then analysed in a gradient containing 5 mM Mg^{2+}.

† In our earlier work[5,6] we had used unwashed runoff ribosomes to establish a physiologically significant interaction of DF with a natural component of the cell. But NH_4Cl-washed ribosomes have proved equally satisfactory for DF assays (see also refs. 13 and 17). Evidently this washing completely removes the ligands that protect complexed, polysomal ribosomes from dissociation by DF.

two-fold by 1·0 mM GTP or certain other nucleoside triphosphates, even with an equivalent amount of Mg^{2+} added. Others, however, have observed no effect of GTP with partially purified DF[17] or purified F_3 (ref. 13). With pure F_3, and with recent unpurified preparations, we now find that the stimulatory effect of GTP can be completely suppressed by an equivalent amount of Mg^{2+}. This effect may therefore be accounted for by chelation of Mg^{2+}. The 5 mM Mg^{2+} plus 1 mM GTP in our standard assay is thus equivalent to 4 mM Mg^{2+} alone.

Is F_3 the Only Dissociation Factor?

Although pure F_3 has DF activity and pure F_1 and F_2 have not, when a crude initiation factor preparation is fractionated on a DEAE–cellulose column by the procedure of Iwasaki *et al.*[9] the F_1 fraction (that which comes through unadsorbed) contains about 25 per cent of the total DF activity, the remainder being found in the F_3 cut[6,11]. But when the DEAE–cellulose fractionation was carried out as described by Sabol *et al.*[13] the entire DF activity was eluted at the position expected for F_3. It seems likely, therefore, that in the NH_4Cl extract of ribosomes, F_3 is the only protein with DF activity. Because it is a basic protein the variation in its adsorption on DEAE–cellulose could be accounted for by a dependence on association with anions.

Miall *et al.*[18], using a buffer with 6 M urea, found that all the DF activity passed through a DEAE–cellulose column, and they concluded that their final, purified DF might be F_1. We have found, however, that in the procedure of Sabol *et al.*[13] the DF activity is no longer retained by the column when the buffer contained 6 M urea. Thus the purification of DF by Miall *et al.* could have concentrated factor F_3, but because the product was less active than the F_3 of Sabol *et al.* and was reported to have a lower molecular weight (9,000 as against 21,200), it seems likely to have been a mixture of F_1 and F_3.

DF Content of Ribosomal Extracts

Our model[5] for the ribosome–polysome cycle requires the presence of a molecule of DF on every native 30S subunit to prevent spontaneous reassociation with a 50S subunit. Yet the 1 M NH_4Cl extract from 100 mg of ribosomal pellet (and thus from 10–20 mg of subunits) dissociates only 1 mg of runoff ribosomes in our standard assay[5]; and the low apparent yield is not the result of incomplete extraction, because re-extraction yields negligible activity (unpublished observations). But because the efficiency of the DF response in our standard assay is only about 5 per cent, the recovery of DF is consistent with the proposed model. (These data are not precise enough, however, to exclude the possibility that part of the "native" 30S peak may consist of immature or damaged subunits.)

The above calculation derives the content of F_3 (DF) in an extract from its activity. A similar content can be calculated from the data of Sabol *et al.*[13] on the recovery of F_3 as a protein, without assumptions concerning its activity. According to their Table 1, 3·4 kg of cells yielded 15 mg of pure F_3, with 19 per cent recovery, corresponding to 79 mg in the initial extract. Because *E. coli* cells yield about 40 mg of ribosomes per g, the extract would have been made from about 136 g of ribosomes. If each ribosome had yielded 1 molecule of F_3 the content in the extract would have been $(21{,}200/2{\cdot}6\times10^6)\times136$, or 1·1 g of F_3. The actual content (79 mg) is thus much too low to correspond to 1 molecule per ribosome, but it corresponds quite nicely to 1 molecule per native subunit.

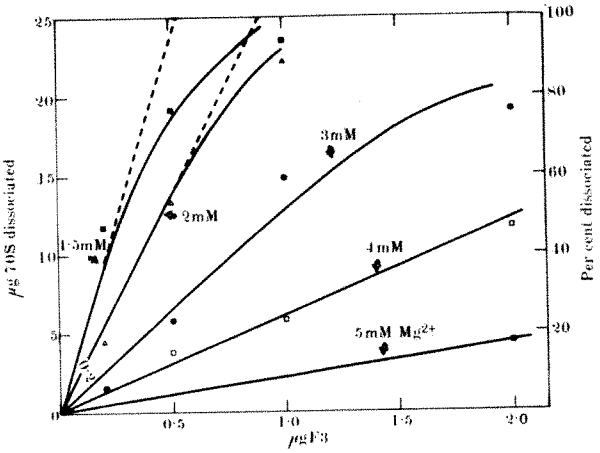


Fig. 3. Linearity of the dissociation response to increasing amounts of F_3 at various Mg^{2+} concentrations. The data are those of Fig. 2, with 25 μg of 70S ribosomes corresponding to 100 per cent.

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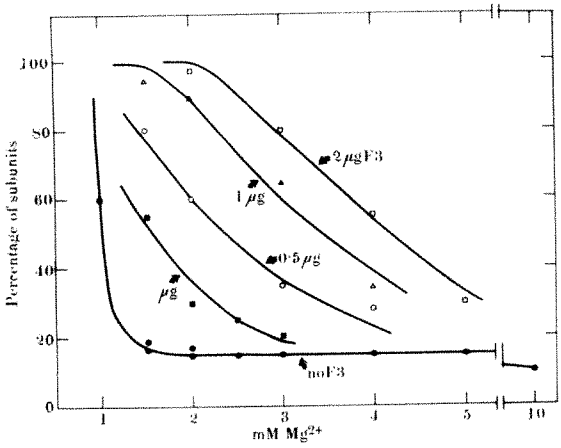


Fig. 2. Dissociation of 70S ribosomes by F_3 at various Mg^{2+} concentrations. The procedure was as in Fig. 1 except for variations in the concentrations of F_3 and magnesium acetate. The per cent dissociation was determined by cutting out and weighing a tracing of the peaks; the background level of dissociation, without added F_3, is not subtracted, and so 100 per cent represents dissociation by F_3 of 25 of the 30 μg of ribosomes present.

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Novel Subunit in Secretory IgA

by

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A new component, "J" (for joining), has been found in rabbit and human secretory IgA in addition to the S, α, and light chains already characterized. J has a molecular weight of approximately 23,000 and, by contrast with the S component, is disulphide-linked to the α chain. It is present in the dimer but not the monomer form of human IgA myelomas, and therefore seems to be responsible for the dimer structure characteristic of secretory IgA.

Secretory IgA possesses a polypeptide chain designated secretory or S component[1] which is not found in serum IgA[2]. This chain is known to be synthesized by epithelial acinar cells rather than the plasma cells responsible for light and heavy chain synthesis[2,3]. The two cell interaction required for secretory IgA synthesis has focused intense interest on the assembly of the IgA macromolecule.

Cebra and Small[4], working with rabbit colostral IgA, concluded that the light and heavy chains are assembled into two IgG-like monomeric units, and the S component is linked non-covalently to the molecule. Their data suggested that the S component is composed of two 25,000 molecular weight subunits linked by a disulphite bond. The occurrence of S subunits was deduced from electrophoretic studies which showed a fast-moving band migrating ahead of light chain in preparations of completely reduced and alkylated IgA. These conclusions are somewhat at variance with those of Tomasi and Bienenstock[5], who found that in human secretory IgA only a fraction of S component was non-covalently bound to the molecule, the majority being linked by disulphide bonds. In addition, the human S component could not be broken down into subunits following treatment with reducing agents. The discrepancies in the two studies were ascribed to species differences in the IgA.

The structural studies presented here were carried out on IgA isolated from rabbit and human colostrum. The rabbit IgA was purified by the method of Cebra and Robbins[6] and the human IgA by the method of Tomasi and Bienenstock[5]. The purity of the preparations was tested by immunoelectrophoresis against goat anti-rabbit whey and serum and goat anti-human serum. At a concentration of 10 mg/ml., the preparations exhibited no other precipitin arc than that corresponding to the respective IgAs.

As the first step in its fractionation, the rabbit IgA was treated with detergent to dissociate the non-covalently bound S component. The dissociation seemed to be complete, for no further S component was obtained on subsequent treatment with a combination of 8 M urea and detergent. The S component could then be separated by chromatography on a 'Sephadex G-200' column equilibrated with 0·05 M decyl sulphate or by electrophoresis on sodium dodecyl sulphate (SDS) polyacrylamide gels[7]. The latter results are illustrated in Fig. 1. The complex of covalently bound heavy and light chains barely penetrated the gel (Fig. 1*b*), while the S component migrated with a slightly lower mobility than bovine serum albumin (compare Fig. 1*b* with Fig. 1*a*). From a comparison of the positions of the S component and albumin, the S component seemed to have a molecular weight of approximately 70,000.

To determine its subunit structure, the isolated rabbit S component was completely reduced in the presence of 10 M urea and the resulting SH groups were alkylated with iodoacetamide. Subsequent electrophoresis on SDS gels revealed only a single component (Fig. 1*d*) which had essentially the same molecular weight as the untreated S (Fig. 1*c*). Furthermore, when the reduced and alkylated S component was chromatographed on 'Sephadex G-200' equilibrated with decyl detergent (Fig. 2), the protein emerged as a single peak slightly behind the elution volume of non-reduced S. The absence of smaller molecular weight material eliminated the possibility that the S component is composed of subunits. The slight difference in the elution volume of reduced S, corresponding to a change in molecular weight from 70,000 to 64,000, suggested that its conformation was altered during the reduction of the disulphide bonds.

Similar methods were applied to investigate the subunit structure of the covalently bonded complex remaining after the dissociation of the S component. When the completely reduced and alkylated material was electrophoresed on SDS gels or subjected to chromatography on 'Sephadex G-200' only two fractions were obtained which exhibited the behaviour typical of α and light chains. When the reduced material was electrophoresed on an alkaline urea gel[8], however, a densely staining component was seen migrating in the leading edge of the light chain region (Fig. 3*b*). The same densely staining component was observed when the isolated light chain fraction, but not when the isolated heavy chain fraction was subjected

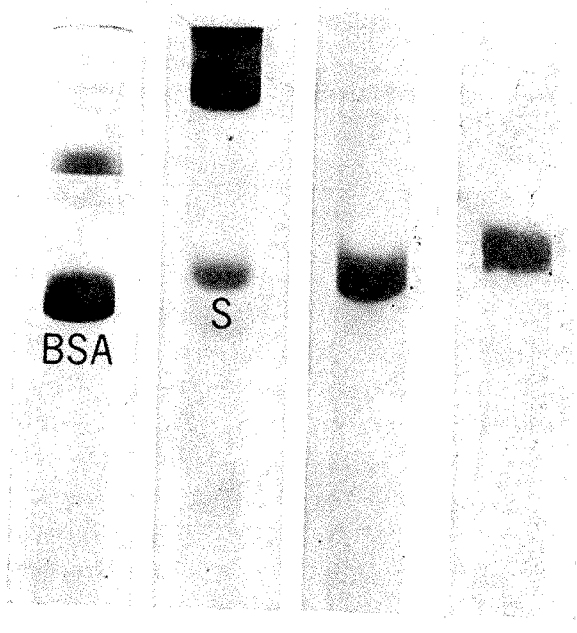


Fig. 1. Electrophoretic patterns obtained on 5 per cent polyacrylamide gels *p*H 8·1 with 0·1 per cent SDS. Electrophoresis for 3 h at 12 V/cm. *a*, Bovine serum albumin (BSA); *b*, rabbit secretory IgA; *c*, rabbit S component; *d*, rabbit S component reduced in the presence of 10 M urea with 0·15 M dithiothreitol and alkylated with 0·45 M iodoacetamide.

to electrophoresis on alkaline gels (Fig. 3*d*). Thus the co-chromatography with the light chain indicated that the fast-moving component had a molecular weight of approximately 23,000.

This fast-moving component had previously been shown to be peculiar to secretory IgA[9] and was thought[3] to represent the subunit of S. Such an interpretation is ruled out by the findings reported here that S has a single chain structure and that the fast-moving component is present in IgA freed of S. Two alternative explanations are consistent with these findings: either the fast moving component represents a light chain type peculiar to IgA, or it represents an additional subunit in the IgA macromolecule. (From now on, the letter "J" will be used to designate the fast-moving component.)

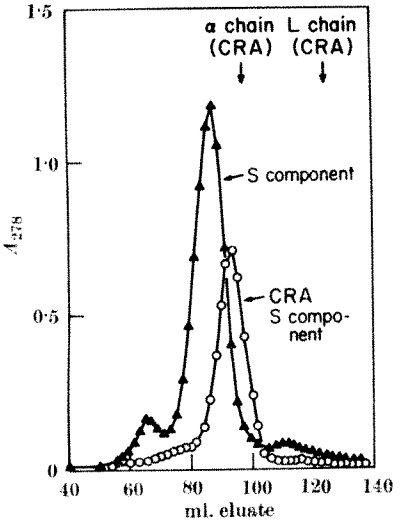


Fig. 2. Chromatography on 'Sephadex G-200' equilibrated with decyl detergent of S component and S component (CRA) after complete reduction and alkylation (10 M urea, 1 M mercaptoethanol and 1·05 M iodoacetic acid).

Evidence suggesting that J is not a light chain was provided by amino-acid analyses of the material eluting at the light chain position when completely reduced and alkylated IgA was chromatographed on 'Sephadex G-200'. The average composition of these fractions was compared with the composition of IgC light chains isolated from the same animals and prepared in identical conditions. As shown in columns 1 and 2 of Table 1, the composition

Table 1. COMPARISON OF THE AMINO-ACID COMPOSITION* OF IgA AND IgG LIGHT CHAINS

Amino-acid	IgG CRA†	IgA CRA†	Δ‡	IgA MRA§	IgA residual CRA‖	Δ
Lys	8·8 ±0·11‡	8·5 ±0·10¶		8·6	7·8	1·0
His	1·4 ±0·034	1·4 ±0·028		1·3	1·3	
Arg	3·3 ±0·057	4·5 ±0·098	1·2	3·3	5·4	2·1
Asp	20·0 ±0·18	21·7 ±0·17	1·7	20·2	22·1	2·1
Thr	30·6 ±0·47	29·7 ±0·27		31·1	27·4	3·2
Ser	20·9 ±0·074	19·1 ±0·090	1·8	20·6	17·1	3·8
Glu	21·2 ±0·067	20·8 ±0·096		21·4	19·8	1·4
Pro	12·0 ±0·067	12·3 ±0·11		12·3	12·6	
Gly	19·7 ±0·15	17·3 ±0·13	2·4	19·6	15·2	4·5
Ala	16·0 ±0·10	14·6 ±0·12	1·4	16·4	13·0	3·0
Val	21·4 ±0·35	19·8 ±0·11	1·6	21·7	18·7	2·7
Met	0·72 ±0·069	0·78 ±0·061		0·72	0·78	
Ile	7·2 ±0·055	7·7 ±0·056	0·5	7·3	8·3	1·1
Leu	11·0	11·0		11·0	11·0	
Tyr	10·2 ±0·11	9·7 ±0·14		10·5	9·0	1·2
Phe	6·7 ±0·038	6·4 ±0·053		6·5	6·1	
CMcys	7·3 ±0·11	7·4 ±0·23		0·99	8·0	
Gln	0			Trace		

* After 20 h hydrolysis; results normalized to a leucine value of 11.
† Light chain obtained after complete reduction and alkylation.
‡ Δ = (IgG − IgA).
§ Light chain obtained after mild reduction and alkylation.
‖ Residual light chain obtained after complete reduction and alkylation of aggregated material eluted in preparation of mildly reduced and alkylated light chain.
¶ Standard error of the mean. IgG light = average of five preparations. IgA light = average of seven preparations.

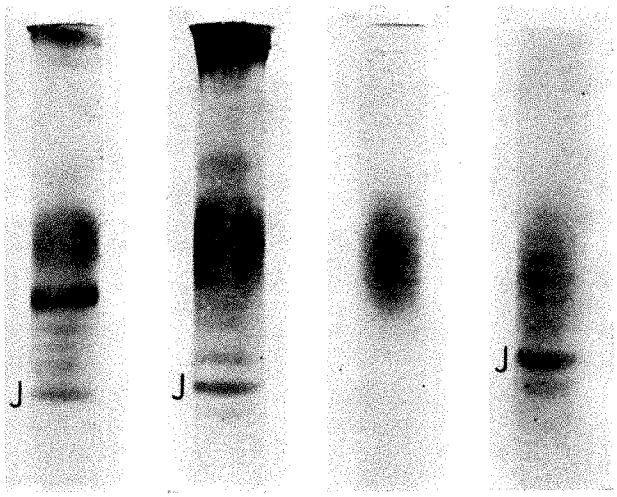


Fig. 3. Electrophoretic patterns obtained on 4 per cent polyacrylamide gels pH 8·7 with 8 M urea. Electrophoresis for 2 h at 40 V/cm. *a*, Rabbit secretory IgA reduced in the presence of 10 M urea with 0·15 M dithiothreitol and alkylated with 0·45 M iodoacetamide; *b*, rabbit IgA freed of S component treated as in *a*; *c*, material which eluted in the light chain position of a 'Sephadex G-200' chromatogram of mildly reduced and alkylated rabbit secretory IgA (0·1 M mercaptoethanol and 0·105 M iodoacetamide); *d*, material which eluted in the light chain position after complete reduction and alkylation of aggregated material eluted in preparations of mildly reduced and alkylated rabbit IgA (10 M urea, 1 M mercaptoethanol and 1·05 M iodoacetamide).

of the IgA light chain fraction differed considerably from that of the IgG. Statistical analyses showed that the content of seven amino-acids, arginine, aspartic acid, serine, glycine, alanine, valine, and isoleucine, was different in the IgA and IgG light chain fraction ($P < 0{\cdot}01$). Moreover, the IgA light chain fraction always contained residual glucosamine while the IgG light chains contained none.

The disulphide bond(s) linking J chain were found to be less susceptible to reductive cleavage than most of the light–heavy interchain disulphide bonds. When secretory IgA was mildly reduced in conditions which freed approximately 65 per cent of the total light chain present, only light chains of IgG-like composition and electrophoretic behaviour were obtained (Table 1, column 4, and Fig. 3*c*). When the remaining 35 per cent of the light chain bound to heavy chain was released by complete reduction and alkylation, J chain appeared in the fraction eluting at the light chain position. Furthermore, J comprised a relatively greater proportion of the total material in the fraction as shown by the denser staining of the anodal band (compare Fig. 3*d* with Fig. 3*a*) and by the more anomalous composition of the mixture (Table 1, column 6). The finding that a high percentage of normal light chain could be separated from the J chain suggested that J is linked to heavy chain.

When these investigations were extended to human colostral IgA, evidence was obtained for a comparable J chain. The characteristic fast-moving bands were found to be present in the light chain fraction, indicating that human J also had a molecular weight in the order of 23,000. The electrophoretic mobility of the bands was similar to that exhibited by the rabbit J, but the human J could be resolved more easily because of the slower mobility of the human light chains. These data are illustrated in Fig. 4, in which the banding of the light chain fractions from completely reduced and alkylated human IgA and human IgG are compared (compare Figs. 4*a* and *b*). Furthermore, the average compositions of the human IgA light chain fractions differed significantly from the control IgG fractions and many of the differences were in the same direction as those observed in the analyses of the rabbit material.

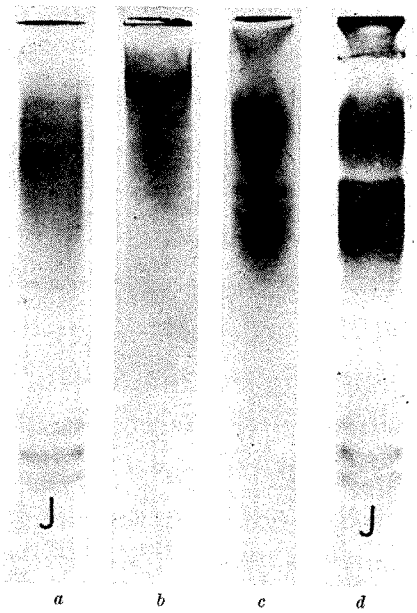


Fig. 4. Electrophoretic patterns obtained on 4 per cent polyacrylamide gels *p*H 8·7 with 8 M urea. Electrophoresis for 2 h at 40 V/cm. *a*, Light chain fraction from totally reduced and alkylated human secretory IgA; *b*, completely reduced and alkylated human IgG; *c*, completely reduced and alkylated monomer from a $\gamma A_2(\lambda)$ myeloma; *d*, completely reduced and alkylated dimer from the same myeloma.

Although these studies of rabbit and human secretory IgA supported the interpretation that J is a novel subunit, the data were not sufficient to eliminate the possibility that J is a special light chain more resistant to reductive cleavage than most light chains. To obtain more conclusive evidence, investigations were carried out on human IgA myelomas in which both monomer and dimer forms were produced (myeloma proteins purified from human serum kindly provided by Drs Hugh Fudenberg, Al Levin and Richard Pink). Three pairs of proteins were studied, one pair of the γA_1 subclass, containing $\varkappa$ light chains, a second of the same subclass but containing λ light chains, and a third of the γA_2 subclass containing λ chains. When the protein pairs were completely reduced and alkylated and electrophoresed on alkaline urea gels, fast moving bands were found to be present in all the dimers but absent from all the monomers. The typical patterns obtained are illustrated in Figs. 4*c* and *d* by the $\gamma A_2(\lambda)$ set. It may be seen that the J from the myeloma dimer had the same mobility and charge heterogeneity as the human colostral J (compare Figs. 4*d* and *a*). At the same time the banding of the heavy and light chains was identical in the monomer and dimer, indicating that one clone was synthesizing both forms of the IgA. Any one clone of plasma cells is known to produce only a single light chain, so these results demonstrate that J is a novel subunit.

In the case of the myeloma IgA, J would appear to be synthesized in the same plasma cell as the heavy and light chains and to function by linking the monomeric units. Rabbit colostral IgA has also been shown to be secreted from the plasma cell as a dimer[10], so a similar process presumably occurs in the synthesis of normal secretory IgA. The mechanism of J is obtained and its molar ratio in the dimer IgA determined.

The role of J in the final assembly of the dimer and the S component is intriguing. J may provide the binding site for S, either directly through the contribution of groups to the site, or indirectly through an induced conformational change in the α chains. J may further provide the recognition signal for the interaction between the plasma cell synthesizing the J–dimer complex and the epithelial cell synthesizing the S component. Studies are in progress to evaluate these possibilities.

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In vitro Combination of Human and Bovine Free Secretory Component with IgA of Various Species

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The free form of the secretory component usually associated with secretory IgA can be isolated from human and bovine milk. These free secretory components of different origin combine *in vitro* with human polymeric myeloma IgA, with mouse myeloma IgA, and with the serum IgA of nine different mammalian species.

Since the secretory component (SC) has been identified as a part of the secretory IgA (SIgA) and shown to be detachable from the IgA molecule in certain conditions[1–5], many attempts have been made to recombine the two separated parts of the SIgA *in vitro*[6,7]. Most of these experiments were done with an SC cleaved from SIgA by reduction and alkylation, and no study of the binding involved in this recombination has been reported. Combination of the free form of the secretory component with an IgA lacking SC would represent a test of greater functional significance. Free secretory component (FSC) is known to be present in human colostrum[4,5,8] and an analogous protein has been identified in bovine, ovine and caprine secretions[9–11]. This study deals with the combination of purified human and bovine FSC with polymeric human myeloma IgA (Fig. 2) and with IgA from the sera of nine different mammalian species (Fig. 3 and Table 1).

Purification of FSC from Human and Bovine Milk

This purification was based on the original observation that these two components are not retained by DEAE-

cellulose equilibrated with 0·01 M phosphate buffer (*p*H 7·4). The starting materials were 5th–10th day maternal milk and mature cow milk from which the fats and the casein had been removed by centrifugation at 45,000*g* for 1 h. The proteins were precipitated with 50 per cent saturated $(NH_4)_2SO_2$ at room temperature. The precipitation was performed twice in order to remove most of the lactoferrin remaining in the supernatant. The precipitate was redissolved, dialysed against 0·01 M phosphate buffer (*p*H 7·4) and applied to a DEAE-cellulose column equilibrated with the same buffer. In these conditions the secretory components were not retained by DEAE-cellulose and were obtained contaminated only with small amounts of IgG. These fractions were further purified by gel filtration on 'Sephadex G-200' and preparative ultracentrifugation on a linear 5 per cent to 20 per cent sucrose density gradient (45,000 r.p.m. for 15 h at 4° C in a Beckmann Spinco model L2.65 B ultracentrifuge).

The purified proteins gave only one line on immunoelectrophoresis with a polyvalent antiserum (Fig. 1*a*, *c*). When revealed in whole colostrum by anti-SIgA antiserum the human and the bovine FSC gave a β_2-line fusing partially with SIgA (Fig. 1*b*, *d*). Tested by double diffusion with an antiserum against SIgA, purified human and bovine FSC showed a partial identity with human and bovine SIgA respectively and no common antigenicity with human myeloma IgA and bovine serum IgA (Fig. 1*e*, *f*). Their molecular weights were determined by electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate (SDS) according to Weber and Osborn[12]. The values obtained were 85,000 for human FSC and 75,000 for bovine FSC (Fig. 1*g*). These results are consistent with those of Newcomb *et al.*[4], who reported a molecular weight of 76,000 for human SC.

Combination of FSC with Human Myeloma IgA

One hundred μg of each of the above purified FSC, labelled with ^{125}I by the chloramine T method[13], were incubated for 1 h at 37° C (in 0·5 ml. of 0·14 M NaCl adjusted to *p*H 7·4 with 0·01 phosphate buffer) either with 5 mg of purified human IgG or with 2 mg of purified human myeloma IgA of polymeric form. After incubation, these proteins were applied to a 'Sephadex G-200' column. When incubated with IgG, 96 per cent of the labelled FSC protein was eluted just after the 7S peak (Fig. 2*a*), indicating that it remained in its free form. On the other hand, when incubated with myeloma IgA, 86 per cent of the labelled FSC was eluted with the IgA protein in the exclusion volume of the 'Sephadex G-200' column (Fig. 2*c*), suggesting that most of the labelled FSC had been bound to the polymeric IgA. Similar results were obtained by ultracentrifugation of the incubated protein on sucrose density gradients.

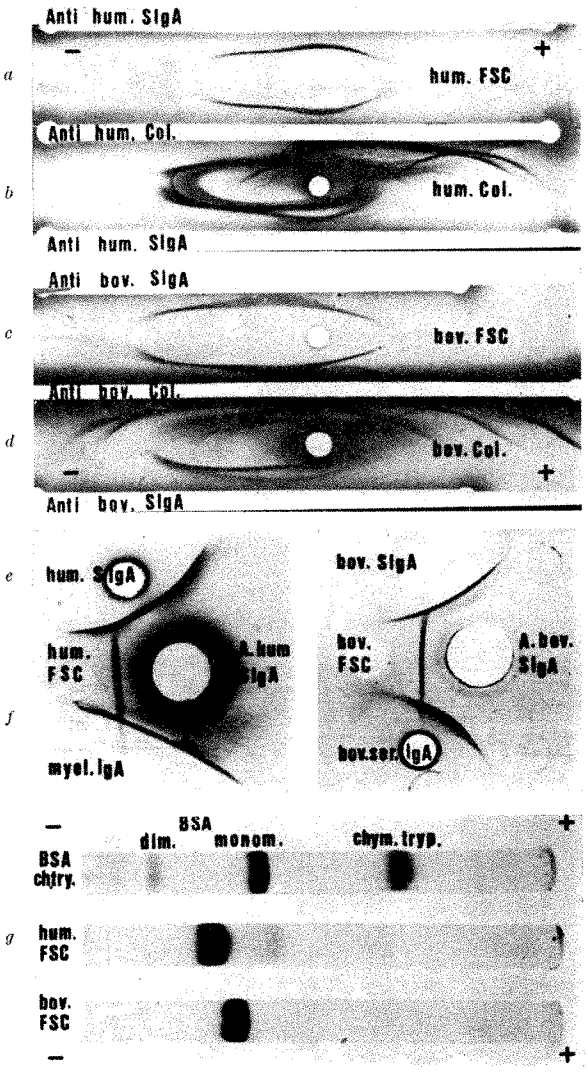


Fig. 1. Immunoelectrophoresis, Ouchterlony plates and polyacrylamide gel electrophoresis of human and bovine free secretory component (FSC). Antigens: *a–f*, hum. FSC = purified human FSC; hum. Col. = whole human colostrum after removal of fats and casein; bov. FSC = purified bovine FSC; bov. Col. = whole bovine colostrum after removal of fats and casein; myel. IgA = purified human myeloma IgA; bov. ser. IgA = whole bovine serum. Antisera: rabbit antisera were used throughout as indicated in the picture. *g*, Electrophoresis in polyacrylamide gels containing SDS according to Weber and Osborn[12]. (BSA dim. monom. = bovine serum albumin dimers monomers; chym. tryp. = α-chymo-trypsinogen; hum. FSC, bov. FSC = human and bovine purified FSC. Migration from cathode (left) to anode (right).

Table 1. COMBINATION OF HUMAN AND BOVINE FSC WITH SERUM IGA OF LIVE MAMMALIAN SPECIES

Species	Per cent of combination in whole serum	Labelled FSC attached to* IgA	IgM	IgG	Antiserum used to precipitate IgA
Human	76	+ + +	+	Traces	Rabbit anti-human IgA
Bovine	57	+ +	+ +	0	Rabbit anti-bovine SIgA absorbed bov. FSC†
Sheep	55	+ + +	+ +	0	
Goat	53	+ + +	+ +	0	
Pig	64	+ + +	0	0	Rabbit anti-human IgA ‡
Dog	48	+ + +	+	0	
Horse	52	+ +	+	0	
Guinea-pig	26	+	?	0	Rabbit anti-guinea-pig SIgA§
Mouse	27	+	Traces	0	Rabbit anti-mouse myeloma IgA‖

* Determined by the intensity of the lines on autoradiography of immunoelectrophoresis technically identical to the ones shown in Fig. 2.

† The bovine, ovine and caprine IgA were previously identified and shown to be homologous to human IgA[10,11,17].

‡ Similar cross-reactions of antiserum against human IgA with the IgA of these species have been reported[17].

§ This antiserum was obtained from Dr A. Asofsky, of NIH, Bethesda, USA. It did not react with the human and bovine FSC preparations used in the combining experiment.

‖ This antiserum was obtained from Dr H. Jaquet, Institut de Biochimie, Lausanne, Switzerland[24].

Binding to IgA was confirmed by autoradiography taken from immunoelectrophoresis[14] and single radial diffusion analysis[15] of the fractions eluted from the 'Sephadex' column, using an antiserum monospecific for IgA. This antiserum did not react either with the small peak of FSC remaining in the free form or with the whole labelled FSC preparation. Bovine and human FSC were bound to human IgA equally well. In order to show that polymeric myeloma IgA was not binding any protein nonspecifically, purified human serum albumin (HSA) was labelled and tested for combination with myeloma IgA in an identical experiment. There was no *in vitro* combination between IgA and HSA. HSA was chosen for this test because a small amount of it has been found attached to myeloma IgA or serum IgA[16,17]. The combination of FSC was confirmed with polymeric myeloma IgA obtained from five different patients. These IgA myeloma preparations were purified by DEAE-

cellulose chromatography and the polymeric form selected by filtration on 'Sephadex G-200'. When tested with polyvalent antiserum, they gave only one line on immunoelectrophoresis. Examination by analytical ultracentrifugation showed them to be heterogeneous and composed of several polymers with sedimentation coefficients ranging from 10S to 15S.

When a 7S IgA fraction (selected by 'Sephadex G-200' filtration from a serum containing a myeloma IgA mostly of monomeric form) was incubated with FSC in an identical experiment, no combination occurred. Thus the polymeric form of IgA was necessary for the *in vitro* combination. This is in agreement with the present concept of the *in vivo* combination, which is based on the recent observation that rabbit as well as human SIgA have symmetrical light chains and should therefore be synthesized by a single plasmocyte[18–20]. This synthesis of IgA dimers by single plasmocytes has to occur before combination with FSC.

Another combination test was done with 7S myeloma IgA polymerized by reduction followed by reoxidation in acetate buffer at *p*H 5, according to the method of Abel and Grey[21]. Polymerization of 40 per cent of the 7S IgA was obtained but the FSC did not combine with it. This shows that the artificially prepared polymers are not identical with the native polymers. The details of these experiments have been reported at the fourth Immunopathology Symposium[22]. Heat aggregated IgG did not show any more binding ability than did 7S IgG. On the other hand, purified IgM obtained from patients with Waldenström's disease was able to bind FSC as well as polymeric myeloma IgA. Whether or not this binding is due to polymers of IgM or to the monomeric form is under investigation. The *in vitro* combination of FSC with purified IgM agrees with the recent observation of Thompson, who demonstrated the presence of secretory antigenic determinants on the IgM of patients with IgA deficiency[23].

Denaturation of IgA combined with FSC

The strength of the binding between FSC and IgA was tested by exposing the combined proteins excluded from 'Sephadex G-200' (Fig. 2*c*) to different denaturing agents for 6 h at room temperature and then chromatographing the proteins on a 'Sephadex G-200' column equilibrated with the same denaturing agent. The results show that when treated with 0·5 M propionic acid, the combined proteins remained almost entirely bound (Fig. 2*d*); when treated with 6 M guanidine, 21 per cent of the labelled SC was detached (Fig. 2*e*). This last result corresponds to what has been observed when native human SIgA was treated with 6 M guanidine[6] and suggests that the *in vitro* combination is similar to the one which occurs *in vivo*. The bovine FSC combined with human myeloma IgA gave similar results when tested with denaturing agents. Preliminary results indicate that the binding of FSC with IgM is as resistant to denaturation as the binding with IgA.

Reduction and Alkylation

The binding involved in this *in vitro* combination was further studied by reduction and alkylation. When

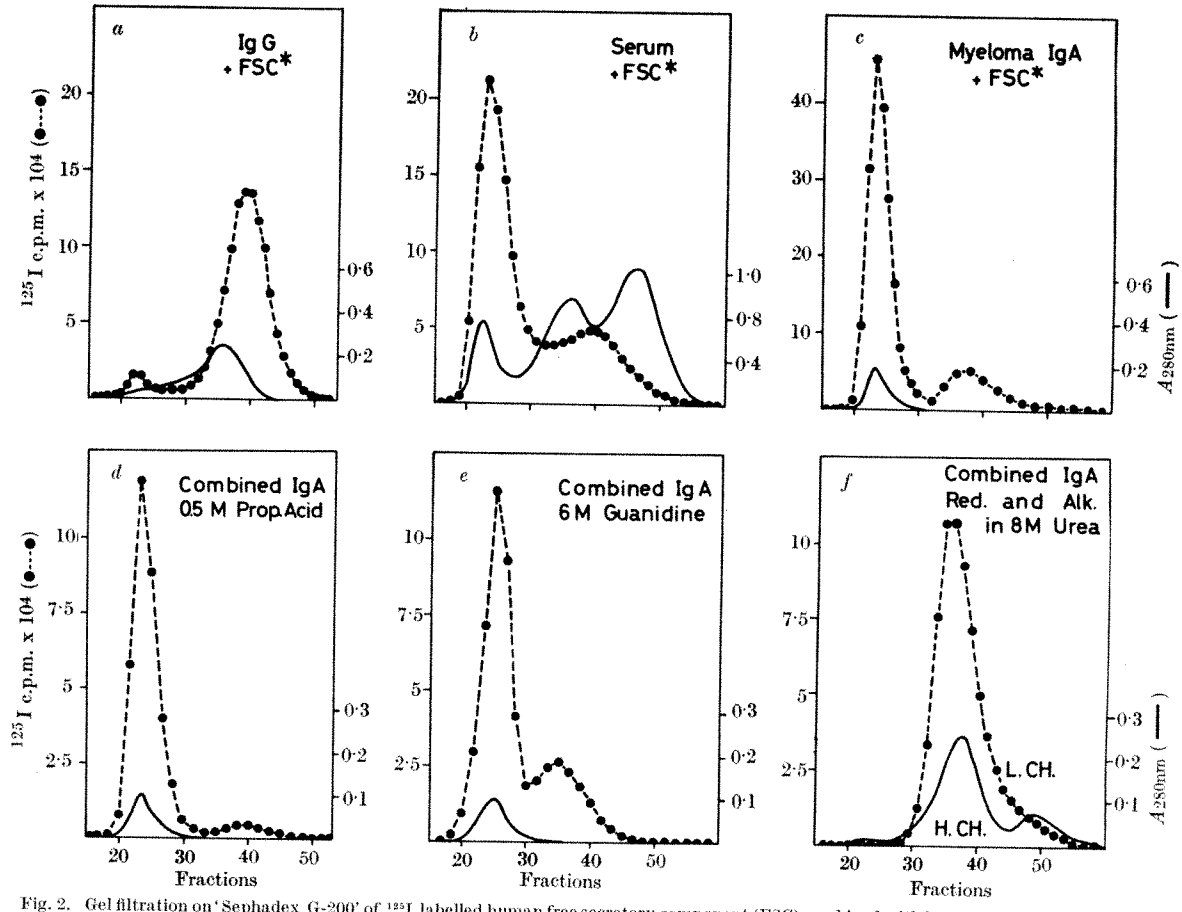


Fig. 2. Gel filtration on 'Sephadex G-200' of ^{125}I labelled human free secretory component (FSC) combined with human serum and polymeric myeloma IgA. The dotted line represents the ^{125}I counts per minute $\times 10^4$ (scale on the left of the graphs); the full line, the absorbance of the proteins at 280 nm (scale on the right). *a*, Control showing unbound FSC (incubated with human IgG as indicated in the text) eluted after the 7S IgG peak; *b*, elution pattern of FSC after incubation with normal human serum; *c*, elution pattern of FSC after incubation with human polymeric myeloma IgA; *d–f*, filtration of polymeric myeloma IgA combined with FSC (taken from the first peak of *c*) in *d*, after denaturation by 0·5 M propionic acid, in *e*, after denaturation by 6 M guanidine, in *f*, after reduction and alkylation in 8 M urea as indicated in the text. In *a–c* the 'Sephadex G-200' was equilibrated with 0·05 M Tris–0·2 M NaCl solution adjusted to *p*H 8 by HCl; in *d–f* with the above-mentioned denaturing agents. In *f*, H.CH = heavy chain and L.CH = light chain.

the "combined IgA" (first peak of Fig. 2*c*) was only partially reduced and alkylated, without the use of denaturing agents, the two proteins were separated. The IgA was depolymerized and eluted from 'Sephadex G-200' as 7S IgA, before the labelled SC which was eluted as the native FSC. This separation by partial reduction and alkylation, contrasting with the resistance to denaturing agents, seems to indicate that disulphide bridges are involved in the *in vitro* combination. (The reduction was performed by 0·02 M dithiothreitol in Tris 0·05 M–NaCl 0·20 M solution adjusted to *p*H 8 by HCl, for 1 h at 37° C and the alkylation by 0·04 iodoacetamide for 1 h at 37° C, the *p*H maintained at 8 by the addition of solid Tris.) When the "combined IgA" was completely reduced and alkylated using the same reagents in the presence of 8 M urea, the IgA chains were dissociated but the labelled SC remained apparently intact (Fig. 2*f*), suggesting that it is made of a single polypeptide chain. The complete reduction and alkylation of unbound human and bovine FSC gave identical results.

FSC combines with Serum IgA of Different Species

One hundred μg of labelled FSC was incubated with 1 ml. of serum for 1 h at 37° C and applied to a 'Sephadex G-200' column. The elution pattern of human serum combined with human FSC after filtration on 'Sephadex G-200' is shown in Fig. 2*b*. About 78 per cent of the labelled protein was eluted with the exclusion volume, suggesting a combination of FSC with a rather large protein. When human FSC was incubated in identical conditions with the serum of a completely agammaglobulinaemic patient or with human cord serum (which contains IgG but neither IgA nor IgM), less than 5 per cent of the labelled material was eluted in the exclusion volume and 95 per cent remained unbound, giving a similar elution pattern to that of the control with IgG (Fig. 2*a*). These controls indicate that, in the case of the normal serum, the combination must have occurred with IgA or IgM. Single radial diffusion and autoradiography of the eluted fractions, using antisera monospecific for IgA, IgM and IgG, showed that the FSC combined mostly with IgA. The preferential affinity for IgA was further demonstrated by immunoelectrophoresis and autoradiography of the combined proteins as shown in Fig. 3*a*. Bovine FSC was combined with human serum IgA as well as human FSC (Fig. 3*b*). In the light of this observation combinations with the sera of other species were tried. Table 1 summarizes the results obtained with the serum of nine mammalian species. It indicates the percentage of labelled FSC, either human or bovine (average from four determinations), combined with pooled normal serum from the species tested, the relative amount of FSC attached to IgA, IgM and IgG, roughly evaluated from the intensity of the lines on autoradiography of immunoelectrophoresis, and the antiserum used for precipitation of the protein homologous to human IgA (similar cross-reacting antisera were used to reveal IgG and IgM). These results show that in the nine species tested, FSC was bound mostly to IgA. In a few species, namely the ruminants which have a low level of serum IgA[10,11], FSC was also attached to IgM (Fig. 3*c*, *d*). In no species did the FSC bind significantly to IgG. A particular case was the sera of mice with IgA myeloma (either C3H × 5647 or BALB/c MPC-1)[25,26]. In these sera 70 per cent of the FSC preparations were bound specifically with the mouse IgA paraprotein (Fig. 3*e*). Mouse myeloma IgA is known to have many common properties with human myeloma IgA, the same size heterogeneity, a high carbohydrate content[27] and a similar C-terminal tripeptide[28]. Now it is shown that it binds equally well human FSC.

In conclusion, the similar ability of the serum IgA of all nine species tested to bind FSC suggests that this is a property of the IgA of all mammals. Only two non-mammalian species, chicken and duck, were tested for

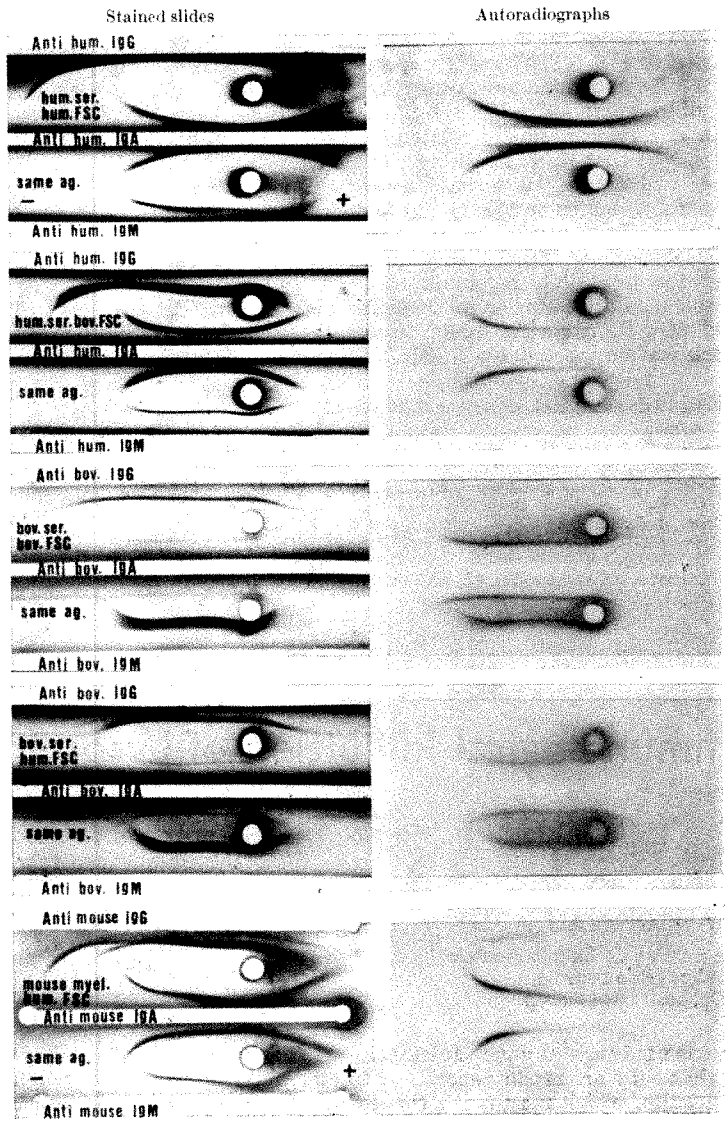


Fig. 3. Immunoelectrophoresis and autoradiographs showing the combination of human and bovine labelled free secretory component (FSC) with human and bovine serum IgA or IgM and with mouse myeloma IgA (C3H ×5647)[25]. Antisera: pig and rabbit antisera, monospecific for human bovine or mouse IgG (upper trough), IgA (middle trough) and IgM (lower trough), were used as indicated in the pictures. In *c* and *d* upper trough the antiserum is monospecific against bovine IgH_1 (IgG_2 was tested on other slides and found also unable to bind FSC). (Only one antiserum, against mouse IgG applied in the upper trough of *e*, was not monospecific and revealed partially the myeloma IgA appearing in autoradiograph as a short labelled line.) Antigens: combined fractions—first radioactive peak from 'Sephadex G-200' plus part of the 7S IgG peak (the second radioactive peak of unbound FSC was discarded), of human, bovine and mouse (with IgA myeloma) serum incubated with either human or bovine labelled FSC, were applied to the wells as indicated in the picture. The autoradiographs, shown on the right, were obtained by exposing photographic film (Kodak Royal Pan), for 24 h in the dark, to the immunoelectrophoretic slides shown on the left of the picture.

combination and their sera exhibited a very low binding ability for human FSC (6 per cent and 3 per cent respectively). The fact that, in addition to IgA, the IgM of some mammals was able to bind FSC *in vitro* should be emphasized in the light of the hypothesis that IgA is phylogenetically related to IgM[29]. Finally, it is suggested that this combining test should be standardized and used as one of the criteria for the identification of immunoglobulins in newly studied species.

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Immunological Inhibition of Lysosome Function

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Antibodies directed against soluble components from purified liver lysosomes are taken up by fibroblasts and to impair lysosomal function. As a result, lysosomal hydrolases are inhibited, the uptake and digestion of foreign protein are impaired and there is a marked increase in the size and number of lysosome-like cytoplasmic bodies —resembling the condition seen in many storage diseases.

Lysosomes are unique among intracellular organelles in that they are readily accessible to extracellular macromolecules, which may reach them through the operation of endocytosis followed by fusion of the endocytic vacuole with a lysosome[1]. In the past, this property has been exploited successfully for extending the digestive capacity of lysosomes to a new substrate (sucrose) by means of the appropriate enzyme (invertase)[2]. The opposite end—curtailment of lysosomal digestion—has been pursued in the present experiments, in which cells were exposed to antibodies inhibitory to their lysosomal hydrolases. We describe here the first results of these experiments which provide evidence, both biochemical and morphological, that the antibodies are taken up within the lysosomes and cause a severe impairment of their functioning. As a test system, we used rat fibroblasts obtained by trypsinization from 18–20 day old embryos, and cultured at 37° C in a modified Eagle–Dulbecco medium[3] containing 10 per cent of heat-inactivated calf serum. The cultures used in our experiments were essentially in the stationary phase, and contained about 0·15 mg fibroblast protein per ml. of medium. For harvesting, the cells were detached with 0·02 M EDTA, collected by centrifugation, and washed several times with ice-cold 0·25 M sucrose.

Preparation and Properties of γ-Globulin

Immunoglobulins directed against the soluble components of rat liver lysosomes (anti-LS globulins) were prepared essentially as described by A. T. In brief, lysosomes were separated by flotation from the livers of rats injected with Triton WR-1339, ruptured by dialysis against 1 mM sodium bicarbonate and divided by high speed centrifugation into a soluble and an insoluble fraction. The soluble fraction, containing the bulk of the lysosomal hydrolases, was used for the immunization of rabbits. γ-Globulins were purified from the antisera by precipitation with 50 per cent ammonium sulphate, followed by chromatography on DEAE-cellulose with 0·08 M Tris-HCl buffer (*p*H 7·4). Non-immune rabbit sera were processed identically for the preparation of control γ-globulins. γ-Globulins were conjugated with fluorescein according to McKinney *et al.*[5], before the chromatographic step which was then performed with phosphate buffer (*p*H 6·3), the fraction eluting between 0·05 and 0·10 M phosphate being retained.

It has been shown by A. T.[4] that anti-LS globulins inhibit several acid hydrolases, not only of rat liver but also of rat bone and fibroblasts. Sera from control rabbits or from rabbits immunized against other subcellular fractions, including the insoluble lysosomal subfraction, had little or no effect. Fig. 1 illustrates some inhibitory effects of the anti-LS globulins used in the present work. The strong inhibition of cathepsin D, also observed by Weston *et al.*[8] with an antiserum against the purified protease, and that of acid deoxyribonuclease are particularly noteworthy, in agreement with the general finding that immunological inhibition of enzymes increases with the molecular size of their substrates[9]. In view of the importance of the hydrolases acting on macromolecules as initiators of digestion, it is likely that the anti-LS globulins are capable also of inhibiting strongly the breakdown of many of the complex materials that are the natural substrates of lysosomal digestion.

Uptake and Processing by Fibroblasts

Fibroblasts were cultured for 30 h in a medium containing 0·1 mg fluorescein-labelled control γ-globulins per ml. They were harvested, washed thoroughly, dissolved in 0·03 M NaOH containing 0·65 per cent sodium deoxycholate, and analysed fluorometrically at 520 nm, with an excitation wavelength of 480 nm, in a Zeiss ZFM 4C spectrofluorometer. Their average content of fluorescent material corresponded to 8·6 μg of labelled globulins per mg of protein. Examination of the cells in the fluorescence microscope showed the label to be concentrated within cytoplasmic granules (Fig. 2). The nature of these granules was investigated by isopycnic density gradient centrifugation. For this purpose, a homogenate of fibroblasts exposed for 30 h to fluorescein-labelled normal γ-globulins was prepared in 0·25 M sucrose and freed of nuclei by centrifugation for 10 min at 600*g*. The resulting cytoplasmic extract (10 ml.) was layered over a 32 ml. linear sucrose gradient (density 1·10–1·25), itself resting on a 6 ml. cushion of density 1·34, in the automatic zonal rotor of Beaufay[10]. After centrifugation for 3 h at 35,000 r.p.m., which sufficed to bring most particles practically to their equilibrium position in the gradient, the contents of the rotor were collected into thirteen separate fractions, which were assayed for various enzymes and for fluorescent material. The average density of each fraction was also measured, so that the results could be represented in the form of density distribution histograms of each component[11]. Fibroblast lysosomes, as detected by three acid hydrolases, are distinctly denser than are the other cytoplasmic particles identified by catalase (peroxisomes ?), cytochrome oxidase (mitochondria), and inosine diphosphatase (microsomes ?) (Fig. 3). The fluorescent material clearly follows the distribution of acid hydrolases, and is largely concentrated, together with these enzymes, in a region of the gradient practically free of the other marker enzymes.



Fig. 2. Fluorescence micrograph of fibroblasts cultured for 30 h in the presence of fluorescein-labelled normal γ-globulins (×1,400; excitation wavelength 420 nm). Cells incubated with unlabelled globulins showed no detectable fluorescence.

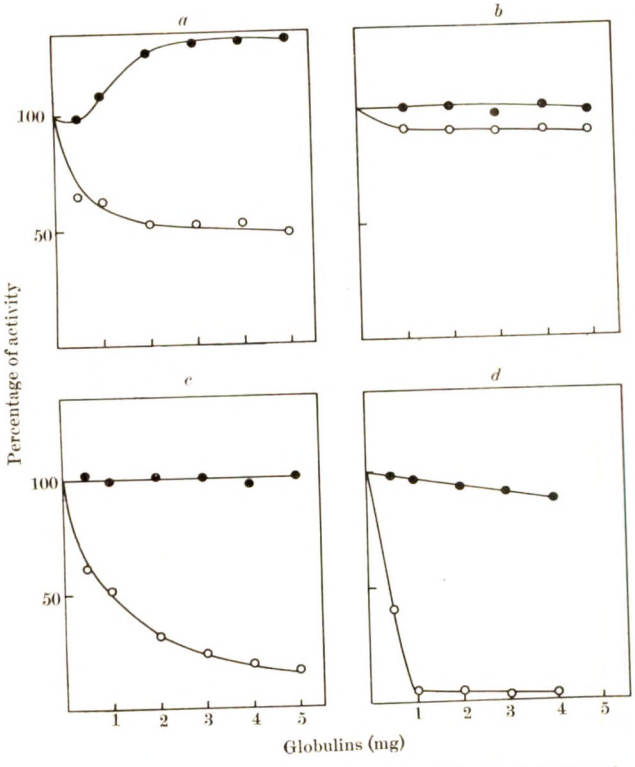


Fig. 1. Immunological inhibition *in vitro* of fibroblast lysosomal enzymes. Cultured cells were homogenized in 0·25 M sucrose with a Dounce homogenizer and then frozen and thawed several times. Amounts of homogenate containing 0·2 mg of protein were preincubated for 1 h at 37° C with the quantities of γ-globulins indicated in the abscissa, and then assayed for (*a*) β-N-acetyl-glucosaminidase[6], (*b*) acid β-glycerophosphatase[7], (*c*) acid deoxyribonuclease[7], and (*d*) cathepsin D[7] (the latter enzyme at *p*H 5·0). The enzymatic activities were measured in the absence of 'Triton X-100'. ●, Control globulins; ○, anti-LS globulins.

In another experiment, fibroblasts were kept for 4 days in a medium containing 0·1 mg of fluorescein-labelled control γ-globulins and 0·3 mg of unlabelled control γ-globulins per ml. At the end of the incubation, the medium was collected and fractionated on a column of 'Sephadex G-50' with 0·15 M NaCl–2 mM phosphate buffer (*p*H 7·4). The fractions were analysed by fluorometry. The cells were disrupted by freezing and thawing and treated with 0·1 per cent 'Triton X-100'. The supernatant recovered from this suspension by centrifugation at high speed, which contained all the intracellular fluorescent material, was also fractionated on 'Sephadex G-50' for fluorometric analysis. 2 per cent of the total fluorescent material was intracellular, 98 per cent extracellular (Fig. 4). Furthermore, 18 per cent of the intracellular and 36 per cent of the extracellular material were retarded on the column. In contrast the fluorescent γ-globulins, when incubated for the same length of time at 37° C with medium alone or with cell-free medium collected from a 4 day old culture, came out entirely with the void volume.

It seems from these results that fibroblasts take up fluorescein-labelled γ-globulins from the medium, break them down, and return conjugated fragments of the protein molecule to the medium. In all likelihood, this traffic takes place according to the generally accepted scheme of endocytosis and lysosomal digestion[1], which involves pinocytosis of the proteins, fusion of the pinocytic vacuole with a lysosome, and digestion of the proteins within the resulting secondary lysosome. The products of digestion are believed to diffuse or be transported across the lysosomal membrane, and to be metabolized in the cytoplasm. How the fluorescein-labelled fragments reach the extracellular medium in the present case, whether by diffusion or by some more specific excretion mechanism, is not known.

Effects of Anti-LS γ-Globulins on Fibroblasts

Fibroblasts were maintained for 4 days in the presence of 0·5 mg/ml. of either anti-LS globulins or control rabbit

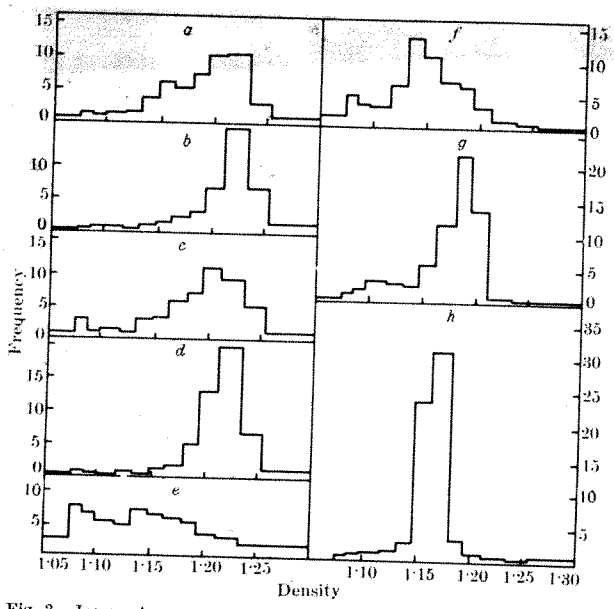


Fig. 3. Isopycnic centrifugation of a cytoplasmic extract (post-nuclear supernatant) obtained from fibroblasts cultured for 30 h in the presence of fluorescein-labelled control γ-globulins (0·1 mg/ml.). Fractions are analysed for (*e*) proteins[12], (*h*) cytochrome oxidase[7], (*g*) catalase[13], (*f*) inosine diphosphatase[14], (*a*) acid phosphatase[7], (*b*) β-N-acetyl-glucosaminidase[6], (*c*) cathepsin D[7] and (*d*) fluorescein labelled γ-globulins (or labelled γ-globulin fragments).

γ-globulins. The cells were then harvested, washed carefully, homogenized, and assayed for various enzymes. The activities of the cells exposed to the anti-LS globulins, expressed in terms of those of the control preparation, were 98 per cent for cytochrome oxidase and catalase, 82 per cent for acid phosphatase, 76 per cent for β-N-acetyl-glucosaminidase, 73 per cent for acid deoxyribonuclease, and 49 per cent for cathepsin D. The decrease in activity of the hydrolases parallels in relative magnitude that observed in a homogenate preincubated with anti-LS globulins (Fig. 1). This suggests that we are dealing, as in the latter case, with an immunological inhibition of the enzymes. But whereas some 0·3 to 0·4 mg of anti-LS globulins had to be added *in vitro* to 0·2 mg of fibroblast protein to produce the degree of enzyme inhibition observed in the *in vivo* experiment (Fig. 1), nothing near this amount of antibody could have been present in the washed cells (if the cells do not discriminate between fluorescein-labelled and unlabelled γ-globulins, their content of anti-LS globulins would be of the order of 80 μg/mg fibroblast protein according to the results given here). If, therefore, immunological inhibition were responsible for the decreased activities of lysosomal hydrolases in the homogenate of cells exposed to anti-LS globulins, interaction between the antibodies and the enzymes must have taken place in the living cells before homogenization, that is, within their lysosomes. Pinocytosis of the antibodies would indeed allow such a phenomenon to occur.

If the antibodies inhibit the lysosomal hydrolases within the lysosomes, digestion of pinocytized materials should be impaired. As a test of this possibility, fibroblasts were maintained for 4 days in the presence of 0·1 mg/ml. of fluorescein-labelled normal γ-globulins, as in the experiment of Fig. 4, but with 0·3 mg/ml. of anti-LS γ-globulins in the medium instead of normal γ-globulins. As shown in Fig. 5, degradation of the labelled protein was almost completely inhibited in the cells exposed to anti-LS globulins. It is, however, remarkable that the amount of undegraded fluorescein-labelled globulins stored in the cells hardly exceeded that found in non-inhibited cells (15·4 against 12·1 μg per mg fibroblast protein). This finding, for which we have at present no explanation, indicates either that pinocytosis was severely inhibited or that it was followed by considerable regurgitation, in the antibody-treated cells. It also suggests an alternative explanation for the lack of protein degradation, which could be a consequence of inhibited uptake rather than of impaired digestion. The almost complete absence of degradation products in the treated cells themselves, however, indicates that intralysosomal inhibition of the proteases by pinocytized antibodies played a significant part in the chain of events.

Another striking effect of the antibodies was observed on the glucose consumption of the cells, which amounted to 3·2 mg of glucose per day per mg fibroblast protein in the treated cells, against 1·3 mg per day per mg protein in the control cells. This effect was accompanied by a more rapid acidification of the medium, suggesting that part at least of the glucose consumed in excess was glycolysed to lactic acid.

Morphological Effects

Fibroblasts cultured for 4 days in medium containing either control rabbit γ-globulins or anti-LS globulins, as in the experiments of Figs. 4 and 5, were collected after trypsinization, washed with cold saline and fixed with 1 per cent osmium tetroxide in 0·1 M phosphate buffer (*p*H 7·4), in the presence of a minute amount of plasma proteins. The fixed cells were centrifuged and the pellet dehydrated and embedded in 'Epon'. Ultrathin sections were obtained with a Reichert OmU2 ultramicrotome. They were stained both with uranyl acetate and with lead citrate, and examined with a Siemens 'Elmiskop 1' at 80 kV. Typical micrographs are depicted in Fig. 6.

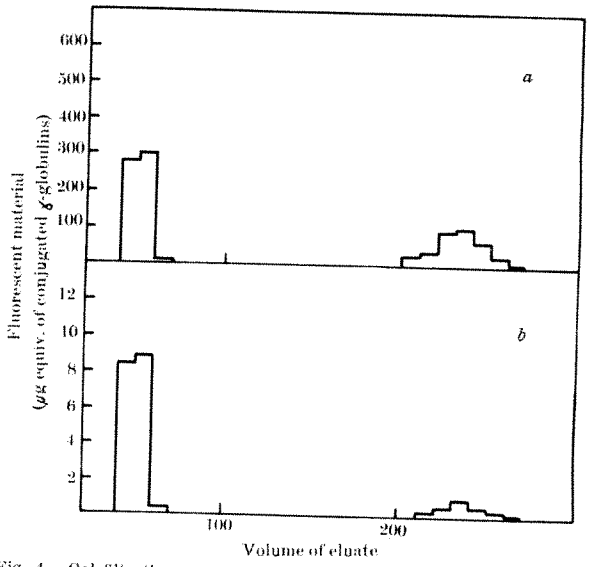


Fig. 4. Gel filtration on 'Sephadex G-50' of culture medium (*a*) and soluble cell extract of fibroblasts (*b*). The cells were cultured for 4 days in the presence of fluorescein-labelled control γ-globulins (0·1 mg/ml.) plus unlabelled control γ-globulins (0·3 mg/ml.). The initial peak corresponds to intact labelled γ-globulins, whereas the retarded peak is composed of low molecular weight products.

In the cells exposed to anti-LS antibodies, the cisternae of the endoplasmic reticulum are much less dilated than they are in the control fibroblasts. On the other hand, the treated cells show a striking increase in the size and number of cytoplasmic bodies of lysosomal type. In the control cells, these bodies are small and scattered irregularly in the cytoplasm. By contrast, the antibody-treated cells are literally packed with large granules of similar appearance. In both cells, the granules are bounded by a single membrane and have a pleiomorphic content.

The condition of "congestive enlargement of the lysosomes"[15] induced by the antibodies recalls that observed

in many congenital storage diseases, except that in the latter the lysosomal contents are made up of only a few specific chemical species which often have a characteristic appearance in the electron microscope[16]. Many storage diseases are now known to arise from a severe deficiency of a lysosomal hydrolase, essential for the further digestion of the substances that accumulate in the lysosomes. It is tempting to assume that we have artificially created here some sort of generalized storage syndrome by widespread inhibition of the lysosomal hydrolases by pinocytized antibodies. Because no accumulation of exogenous substances was revealed by the use of fluorescein-labelled globulins as indicator, it is most likely that the lysosomes are filled largely with endogenous constitutents segregated by autophagy. It remains to be seen, however, to what extent an enhancement of cellular autophagy, which is known to be stimulated by many metabolic stresses[15], may have contributed to the development of the syndrome.

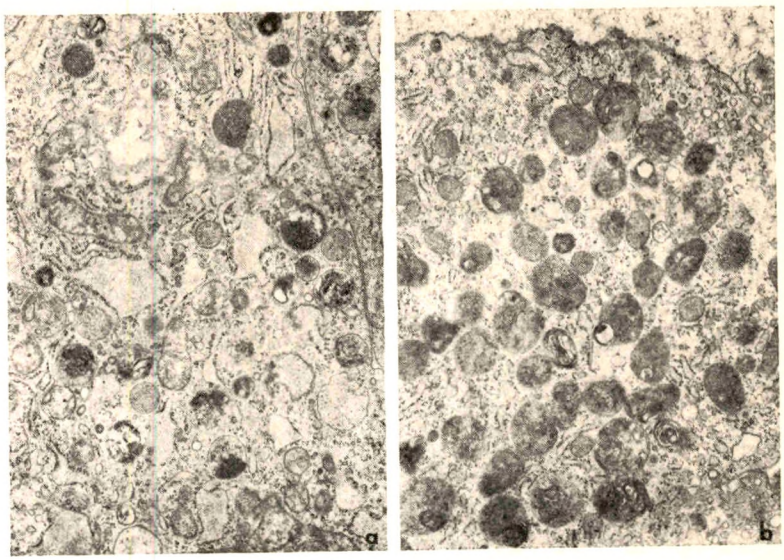


Fig. 6. Electron micrograph of cultured fibroblasts taken at the same magnification (×10,265). Cells maintained for 4 days in the presence of 0·3 mg/ml. of control γ-globulins (*a*) or of anti-LS γ-globulins (*b*).

Immunological Inhibition

Our results indicate that severe impairment of both the heterophagic and the autophagic functions of lysosomes[15] can be induced by exposure of cells to immunoglobulins inhibitory to their lysosomal hydrolases. In all likelihood, the antibodies exert this inhibitory effect within the lysosomes of the living cells, which they reach by the well established channel of pinocytosis followed by fusion of the pinocytic vacuoles with lysosomes.

It is, however, doubtful that this mechanism can account for all of our findings, in particular for the inhibited uptake of the conjugated protein. Other unresolved questions are the mechanism whereby the anti-LS globulins stimulate glycolysis and their possible influence on the rate of cellular autophagy. Also puzzling is the fact that cathepsin D was inhibited only by about 50 per cent in the homogenate of antibody-treated cells, whereas the breakdown of an exogenous protein by the same cells when intact was almost fully blocked. The conditions of the *in vitro* assay, however, are so far removed from those prevailing within lysosomes that too much significance should not be attached to this discrepancy. It is also possible that the primary attack of the conjugated γ-globulins involves an enzyme other and more strongly inhibited than cathepsin D, or that part of the protease is located within primary lysosomes that are neither accessible to pinocytized materials nor involved in intracellular digestion.

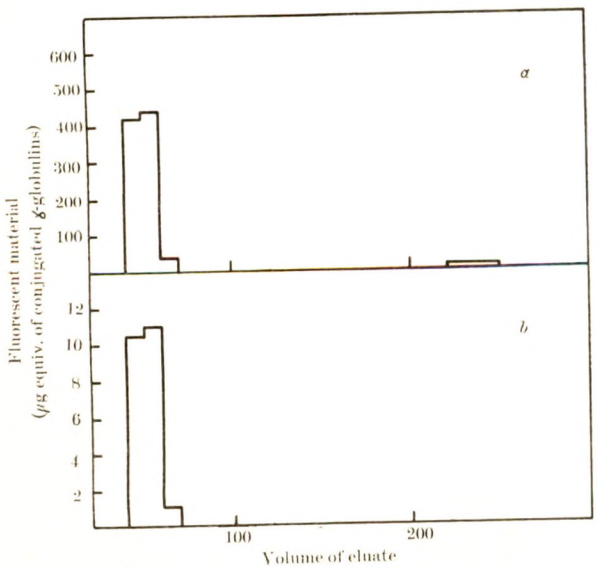


Fig. 5. Gel filtration on 'Sephadex G-50' of culture medium (*a*) and soluble cell extract of fibroblasts (*b*). The experiment was performed like that of Fig. 4 except that unlabelled anti-LS γ-globulins (0·3 mg/ml.) were substituted for the unlabelled control γ-globulins.

In spite of these complications, there seems little reason to doubt that a true immunological inhibition of lysosomal digestion has been achieved in these experiments. We therefore have a very valuable tool with which to explore a number of situations in which a role of lysosomes is suspected, but has not been demonstrated or assessed quantitatively. Our results also illustrate a new type of immunological cytotoxicity, not involving complement and dependent on an interaction with intracellular antigens.

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Incidence in River Water of *Escherichia coli* containing R Factors

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Essex

Large numbers of *Escherichia coli* with antibiotic resistance transmissible to pathogenic organisms have been found in some British rivers. The principal source was insufficiently treated human sewage.

LARGELY as a consequence of the widespread use of antibiotics, R factors, which transfer antibiotic resistance among enterobacteria, have become common in the non-pathogenic *Escherichia coli* of the alimentary tract of man and domestic animals in the British Isles. Many R^+ organisms, especially those of human origin, would be expected ultimately to enter sewage and from this, possibly, rivers. I have therefore determined the incidence of such organisms in British rivers, paying here particular attention to *E. coli* with transmissible resistance to chloramphenicol, which is considered to be potentially the more dangerous because its resistance might be transmitted to *Salmonella typhi* and so render the treatment of typhoid fever more difficult. I also investigated the effect of sewage treatment on the survival of R^+ *E. coli*.

Coliform Counts

Samples of river water and sewage (100 ml.) were collected in sterile glass containers, and viable coliform organisms were counted by the method of Miles and Misra[1], using ordinary MacConkey's agar. Counts were also made on MacConkey's agar containing 15–20 μg/ml. of streptomycin, oxytetracycline, ampicillin, chloramphenicol, neomycin or sodium nalidixate to estimate the numbers of coliform organisms that were resistant to each antibiotic. Organisms were classed as coliform if they were Gram-negative and formed red colonies on MacConkey's agar similar in size to those of *E. coli*. When the number of these organisms in any particular specimen was expected to be low, one Petri plate of medium of each kind was also inoculated with 1·0 ml. of that specimen. A more intensive search was made for organisms resistant to chloramphenicol; where necessary, 20 ml. of each specimen was centrifuged and all the deposit was inoculated on to a plate of MacConkey's agar containing that antibiotic.

Faecal *E. coli* were identified by their ability to produce acid and gas in MacConkey's broth at 44° C (Eijkman's test), and then submitted to an antibiotic-sensitivity test; resistant organisms were further examined for transferable resistance.

To test for sensitivity and transferable resistance I used the methods of Smith[2]. Nalidixic acid-resistant mutants of a *lac*⁻ *Escherichia coli* K12F⁻, of *Salmonella typhimurium* of phage type 29, and *Salmonella typhi*, type A, were used as prospective recipient strains in the transfer tests. To test for transferability of nalidixic acid resistance, ampicillin-resistant mutants were used as prospective recipient strains instead of nalidixic acid-resistant strains.

Bacteriological Examination

Specimens of water for bacteriological examination were collected at ninety-eight sites on fifty-four British rivers or their tributaries. The results of some of these examinations are summarized in Table 1. The rivers Taff (Table 2), Trent, Severn and Thames were sampled at ten or more places. There was a great variation in the concentration of antibiotic-resistant coliform organisms in specimens examined from different sites, but there was a good degree of consistency in specimens collected from the same site at different times. Specimens containing low concentrations of antibiotic-resistant coliform bacteria had been taken from rivers that flowed almost entirely through rural areas, such as the Great Ouse and the Jed, or from places near the end of a long stretch flowing through a rural area, such as the Severn before Stourport and the Avon before Bristol. Specimens with high concentrations of resistant bacteria had usually been obtained from rivers after they had flowed through urban areas, such as the Ribble at Preston, the Avon after Bristol and the Tame

Table 1. CONCENTRATION OF ANTIBIOTIC-RESISTANT COLIFORM ORGANISMS IN SPECIMENS OF RIVER WATER

Origin of specimen	Viable coliform organisms/ml. resistant to C	T	S	Neo	Nal	A	Total
Tame, after Birmingham	30	200	80	20	1	200	5,000
Cole, after Birmingham	30	100	80	15	2	400	5,000
Don, after Thorne	12	100	200	8	20	400	800
Ribble, at Preston	16	50	25	0	6	250	2,000
Groom, at Groomsbridge	10	200	150	4	0	5,000	15,000
Lyme, at Newcastle	5·5	130	80	2	0	500	4,000
Ebbw, at Bassaleg	10	50	50	4	1	100	200
Douglas, at Rufford	2	70	100	5	0	800	2,500
Avon, before Bristol	0	0	4	0	0	40	150
Avon, after Bristol	10	150	200	6	6	5,000	75,000
Severn, before Stourport	0·15	2	8	0	0	30	50
Severn, after Stourport	2·2	40	60	0	1	400	800
Stour, at Stourport	25	350	800	15	5	3,000	10,000
Thames, at Westminster	0·4	6	4	0	0	100	400
Thames at Westminster*	4	30	30	0	1	300	1,000
Great Ouse, before Huntingdon	0	0	0	0	0	0	150
Jed, at Jedburgh	0	0	0	0	0	0	50
Cam, at Cambridge	0	0	3	0	0	20	50
Leader, at Leader	0	0	0	0	0	3	10

C, chloramphenicol; T, tetracyclines; S, streptomycin; Neo, neomycin; Nal, nalidixic acid; A, ampicillin.
* Collected in October 1970 during a partial strike of sewage works.
A sample (20 ml.) of each specimen was examined to determine its content of chloramphenicol-resistant coliform organisms; 1 ml. was examined to determine its content resistant to the other antibiotics.

Table 2. CONCENTRATION OF ANTIBIOTIC-RESISTANT COLIFORM ORGANISMS IN SPECIMENS OF WATER COLLECTED FROM THE RIVER TAFF AND ITS TRIBUTARIES

Collection site	Viable coliform organisms per ml. resistant to C	T	S	Neo	Nal	A	Total
Before Merthyr Tydvil	0	0	0	0	0	0	0
At Troedyrhiw	0	0	1		0	10	20
Before Aberfan	0·05	1	5	0	0	4	50
After Aberfan	4	40	20	1	0	200	250
After Abercynon	50	500	1,000	50	2	10,000	20,000
At Cilfynydd	10	100	150	20	0	500	3,000
Before Pontypridd	5	150	200	8	0	1,000	4,000
After Pontypridd	4	80	300	1	0	1,500	4,000
At Upperboat	3	50	70	3	0	300	1,000
At Taffswell	2	40	100	10	0	500	5,000
At Whitchurch	1·5	30	30	0	0	100	500
At Cardiff Castle	0·2	6	20	0	0	100	500
Before Cardiff docks	0·15	6	15	0	0	40	150
Tributaries							
Rhondda Fawr, before Tynewydd	0	0	0	0	0	0	0
Rhondda Fawr, at Tonypandy	0·3	30	12	1	0	80	1,000
Rhondda Fawr, at Porth	0·75	70	200	3	0	500	2,500
Rhondda Fawr, at Pontypridd	1	30	80	0	0	80	1,500
Rhondda Fach, before joining Taff	0	0	10	1	0	60	100
Cynon, before joining Taff	1	20	5	1	0	20	150

For other details see Table 1.

and Cole after Birmingham. Specimens from the Stour at Stourport and the Severn before and after entering this town demonstrated how a comparatively small heavily contaminated tributary can increase significantly the concentration of antibiotic-resistant coliform organisms of the river into which it flows. The appearance of the Stour and many of the other rivers containing large concentrations of antibiotic-resistant coliform organisms resembled that of raw sewage. All specimens taken in normal conditions from the river Thames, into which large quantities of treated sewage, particularly at London, are discharged, yielded comparatively few antibiotic-resistant coliform organisms. The concentration was considerably higher in water collected at Westminster during a partial strike of sewage workers than in water collected at the same place when sewage works along the Thames were functioning normally.

No coliform organisms were found in the specimen taken from the Taff immediately before Merthyr Tydvil or in that taken from its main tributary the Rhondda Fawr, immediately before Tynewydd (Table 2). Above these two points, both rivers flow through hilly regions inhabited almost solely by a few sheep. For the rest of their courses, they flow through densely populated urban areas containing very few farm animals. There are no licensed abattoirs between Merthyr Tydvil and Whitchurch, the stretch of the Taff from which specimens yielding high concentrations of antibiotic-resistant coliform organisms were taken. Between Aberfan and Cilfynydd sewage was known to be discharged into the Taff and from the appearance of this section of the river it was obviously insufficiently treated. Sewage from Pontypridd and from the other human habitations between this town and Cardiff is discharged directly into the sea. The specimens collected from the Taff after it has passed through Pontypridd revealed a decreasing incidence of antibiotic-resistant coliform organisms.

Very few coliform organisms were found in specimens collected at rural locations of canals in the Midlands.

Faecal *E. coli* in River Water

The Eikjman test was performed on a representative collection of coliform organisms isolated from river water (Table 3). No more than five cultures of the "total" coliform organisms and of those which were resistant to ampicillin and chloramphenicol were examined from any one specimen of river water. All cultures of coliform organisms resistant to the other antibiotics had been isolated from different specimens. On the basis of these tests, most of the coliform organisms resistant to chloramphenicol, tetracycline, streptomycin or neomycin were classified as faecal *E. coli*; only a minority of the ampicillin-resistant coliform organisms and the "total" coliform organisms were so classified.

Of 435 chloramphenicol-resistant strains of *E. coli* isolated from ninety specimens of river water, 373 were found to transfer their resistance to *E. coli* K12F⁻ and 208 to *Salmonella typhimurium* of phage type 29. Of forty strains that transferred their chloramphenicol resistance to *E. coli* K12F⁻, thirty-six also transferred it to *Salmonella typhi*; all forty had been isolated from different specimens of river water. None of the 278 chloramphenicol-resistant strains was resistant to chloramphenicol only; 179 were resistant to five or more antibiotics, the commonest pattern involving resistance to chloramphenicol, ampicillin, streptomycin, tetracyclines and sulphonamides.

Twelve of twenty tetracycline-resistant strains of *E. coli* from different specimens transmitted their resistance to *E. coli* K12F⁻. The corresponding figures for four groups of twenty strains, each group resistant to neomycin, ampicillin, streptomycin or nalidixic acid, were twenty, thirteen, twelve and nil respectively.

Table 3. INCIDENCE OF FAECAL *Escherichia coli* AMONG COLIFORM ORGANISMS ISOLATED FROM RIVER WATER

Strains classed as	No. examined	No. that were faecal *E. coli**
Chloramphenicol-resistant	293	269
Tetracycline-resistant	21	20
Streptomycin-resistant	32	20
Neomycin-resistant	20	20
Nalidixic acid-resistant	40	19
Ampicillin-resistant	182	38
"Total"	340	94

* *E. coli* which reacted positively in the Eijkman test.

Effect of Treatment on Sewage

Numbers of viable antibiotic-resistant coliform organisms in sewage entering and leaving six sewage treatment works in the Basildon area of Essex are summarized in Table 4. At the largest works, which serves an area of some 70,000 inhabitants, the activated sludge method is used. The other five together serve a slightly smaller population and the biological filter method is used; the results for these five are grouped together in the table because they resembled each other closely. In all the examinations carried out at all six works, the concentration of antibiotic-resistant coliform organisms was always much lower in the effluent than in the influent sewage. The distribution of faecal *E. coli* among chloramphenicol-resistant coliform organisms was similar to that found in river water.

Importance of Sewage Treatment

The results of my investigation, expressed as viable organisms per millilitre, shows that some British rivers contain many antibiotic-resistant *E. coli*. Because much of their resistance, particularly to chloramphenicol, was transmissible to *Salmonella typhi* and *typhimurium*, this is an undesirable situation.

Available evidence incriminates human beings, through sewage, rather than animals as the main source of the resistant *E. coli*. For example, the incidence of antibiotic-resistant organisms in general was low in rivers and canals that flowed principally through rural areas and high in rivers that flowed through urban areas; in rivers with the highest incidence it was obvious, to eye and nose, that inadequately treated sewage was being discharged into them. The river Taff provided the strongest evidence incriminating human beings. No antibiotic-resistant *E. coli* were found in this river before it entered the predominantly urban area, whereas many were found in the stretch of the river that received inadequately treated sewage from the upper part of this area, where there were no abattoirs. Progressively

Table 4. EFFECT OF TREATMENT ON THE NUMBERS OF ANTIBIOTIC-RESISTANT COLIFORM ORGANISMS IN SEWAGE

Method of treatment	Material examined	Viable coliform organisms per ml.* resistant to C	T	S	Neo	Nal	A	Total
Biological filter	Influent	1,500 (50–5,000)	7,500 (1,000–50,000)	7,500 (2,500–25,000)	200 (50–1,500)	10 (0–100)	50,000 (2,500–150,000)	125,000 (25,000–500,000)
	Effluent	15 (0–500)	120 (20–1,500)	125 (20–5,000)	1 (0–10)	0 (0–40)	1,200 (80–5,000)	2,500 (750–50,000)
Activated sludge	Influent	2,000 (250–4,000)	10,000 (2,500–120,000)	20,000 (4,000–25,000)	250 (100–2,500)	50 (30–1,000)	50,000 (20,000–150,000)	200,000 (100,000–1,000,000)
	Effluent	20 (10–40)	60 (40–150)	60 (40–200)	8 (3–50)	1 (0–4)	500 (100–7,500)	5,000 (1,500–15,000)

* Median count followed by, in parentheses, the range of the counts for twenty-four influent and twenty-four effluent samples obtained from five "biological filter" sewage works and for seven influent and seven effluent samples obtained from an "activated sludge" sewage works; no more than one influent and one effluent sample were obtained on any one day.
For other details see Table 1.

fewer resistant coliforms were found in specimens collected in the lower part of the urban area, where sewage was not discharged into the river.

Sturtevant and Feary[3] in the United States observed no reduction in the concentration of antibiotic-resistant *E. coli* in sewage as a result of treatment at sewage works. My results were quite different. The difference between the two sets of results could be a consequence of the use of different methods of treatment. Certainly, the biological filter and the activated sludge methods, as applied in the six sewage works in the Basildon area, produced a marked reduction in the concentration of antibiotic-resistant *E. coli*, so much so that it is probable that their universal application in Britain would significantly reduce the antibiotic-resistant *E. coli* entering the rivers.

The presence of antibiotic-resistant *E. coli* in river water is not solely a British problem. R^+ organisms, including those resistant to chloramphenicol, were found in fairly large numbers in water collected from the Hudson river in New York. Incidentally, one strain of *E. coli* obtained from this water was one of the highest transmitters of chloramphenicol-resistance found in this present investigation.

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Differential Reactions of Atlantic and Pacific Predators to Sea Snakes

by

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Predators from the Pacific made no attempt to attack the Pacific sea snake *Pelamis platurus*. Atlantic predators, however, were more inclined to attack, sometimes being wounded by their prey. This suggests that if a canal in Central America facilitated the spread of *Pelamis* to the Atlantic, the species would eventually spread throughout the ocean as selection favoured predators disinclined to attack it.

If a sea level canal is built in Central America[1,2] abundant populations of *Pelamis platurus* could reach the Atlantic Ocean from the eastern Pacific. Very little is known about the behaviour and ecology of this venomous reptile, which is widespread in tropical and subtropical waters from the east coast of Africa to the west coast of Central and South America. Known as the yellow bellied sea-snake, it reached the eastern Pacific relatively recently, presumably in the 2 or 3 million years since the rise of the Isthmus of Panama.

We have attempted to evaluate the relationships of *Pelamis* to Pacific predators and to potential Atlantic predators. We have examined the reactions of eastern Pacific predators to *Pelamis* both in the field and in the laboratory, and studied the response of naive Atlantic predators to *P. platurus*.

Search for Natural Predators

Pelamis is ovoviviporous and spends its entire life at sea. Although individuals can dive for up to 1 h they spend much time at or near the surface. In the sea it will spend more than 30 min, more or less motionless, at the surface. *Pelamis* swims relatively slowly and its yellow and black colour and behaviour make it visible and recognizable from farther away than a similarly sized stick or dull coloured animal would be. Specimens are usually easy to capture—we usually catch them from the surface of drift lines with a dip-net. The sluggish habits of the species would be likely to make *Pelamis* potentially vulnerable to a wide range of fish and avian predators.

Wetmore reports seeing *Fregata magnificens* pick up *Pelamis*, fly off, and then drop them without further interest[3]. We have occasionally captured specimens with healed wounds, shaped as though caused by bird beaks. *Pelamis* have not, however, been found in the stomach contents of any sea bird and in many hours of observations we have never witnessed an attack. Neither have we found any evidence of *Pelamis* in the stomach contents of 420 specimens, including twenty-six species of bony and cartilaginous fishes, taken in Panama Bay (between 7° 30′ and 8° 55′ N latitude and 78° 10′ and 80° 0′ W longitude) usually near drift lines containing *Pelamis*.

Reaction of Captive Pacific Predators

Several carnivorous Pacific fishes (*Ginglymostoma*, *Epinephelus*, *Lutjanus*, *Centropomus* and *Scianus*) were established in 15–70,000 gallon tanks of sea water. They were all willing to attack and feed on living anchovies and carangids and after 10 days of acclimatization they were given only specimens of *Pelamis* for 20 min each day. The predators swam to within approximately 12 inches of the snake, stopped to make an inspection and then usually showed no further interest. During more than 30 days no predatory behaviour developed towards *Pelamis*. In some cases when snakes were left in a tank with a predator the latter starved rather than attempt to feed on them. On one occasion a snapper which had become conditioned to seize live food thrown from the surface ingested a snake as it hit the water, before it could have seen or smelt the fish below the surface. It immediately spat out the snake and paid no further attention to it.

Reaction of Captive Atlantic Predators

To test the reactions of naive Atlantic predators to intact *Pelamis*, relatively large specimens of *Lutjanus* spp. were established in concrete tanks and treated in a similar manner to the Pacific predators before being presented with *Pelamis*.

Pelamis represented a new shape, pattern and smell to these fishes. On the initial encounters the Atlantic predators showed interest, swam close for examination

Table 1. SUMMARY OF TESTS OFFERING LIVE *Pelamis* TO CAPTIVE PREDATORS

	No. of species	No. of specimens	No. of trials	No. of attacks	No. of predator deaths
Pacific	10	21	316	1	0
Atlantic	9	21	383	35	3

and followed the snakes for varying periods. Usually, by the tenth trial the more aggressive of the snappers attacked the *Pelamis*. Attacks were usually directed toward the head and ingestion was rapid, being completed in less than 10 s. Two snakes were taken tail first; one of them bit the fish under the eye just before it was completely swallowed. The fish died in 20 min. On several occasions the Atlantic fishes ingested several snakes consecutively. The survival of Atlantic fishes ingesting *Pelamis* varied. Some fish ate an occasional *Pelamis* with impunity, one was killed when eating its first snake and another relatively efficient predator consumed twenty-two snakes in 31 days before succumbing to a snake bite. Table 1 summarizes the results of the feeding experiments with Atlantic and Pacific predators.

Evolution of *Pelamis* Recognition

The Pacific predators which do not eat sea snakes seem to have been selected for a strong aversion to *Pelamis*. Atlantic predators, on the other hand, will feed on live *Pelamis*, and after the first meal more are accepted without hesitation. One 18 inch snapper ingested four 12 inch *Pelamis* in less than 20 min. On one occasion an Atlantic snapper ingested two sea snakes consecutively. The fish died within 1 h, presumably the victim of an internal bite, for no biting was observed during attacking and swallowing. Both sea snakes were regurgitated after about 1 h and both subsequently survived. This may help to explain the evolution of the highly toxic venom which *Pelamis* possesses. Since the fish victim almost always dies, how then has selection produced a venom more toxic than the terrestrial snakes from which sea snakes have evolved? The food of *Pelamis* is not usually envenomated, so that selection for trophic efficiency is probably not a primary factor. The common explanation that a predator might learn to avoid snakes after surviving a sub-lethal bite does not seem to apply in this case. But if a dying fish which has eaten a sea snake and been bitten occasionally regurgitates a snake, selective mechanism for increasing the potency of the venom may be at work.

What will happen when Pacific predators witness successful predation on *Pelamis* by Atlantic fishes (if the canal is built through the geographical barrier)? One of the most powerful stimuli to feeding in fish is for them to witness feeding by other fishes. When we offered sea snakes to Atlantic and Pacific fishes together, the attack and ingestion of *Pelamis* by Atlantic fishes produced excitement and close inspection by the Pacific predators, but no attempt to eat a snake was observed. Sometimes two or more Atlantic snappers fought for a single snake. A Pacific snapper might be attracted to and follow an Atlantic snapper with a partially ingested snake, but we saw no attempts to take it away.

It is possible that the brilliant and conspicuous colour pattern of *Pelamis* is aposematic and that potential predators have been selected to avoid it. In the most common pattern about 75–85 per cent of the body is uniformly black dorsally, yellow midlaterally and brownish ventrally. The lateral and ventral colour is more similar after shedding and the ventral darkening becomes more pronounced with "ageing" of the skin. The tail is covered with black spots on a yellow background; the size and distribution of the spots are individually specific as a finger print. We have examined the distribution of body pattern uniformity in 167 specimens of *Pelamis*. All degrees of intermediate conditions appear between the "standard type" (15–25 per cent of the body spotted as in the left-hand specimen of Fig. 1) and spotting completely up to and including the head (as in the right-hand specimen of Fig. 1). Yellow specimens with reduced spotting have also been observed (personal communication from Dunson and Voris). The variation in pattern has been observed in all age classes, with about equal frequency, so that deviation from the "standard" does not seem to be particularly deleterious.

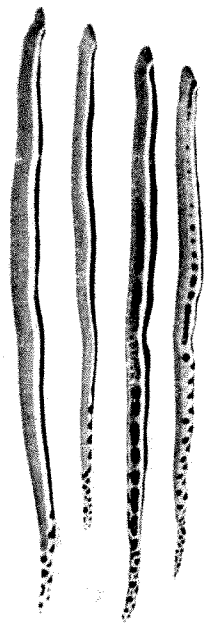


Fig. 1. Examples of pattern variability in *Pelamis platurus* from Panama Bay.

With few exceptions polymorphism (even conspicuous continuous variation) is uncommon in aposematic organisms[4]. The usual explanation for this is that in most circumstances it is not advantageous for aposematic organisms to have variations in pattern which may confuse predators, which if they have been selected for or have learned to avoid a particular colour or pattern may be unable to associate variations with the "standard" and may attack when they should not. If a sensory clue other than vision were important in the predator's recognition of *Pelamis* it might be possible to explain the variation in colour pattern as a relaxation of selection for the "standard" phenotype. In our aquaria two adult Pacific nurse sharks *Ginglymostoma cirratum*, which are essentially scavengers and/or olfactory predators, refused to eat living or dead *Pelamis*. In February 1970, two juvenile *G. cirratum* were captured. One was probably not more than a few weeks old, and it is unlikely that either of them had experienced contact with *Pelamis* in the inshore areas from which they were taken. No *Pelamis* had been reported there for several months. The sharks were quickly trained to accept food from forceps, and offered pieces of fresh and frozen sea snake. All were rejected. They could discriminate between similarly sized and shaped pieces of squid and pieces of sea snake. If *Pelamis* were sandwiched inside the mantle of a squid the sharks would ingest it, mouth the morsel for a few seconds then swallow the squid and expel the snake. These observations suggest that not only visual clues are used by predators to recognize *Pelamis*.

Using felt marking pens we changed the colour of specimens of *Pelamis* to all black or black and red. All of these were rejected by Pacific predators and so were freshly killed and skinned snakes. Feeding frenzies can be produced in captive predators by throwing living anchovies into the tank, but even in these circumstances

of very aggressive, competitive feeding, the fishes discriminated 100 per cent against *Pelamis*. All Pacific predators examined refused to eat living or dead (whole or cut up) *Pelamis* with skins on or off.

Our observations suggest that Pacific fishes can recognize *Pelamis* by visual, olfactory, and/or gustatory clues. The relative importance of the different modalities may, of course, vary from species to species. Aposematic visual aspects seem to be only part of the recognition criteria, although formerly they may have been more important. If conspicuously (but not similarly) coloured ophichtid eels were offered to Pacific fishes they were immediately attacked and eaten. The general serpentine shape and bright colours are thus not necessarily avoided by these predators.

Future Prospects

Pelamis platurus does not have characteristics which are universally avoided by marine fishes. Avoidance of *Pelamis* seems to be specifically selected for in organisms which have direct contact with them. In Central America the avoidance reaction has probably evolved to its present level in less than 3 million years, or rather less than the time since the rise of the Isthmus, after which *Pelamis*, presumably, arrived in the New World.

It is possible to speculate on the colonization potential of *Pelamis* if they are afforded access to the Atlantic. Large numbers of *Pelamis* are periodically stranded or appear swimming along the Pacific shores of Panama and they represent potential propagules through an unobstructed sea level canal. There seems to be no habitat requirement of *Pelamis* which could not be satisfied in the Atlantic. Drift lines of debris attracting the small fishes which constitute much of the food of *Pelamis* are found in both oceans. Drifts of Sargasso weed would probably provide ideal feeding grounds for *Pelamis*.

Invading *Pelamis* would probably be heavily preyed on so that their initial movement would be inhibited. But selection would quickly favour predators that are unwilling to attack *Pelamis* (even if only one out of twenty-two meals results in the death of the predator, this is a very high selection rate). The development of avoidance reaction in predatory species would follow. If a sea level canal were built the probability of these animals eventually colonizing the tropical Atlantic would be great, but predation by Atlantic carnivores would probably reduce the speed of this colonization.

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Evidence that Polywater is a Colloidal Silicate Sol

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Analysis of residues from condensation of "polywater" using the electron probe showed significant amounts of silicon and sodium, suggesting that the unusual properties of "polywater" may be due to the presence of silica or silicate.

Deryaguin and co-workers have presented evidence that a new form of water (anomalous water) is produced when normal water vapour condenses into minute, pristine glass or silica capillaries[1,2]. More recently, Lippincott *et al.*[3] produced water condensate in such capillaries and obtained its infrared and Raman spectra, from which they concluded that the condensate contained a high molecular weight polymeric form of water, or "polywater". A distinction should be made between the claims of Lippincott *et al.* for "polywater" and those of Deryaguin *et al.* for anomalous water. The Russian workers do not claim that anomalous water is a high molecular weight polymeric water. Furthermore, they were extraordinarily careful in preventing electrolyte contamination of the condensate in many of their experiments. The work reported here indicates that salt contamination is an important consideration in the experiments described by Lippincott *et al.*

The Experiments

Capillaries with internal diameters of 50 μm to 5 μm were drawn from acid (HNO_3–H_2SO_4, 1 : 1) cleaned 'Pyrex' and silica (GE 204) tubing. In some experiments the capillaries were loosely placed in 'Pyrex' dishes over distilled water in a desiccator evacuated to about 40 mm Hg and held at 25° ± 1° C. This is the procedure used by Lippincott *et al.*[3]. Other condensation experiments were conducted in an apparatus designed to allow the capillaries to be evacuated independently of the water to reduce the chance of spray reaching the capillaries during the pump-down. The apparatus had a chamber for the capillaries separated from the chamber for water by a grease-free vacuum valve. The water chamber was evacuated first, the valve closed and the capillaries introduced into the other chamber. This section was then evacuated, cut off from the pump and opened to the water.

The capillaries were inspected after 3–10 days' exposure to water vapour. To remove condensate for analysis, the liquid was expelled by air pressure using a gas tight syringe and hypodermic needle. The capillary was sealed into the needle with a drop of melted, purified paraffin. The condensate was expelled on to steel mirror surfaces and the residue examined by light microscopy. For electron probe analysis a pure, low-silicon copper was used instead of steel. The electron probe was an Applied Research Laboratory EMX 200. Infrared spectroscopic determinations were made with a Perkin–Elmer 457 spectrometer.

Silicon and Sodium in Condensate

Condensate was observed in both 'Pyrex' and silica capillaries held over distilled water. For the capillaries in the desiccators the yield (number of capillaries with condensate) was about 50 per cent. In the two-chamber apparatus the yield was 10–25 per cent. Fig. 1*A* illustrates the appearance of condensate columns viewed with

a reflected light microscope. When these capillaries were put in an evacuated chamber or left at 40–50 per cent relative humidity for a few hours, most of the condensate evaporated except for a residue with a gel-like appearance. In Fig. 1*B* the gel had formed before the liquid phase had fully evaporated.

Much of the condensate evaporated very rapidly when expelled on to steel mirror surfaces, leaving behind minute films of residue, some of which have a particulate appearance (Fig. 2). Close examination revealed that many had ridges near the edge, indicating a profile such as in Fig. 3.

Residues from condensate in silica usually had a more homogeneous appearance (Fig. 4) than those from 'Pyrex'. The only particles that could be clearly associated with the silica derived residues are those clustered at the centre in Fig. 4. Other particles in the photograph are dust.

Electron probe analysis of the residues from 'Pyrex' revealed sodium and silicon in easily detectable amounts. Sodium was most strongly evident in the particulate matter within the residue and the silicon level was highest near the edge. These analyses were not pursued in detail because the corrosion of pristine glass surfaces is well known and the presence of sodium and silicon was not unexpected.

The residues from condensate in silica were thinner and therefore more difficult to analyse than the residues from 'Pyrex'. Nonetheless, the electron probe revealed significant levels of sodium and silicon in the thicker

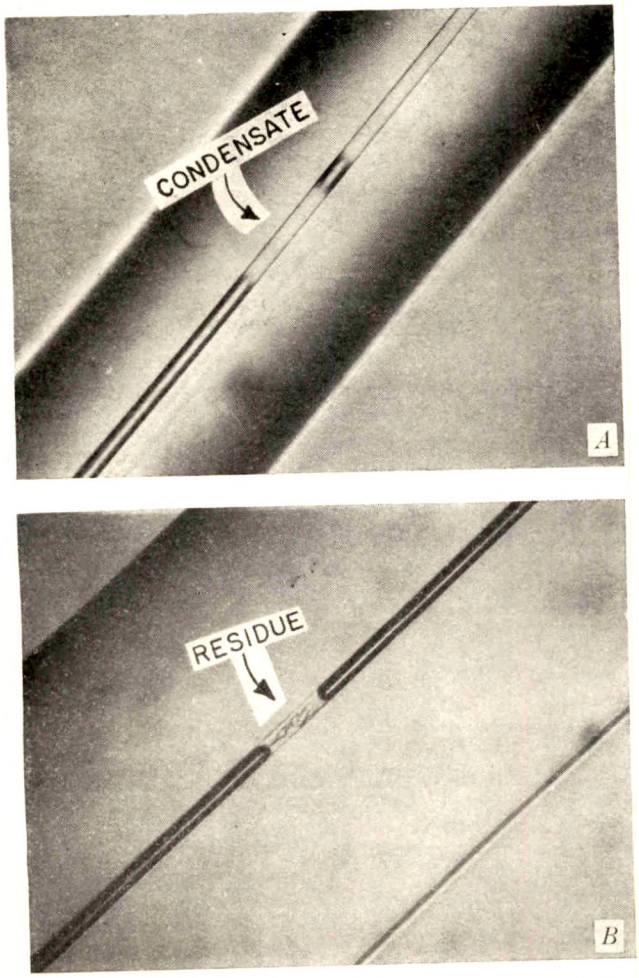


Fig. 1. Appearance of water condensate in a silica capillary immediately after removal from desiccator (*A*) and after most of condensate had evaporated (*B*). Capillary inner diameter is about 30 μm.

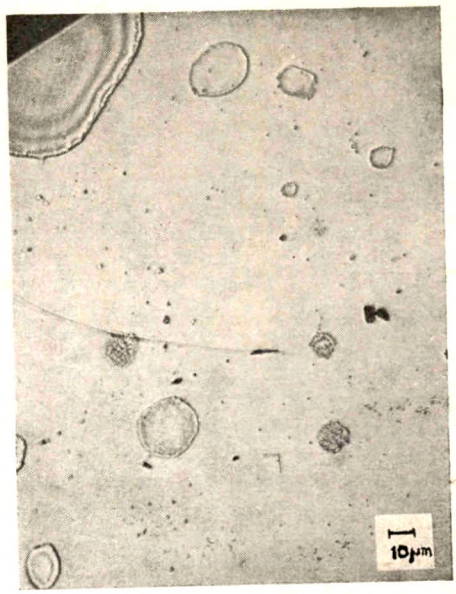


Fig. 2. Residues left by condensate formed in 'Pyrex' and expelled on to a steel mirror.



Fig. 3. Residue profile.

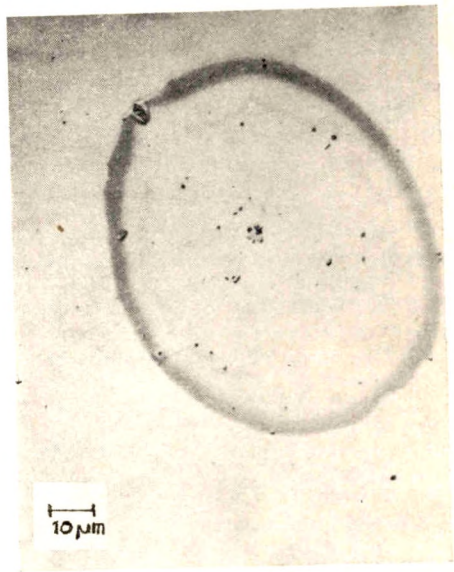


Fig. 4. Residue from condensate formed in silica and expelled on to a steel mirror.

regions near the edges. Fig. 5*A* shows the secondary electron emission for such a region and *B* shows the silicon X-ray (SiKα 7.1252) emission from this same region. The silicon level in this residue is about four times the background level. The relative intensities for silicon and sodium were approximately equal.

Residues of dilute sodium silicate solutions also showed the annular profile drawn in Fig. 3. Analysis of these residues with the electron probe revealed that it was often impossible to detect significant amounts of sodium or silicon in the thin central regions. For example, in Fig. 6 the silicon X-ray emission is displayed for a section of a sodium silicate residue left by a 0·1 μl. drop or a 0·1

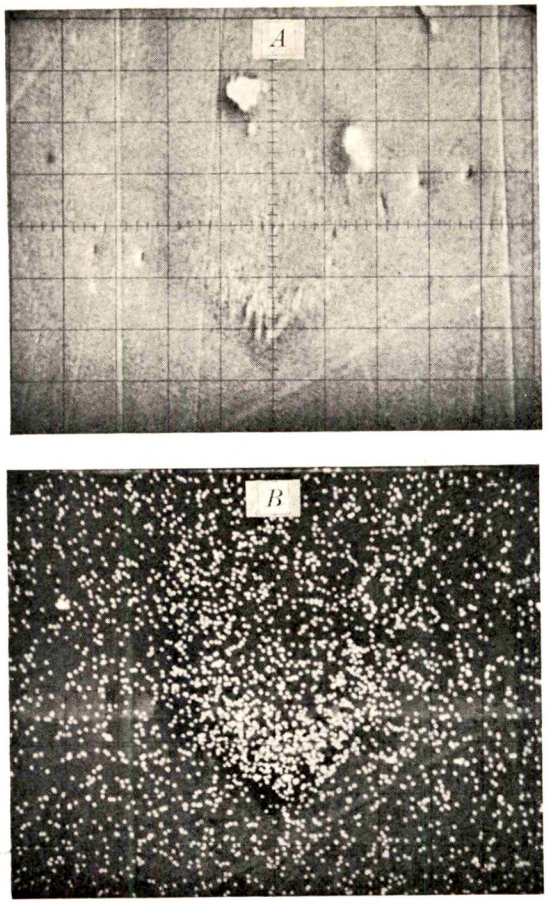


Fig. 5. Electron probe secondary electron emission (*A*) and silicon. X-ray emission (*B*) for a residue of condensate from silica. Cu substrate.

per cent solution. The arc of the rim clearly shows emission due to silicon but no significant emission is observed for the central region even though material was visible with the light microscope.

We conclude from the electron probe results that silicon, sodium and possibly other cations are important if not principal constituents of these condensate residues. Failure to detect these elements in earlier work[3] may have occurred because the residues were too thin.

Infrared Spectra of "Polywater"

If silicates are part of the condensate, then it is possible to offer an alternative explanation for the infrared spectra of "polywater". Silicate solutions are quite alkaline and are thus capable of absorbing CO_2 from the atmosphere. The principal bands in the "polywater" spectrum are at 1,600 cm^{-1} and 1,400 cm^{-1} which are close to the frequencies for the antisymmetric and symmetric O–C–O stretching vibrations. To test this idea infrared spectra were obtained for residues left by drops of bicarbonate–silicate solutions on Irtran 2 plates. A typical result is given in Fig. 7 for the residue from a solution containing 1·5 g/l. of sodium silicate (mole ratio $SiO_2 : Na_2O = 3{\cdot}3$) and 2·6 g/l. of $KHCO_3$. The bands at 1,650, 1,400 and 830 cm^{-1} are caused by bicarbonate and these same bands can be seen in the polywater spectra[3,4]. The broad band at 1,200–1,000 cm^{-1} is caused by silicate and the one between 3,700 cm^{-1} and 2,200 cm^{-1} is probably from residual water.

The spectrum given in Fig. 7 differs from the spectra reported for "polywater", just as the "polywater" spectra differ themselves[3,4]. After a study of the infrared spectra of a variety of bicarbonate–silicate mixtures, the reasons for these differences in the spectra became clear. It was found that the band intensities, positions and shapes were a function of (*a*) total solids concentration, (*b*) ratio of silicate to bicarbonate, (*c*) mole ratio of Na_2O and SiO_2, (*d*) age of the solution and the residue, (*e*) the rate and manner in which the solution dries, and (*f*) the presence of other anions or cations. It is believed that different combinations of these factors gave rise to the observed spectra. For example, the band at 1,650 cm^{-1} in Fig. 7 does not correspond exactly with the 1,600 cm^{-1} band for "polywater"[3,4]. When the bicarbonate–silicate residues were aged for a few days, however, there was a detectable shift of the band to 1,625 cm^{-1} and sometimes lower.

Origin of Silicate Anion

In these experiments the silicate anion could easily originate from a surface corrosion of the capillary walls. The surface hydrolysis of silicate glasses, especially pristine (freshly formed) surfaces, is well known[5]. The process involves the hydration of cationic sites to form an alkaline film capable of hydrolysing the silicate network. Frazer *et al.*[6] describe water adsorption on the inside of freshly blown glass bulbs and observed a visible condensate which they found to be strongly alkaline. They were also able to show that the alkaline film had left the surface rough and porous.



Fig. 6. Electron probe silicon X-ray emission for a sector of a residue left by a drop of sodium silicate solution. Cu substrate.

In the case of condensate from silica capillaries, the electron probe gave clear evidence of sodium as well as silicon which implies contamination because the silica itself does not contain significant amounts of sodium. The principal source of this contamination was probably the glassware holding the capillaries; the formation of a mist or spray from the water during pump-down is believed negligible, especially in the two chamber apparatus. Contamination from the glassware would occur if a water film sufficient to allow surface diffusion or creep of electrolyte on to and into the capillaries developed on the walls. In this connexion, it was noted that in all the condensation experiments there was clear evidence of moisture on the walls of the apparatus, formed, no doubt, during small fluctuations in the room temperature. Moreover, if the 'Pyrex' dish holding the capillaries was replaced with a polyethylene dish where the surface creep should be much reduced, the yield dropped from the usual 50 to about 15 per cent.

Undoubtedly the contaminating salts can contribute to the corrosion of the pristine silica surface but, even with silica, such contamination may not be essential. Silica surfaces offer Si—OH groups for water adsorption and at monolayer coverage and higher ($P/Po > 0{\cdot}4$) the film is mobile[7]. This film is also acidic, for conductivity studies

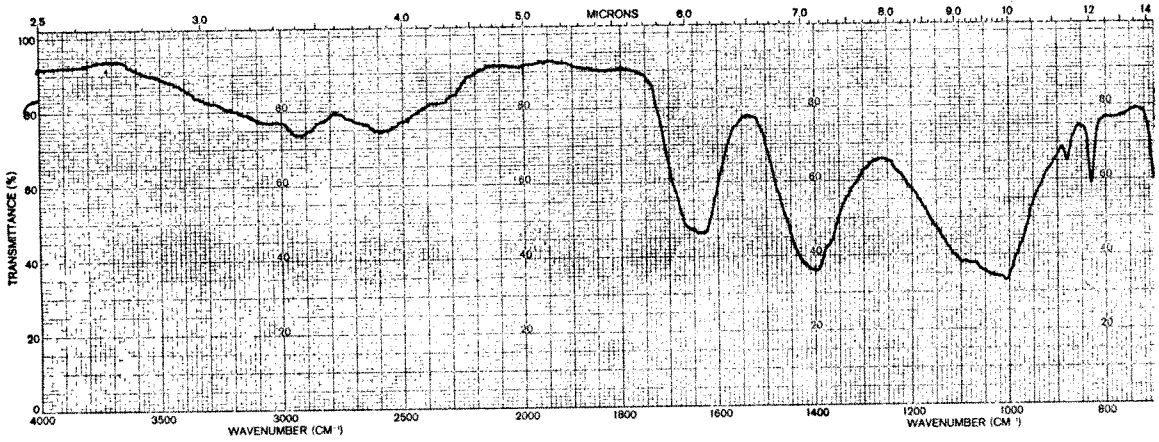


Fig. 7. Infrared spectrum of residue of a sodium bicarbonate–silicate mixture.

indicate the charge carrier is H^+ or H_2O^+[7]. An acid-catalysed surface corrosion may therefore occur, especially on a pristine surface in which many Si—O—Si bonds are strained.

Initially, the corrosion product of silica or 'Pyrex' would be silicic acid (or metal silicate) which would condense quickly into a colloidal silicate sol and ultimately to a colloidal gel if the solution is dried. Many of the properties attributed to "polywater", such as the gel-like appearance[3,8] and the birefringence[8,9] of the condensate residues, certainly suggest silicate. The high refractive indices reported for polywater[9] are also consistent with silicate solutions containing high proportions of metal cation[10].

The results reported here offer alternative explanations for the "polywater" phenomena. Samples of "anomalous water" prepared by the technique of Deryaguin and co-workers have not yet been studied. They obtained condensate in very pure silica capillaries held in a platinum fixture with distilled water as the source of vapour[1]. This experiment would seem to remove any possibility of contamination by electrolyte. An acid-catalysed corrosion of pristine silica by adsorbed water alone, however, may lead to a silica sol, which could explain many of the anomalous properties they observed. We now hope to repeat these experiments and analyse the condensate.

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Plate Tectonics of the Scotia Sea Region

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A newly defined small lithospheric plate in the eastern Scotia Sea accounts for the intense seismicity at the South Sandwich Trench.

THE relative motion of the South American and Antarctic lithospheric plates is not exactly known, and at present the distribution of earthquake epicentres[1,2] cannot alone be used to determine the precise path of the boundary between them. A model for the plate boundaries and motions is presented below which is based additionally on recently published marine geophysical data from the Scotia Sea region[3–7]. These data also bear on the origin of the Scotia Ridge which has long been at issue[8–11].

I presume that the boundary between the South American and Antarctic plates runs between the point where the Chile Rise[12,13] meets the Peru–Chile Trench at 45° S, 76° W, and the Mid-Atlantic Ridge at 55° S, 1° W. Three earthquake epicentres on Fig. 1 (part of Plate 3 from ref. 2) lie along the west coast of Chile, south of 45° S; three others are associated with the north Scotia Ridge, rather more with the southern limb, and others lie in deep water areas west of the South Sandwich Islands and in the far west Scotia Sea (Drake Passage). Intermediate depth shocks, down to 170 km[2,14], supplement the anomalous, intense shallow activity associated with the volcanic South Sandwich Island arc, from the southern end of which a zigzag line of epicentres extends eastward to the Mid-Atlantic Ridge. The sparseness and diffuse distribution of earthquake epicentres over most of this region cannot be attributed entirely to the effect of a relatively unfavourable distribution of recording stations; indeed, if only the larger amplitude shocks are being detected then computed epicentres are less easily dismissed as being of secondary importance and plate boundaries are required to be of complex shape.

A total separation of 1·6 cm/year along 290° of the

South American and Antarctic plates close to the South Atlantic triple junction is obtained by combining the known relative motion between South America and Africa[15] with preliminary spreading rate data from the Atlantic–Indian Ridge[16]. This estimate is approximate and does not apply to the more westerly parts of the boundary as the pole of rotation is unknown. But it seems that relative motion is small over the whole of the boundary, which may account for the sparse and discontinuous earthquake epicentre distribution.

Magnetic Lineations

Trends and ages of correlated and dated magnetic anomalies within the Scotia Sea are shown in Fig. 2 (from ref. 4). Dating is with respect to the Magnetic Reversal Time Scale of Heirtzler *et al.*[17].

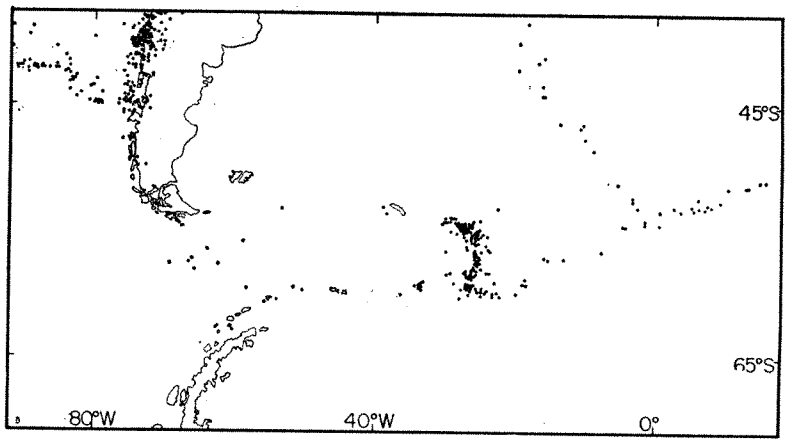


Fig. 1. Distribution of epicentres of shallow focus (< 100 km) earthquakes in the Scotia Sea region[2].

In southern Drake Passage, that part of the Time Scale between 8 and 15 m.y. is recognized in three sections, separated by orthogonal fracture zones which in most places have topographic expression. The easternmost lies on a line joining prominent bathymetric ridges extending north-westward from Elephant Island and south-eastward from near Cape Horn. For convenience the name "Shackleton Fracture Zone" is used for this feature, after the British Antarctic Survey ship aboard which the data were acquired. A spreading rate of 2·4 cm/yr along 130° fits all three sections and Lamont anomaly 6 (21 m.y.) occurs on one profile crossing the easternmost section in a position consistent with extrapolation south-eastward at the same spreading rate. Further extrapolation south-eastward at this rate gives ages of 15 m.y., 20 m.y., and 25 m.y. for the three sea-floor sections at the axis of the trench-like feature which lies in front of the South Shetland Islands between 57° W and 64° W. Unlike Smith Island to the south-west and the Elephant Island group to the north-east, the central South Shetland Islands opposite the trench have a history of late Tertiary andesitic volcanic activity[18]. The isostatic gravity anomaly over the trench[5] is similar to that over island arcs but of smaller amplitude. The lack of earthquake activity shows that the trench is now inactive, but all other features combine to suggest that Pacific crust was being consumed there during much of the late Tertiary.

If these exist, extrapolation along 310°, also at 2·4 cm/yr, would place the three 0 m.y. spreading centres approximately en echelon along a north–south line at 67° W, coinciding with an elevated zone of rough topography separating the Scotia Sea from the deeper, smoother Pacific–Antarctic Basin. I consider, however, that since the South Shetland trench is inactive, this centre is unlikely to be still spreading at 2·4 cm/yr/side, if at all.

East of the Shackleton Fracture Zone, anomaly shapes are apparently much less consistent along the strike, and navigational uncertainties greater. The trend of anomalies here lies parallel to those farther west, and to a double bathymetric ridge lying roughly centrally in eastern Drake Passage. The central valley of this ridge, shown by closed circles in Fig. 2, is followed by a single magnetic anomaly for much of its length, and on two profiles a limited symmetry about this anomaly can be seen. Anomaly offsets coincide with two of the ridge's transverse dissecting troughs. I can identify in the magnetic profiles, equally inconclusively, several conflicting sequences from the Reversal Time Scale, including one which accepts the possible correlation of Lamont anomaly 6 across the Shackleton Fracture Zone. Some of the correlations are of poor quality, but probably the general anomaly trend is correct and the central double bathymetric ridge will prove to be or to have been a spreading centre. Provisionally, a late Tertiary to Recent age for this spreading is assumed, by comparison with the dated sea floor to the west. A lower spreading rate than that measured to the west is consistent with the absence of a trench at either margin and perhaps explains the less consistent anomaly shapes.

Farther east, between South Georgia and the South Orkney Islands, the shape consistency of the anomalies and the navigation are again relatively poor. Trends are different and oceanic anomalies occur mainly to the north of an abrupt boundary at 58° S. Large smooth-surfaced blocks occurring to the south of 58° S show a depth and crustal structure intermediate between that of continent and ocean, and are separated by small areas of apparently normal oceanic crust[4]. Magnetic anomalies here, occurring amid large areas of undisturbed magnetic field, are as likely to be caused by structural disruption of normally magnetized bodies as by the effects of magnetic field reversals. North of 58° S the sea floor lies shoaler than in Drake Passage but with rather less small scale roughness. Asymmetric bathymetric scarps, such as are generally typical of fracture zones, occur here but cannot be correlated between profiles. As in eastern Drake Passage, an approximate symmetry can be seen about a roughly centrally situated anomaly and an unambiguous identification of the Magnetic Reversal Time Scale cannot be made.

In the far east Scotia Sea I tentatively identify an active centre spreading at about 2·7 cm/yr/side along

80°, from within a series of short-wavelength high amplitude anomalies. Where seen, the centre coincides with a generally north–south zone of rough topography along 30° W, apparently continuous between the northern arm of the South Sandwich Trench and the west–east fracture zone lying along 61° S[19], and previously thought to be the Mesozoic inner arc postulated by Trendall[20] as a source for tuffaceous greywackes on South Georgia. In the northern part, anomalies are identified out to 7 or 8 m.y. on both sides, which eastward extend to the South Sandwich Islands themselves. Westward, anomalies beyond 8 m.y. have a similar trend, but correlate less well and cannot be identified. The anomalies are

A model based on the data discussed here must be considered provisional and as such I would propose a pole of relative rotation of the South American and Antarctic plates close to 150° W, 72° S, with an equatorial separation rate of 2 cm/yr, principally to be consistent with the calculated spreading close to the South Atlantic triple junction and with movement along the line of the Shackleton Fracture Zone. The model, shown schematically in Fig. 4, also incorporates the small Sandwich plate and, faintly to emphasize that at present it is one of many alternatives, a scheme for the western Scotia Sea.

Plate convergence is implied at the western margin of southern Chile at rates varying between 1·2 and 1·4

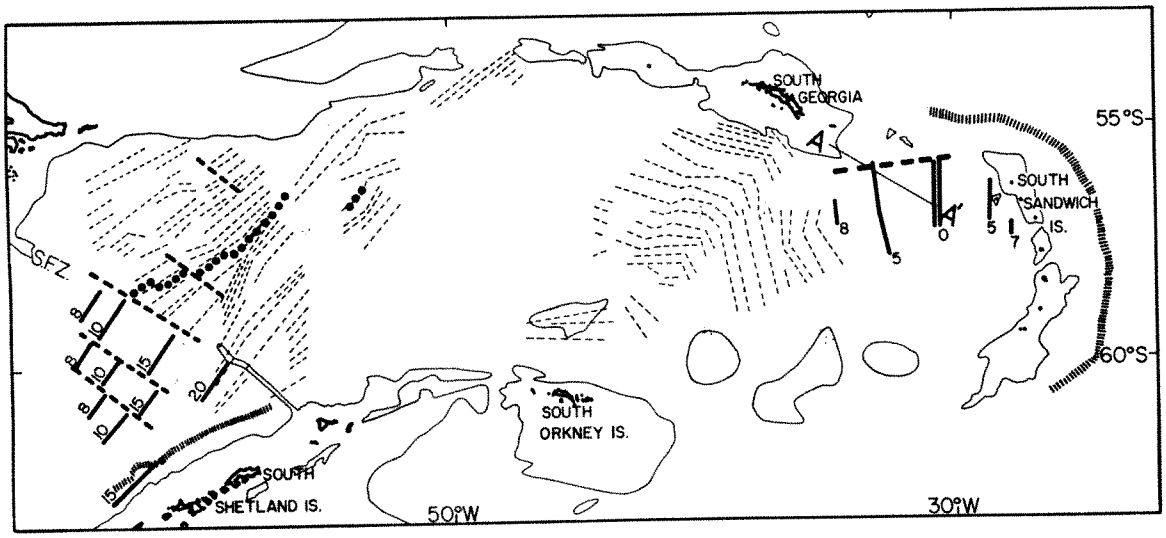


Fig. 2. Magnetic lineations in the Scotia Sea[4]. Dated lineations are shown as thick continuous lines (numbers are ages in m.y.), undated lineations as thin dashed lines. Fracture zones are thick dashed lines (SFZ is Shackleton FZ) and trenches are hatched. Solid circles show the axis of the double bathymetric ridge in the west Scotia Sea and the thin continuous line is the 2,000 m depth contour.

seen on several profiles[4], but no single profile demonstrates all the points made above. For example, profile AA′ of Fig. 3 (located on Fig. 2) compares well with profile EE′ of Eltanin Cruise 19[21] between 0 and 5 m.y. on the western limb of the spreading centre, but westward is affected by a probable fracture zone (marked FZ) and eastward suffers a 90° course alteration.

Present Plate Boundaries and Relative Motion

The major implication of the magnetic lineations for present plate motion concerns this postulated active spreading centre lying to the west of the South Sandwich Islands. With the trench and associated fracture zones it defines a new "Sandwich" lithospheric plate, growing at 2·7 cm/yr at its western margin and overriding Atlantic oceanic crust. The implied age of 7 to 9 m.y. for the crust beneath the islands is consistent with potassium–argon ages of 0·7 and 4·0 m.y. for lavas thought to represent the older parts of the exposed succession[22]. Secondary extensional features are now being found behind many oceanic island arcs[23], so it is probable that the trench formed first, perhaps as a result of a change in the boundaries of the major plates, and that the spreading centre was a secondary development.

The existence of the Sandwich plate removes the requirement that the boundary between the major plates to the west must start from the northern end of the South Sandwich Trench, but the distribution of earthquake epicentres suggests that some relative motion may be occurring along both the northern and southern Scotia Ridge. Bathymetrically, no west–east lineation can be seen along the north Scotia Ridge, whereas a deep, steep-sided trough bisects much of the southern limb. Several epicentres are closely associated with the Shackleton Fracture Zone and others lie to either side between 58° S and 59° S.

cm/yr, approximately along 120°, and at the much greater rate of 5·4 cm/yr at the South Sandwich Trench. Spreading west of the Shackleton Fracture Zone is presumed to have ceased but, to its east within the Scotia Sea, a resultant separation of 1·2 cm/yr must be accommodated by motion at the Drake Passage spreading centre and along the southern Scotia Ridge. If the spreading centre between South Georgia and the South Orkney Islands is real, it does not appear to be active at present.

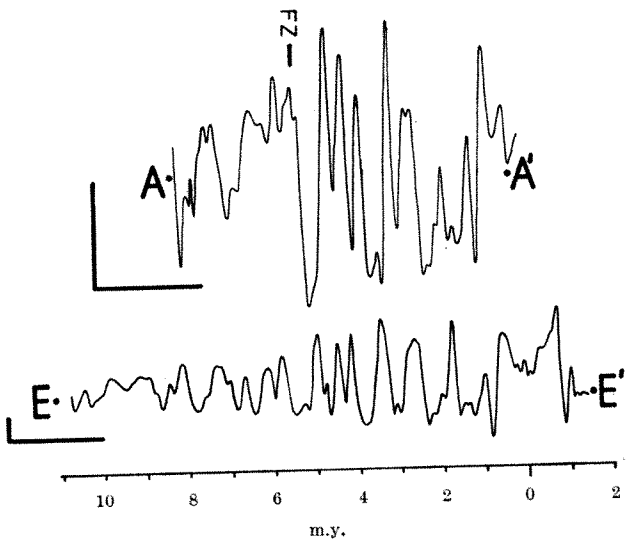


Fig. 3. Magnetic profile AA′, located in Fig. 2, compared with profile EE′ from Eltanin Cruise 19[21]. Position of fracture zone shown (FZ). Vertical and horizontal scale lines represent 250 gamma and 100 kms.

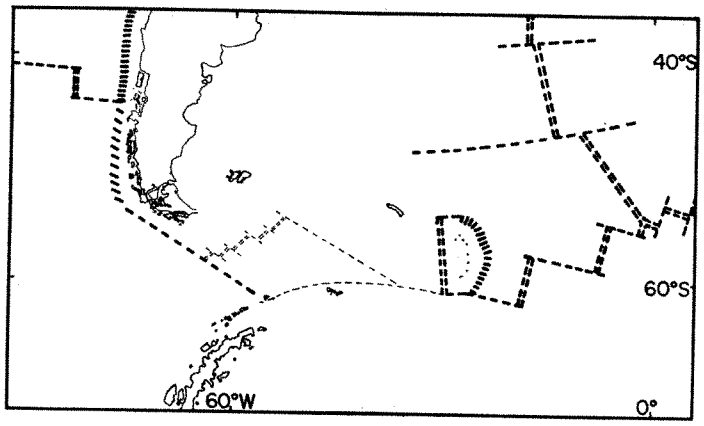


Fig. 4. Postulated boundaries between African, South American, Antarctic and Sandwich plates. Motion between South American and Antarctic plates accords with a pole of rotation close to 150° W, 72° S and an equatorial separation of 2 cm/yr. Plate boundary types are, spreading centre (≡≡≡≡), fracture zone (----) and convergence zone (I I I I). Possible Scotia Sea model is shown faint.

The intense earthquake activity associated with the leading (eastern) edge of the Sandwich plate is attributable in a general sense to the high convergence rate but, more specifically, the 170 km depth to the deepest earthquake is shallower than might be expected[24,25] if plate consumption at 5·4 cm/yr had proceeded for approximately 8 m.y. With this exception, all earthquake activity in the region is seen to occur at boundaries across which relative motion is slow; the lack of activity along other parts of these boundaries may in part result from this slow motion.

Evolution of the Scotia Ridge

The magnetic lineations and recent work on the structure of some of the flat-topped, steep-sided, shallow submarine ridges which make up the Scotia Ridge[3,5–7,26,27] also indicate aspects of the broad structural history of the region. The Ridge abounds with geological structures inconsistent with their present isolated situation and truncated by present "continental" margins, suggesting the fragmentation of a previously continuous continental area. The age of these structures argues a post-Cretaceous fragmentation, which is consistent with the suggested post-Middle Tertiary age for most of the Scotia Sea[4]. Although this latter age is speculative, and parts of the Scotia Ridge have not been examined, sufficient information is available to permit a generalized reconstruction. There is no evidence within the Scotia Sea, east of the Shackleton Fracture Zone, of crustal consumption at northern or southern margins, so that the magnetic lineations of Fig. 2 probably show the true directions of separation of the north and south Scotia Ridge. The indicated reconstruction moves the south Scotia Ridge north-westward along the line of the Shackleton Fracture Zone until Elephant Island lies close to Cape Horn. Simultaneously, South Georgia is moved towards the South Orkney Island block, which with the last movement results in a net westward movement of South Georgia, shortening the north Scotia Ridge. The structure of component blocks of the south Scotia Ridge indicate that it too should be made more compact[5–7], and seemingly the entire Scotia Ridge becomes a continous continental connexion between West Antarctica and South America. The Pacific margin of this block is continuous, cuspate in shape, and comprises only South America as far south as Cape Horn and West Antarctica southward from Elephant Island. Prior to its presumed mid-Tertiary fragmentation this continental area may well have undergone only minor adjustments since the fragmentation of Gondwanaland. If the separation of South America, Africa and East Antarctica has proceeded more or less steadily since their initial break-up it therefore becomes necessary to introduce some relative motion between separate East and West Antarctic plates from then until the Middle Tertiary when, perhaps, these two joined as West Antarctica began to separate from South America. The acceptance of such an initial reconstruction of the Scotia Ridge region and of Mesozoic relative motion between parts of the present Antarctic plate greatly simplifies the problem of Gondwana reconstruction[28,29].

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LETTERS TO NATURE

PHYSICAL SCIENCES

Peculiar Pulse Burst in PSR 1237+25

GENERAL features of the short term variations in pulsars are remarkably steady. It has been found[1], for example, that PSRs 1919+21 and 2016+28 (AP2015) exhibit a marching subpulse phenomenon, and this effect has since been detected in other pulsars. Other pulsars such as PSRs 0950+08 and 1929+10 show no such obvious correlation from pulse to pulse, but do have characteristic statistical properties on the same time-scale. Pulsar 0950+08 is very erratic, often showing 100 per cent modulation of the pulse energy over a single pulse period. On the other hand, 1929+10 has a pulse to pulse modulation of only 50 per cent with the exception of null pulses[2] which recur regularly. Thus the characteristic short-term variations are an additional steady parameter of each pulsar which deserves to be considered along with the period, pulse shape, polarization properties and spectrum as a property of the source. The short-term variations of PSR 1237+25 have been described in detail in two earlier papers[2,3]. This communication describes a peculiar change of mode that occurs infrequently in sequences of pulses from 1237+25.

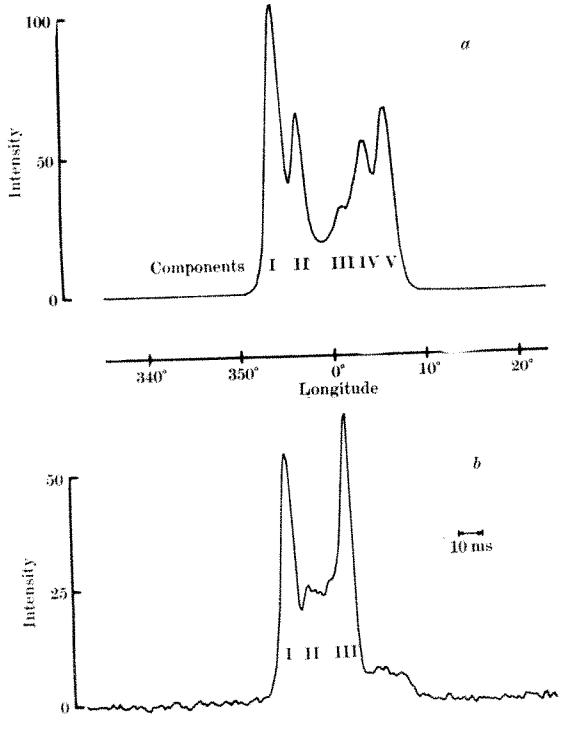


Fig. 1. *a*, A typical average pulse profile from PSR 1237+25 at 318 MHz, showing five distinct components. 1,344 pulses. *b*, The average profile from 45 pulses during the change of mode, showing the weakness then of components IV and V.

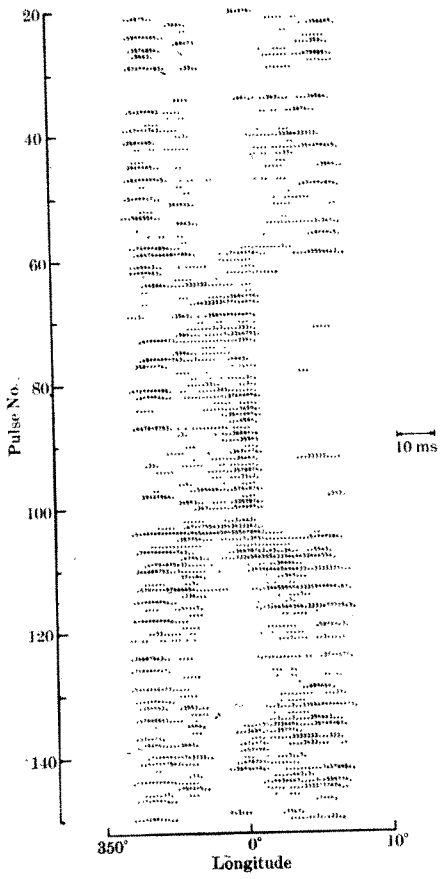


Fig. 2. Computer printout showing an abrupt change of mode at pulse 60 lasting to pulse 104.

It has been shown[3] that the 1237+25 average pulse has five distinct components which comprise two systems of correlated subpulses. Fig. 1*a* contains a typical average pulse profile at 318 MHz which displays these components. Assuming the rotating star model for these objects, the abscissa has been divided into degrees of longitude such that 360° of longitude equals one pulse period and the zero is arbitrary. F. D. Drake has pointed out to me that this longitude is that of the sub-Earth point of the rotating star and may not indicate the relative positions of the radiating regions because of beaming effects. The detailed analysis of a sequence of pulses described in ref. 3 led to the conclusion that components I, II, IV and V participate in a short-period system of subpulse movement, while component III and part of II and IV participate in a long-period system. To confirm these results in the raw data, which are recorded digitally in synchronism with the apparent pulsar period as described in ref. 3, the intensities recorded for a single pulse are scaled and printed by the computer across a single line. Successive pulses are printed on successive lines. An example of this output from an observation of 1237+25 at 318·5 MHz with linear polarization on July 31, 1970, is shown in Fig. 2. An abrupt change of mode occurs at pulse 60 and lasts until pulse 104 where the mode returns to normal just as abruptly. Components IV and V are very weak during this peculiar mode while component III is enhanced considerably. Fig. 1*b* contains the average of these 45 pulses. In both figures it is evident that component III has a very sharp cutoff on the trailing edge.

Similar mode changes have been observed on several occasions. In one case which lasted for about 250 pulses

component II was enhanced while components II and III became much more steady than normal. On another occasion the mode change lasted for about 1,300 pulses. In this case the pulse energies were weak so that detailed information could not be obtained. The phenomenon has been observed at 430 MHz with circular polarization. Mode changes lasting for five to ten pulses have also been detected.

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Molecular Oxygen Absorption of Solar Lyman-α observed by Interkosmos-I

THE satellite Interkosmos-1, a joint project of the Soviet Union, the German Democratic Republic and Czechoslovakia, was launched on October 14, 1969, into an orbit with perigee height 260 km, apogee height 640 km and an inclination of 48·4 degrees. A Lyman-α photometer, designed at this institute, was mounted on the observing platform of the satellite, and directed towards the Sun with an accuracy of ±1 degree. The photometer consisted of a modified ionization chamber of Forsterite ceramics with an LiF window and a filling of NO gas[1], followed by a d.c. amplifier with a time constant of 0·14 s and two output channels with different sensitivities. The spectral range of the photometer was from 1050 Å to 1350 Å[2]. Its absolute calibration, carried out at the Physical Institute of the Academy of Sciences of the USSR, yielded a scale value of $K_{phot} = 0{\cdot}780 \times 10^{11}$ photons $cm^{-2}\ s^{-1}\ V^{-1}$ at 1216 Å in the high-sensitivity channel, with an error of ±3 per cent. During the period of observation, October 15–23, 1969, the measured absolute values of solar Lyman-α flux varied between 3·7 and 4·7 ergs $cm^{-2}\ s^{-1}$, with a mean value of 4·1 ergs $cm^{-2}\ s^{-1}$. One purpose of the experiment was to measure the height-dependent absorption of Lyman-α in the upper atmosphere when the satellite entered the Earth's shadow ("satellite sunset"). The most reliable data of this kind were obtained on October 15 at 1409 UT, when the line-of-sight between the satellite and the Sun touched the Earth's surface at a geographic latitude of about 40° N. For this case, Lyman-α absorption, expressed in terms of optical thickness of the atmosphere as a function of the "grazing height" above the Earth's surface, is presented in Fig. 1. The orientation of the satellite towards the Sun was guaranteed for all grazing heights greater than 10 km.

Theoretical curves of optical thickness at grazing incidence were calculated from the O_2 and N_2 densities given by the CIRA 1965 model atmosphere, which are nearly equal to the mean values between the summer and winter models of the US Standard Atmosphere Supplement 1966. Absorption cross-sections of $\sigma(O_2) = 8{\cdot}4 \times 10^{-21}$ cm^2 for O_2, and $\sigma(N_2) = 6 \times 10^{-23}$ cm^2 for N_2 (ref. 3) were adopted. With these cross-sections, absorption by N_2 contributes less than 3 per cent to the total optical thickness below 120 km. This theoretical height dependence of optical thickness, taking into account the finite diameter of the Sun's disk, is represented by the

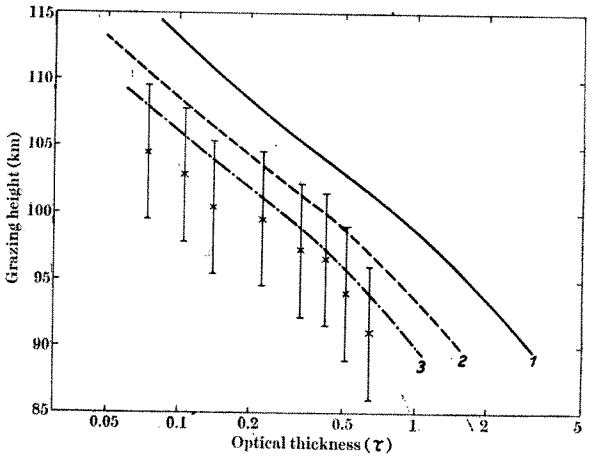


Fig. 1. Optical thickness at grazing incidence for Lyman-α, measured on the satellite (crosses). Theoretical values of optical thickness are computed from the CIRA 1965 model atmosphere (curve 1), and with O_2 densities reduced to a half and a third of the CIRA values (curves 2 and 3, respectively).

continuous curve in Fig. 1. It coincides almost exactly with similar calculations by Thomas and Bowman[4]. Fig. 1 shows that the measured data have nearly the same height gradient as the theoretical curve, but their absolute values are smaller than the theoretical ones by a factor of three or more.

Because it seems improbable that the cross-sections adopted are wrong by a factor of this order of magnitude, we have to conclude that at least in the height interval between 90 and 120 km the O_2 concentration should be only one third of the CIRA model values in order to fit the observed absorption. (The broken curves indicate theoretical optical thickness if the O_2 concentration is reduced to half or one third of the CIRA values, respectively.) This conclusion agrees well with recent rocket absorption measurements around 1450 Å made by Wildman *et al.*[5] in Australia, and with a similar difference between CIRA data and rocket ultraviolet absorption results reported by Weeks and Smith[6]. Mass spectrometer data[7] also indicate that at around 150 km the CIRA O_2 concentrations are considerably too large, whereas the analysis of Solrad-8 Lyman-α absorption by Norton and Warneck[8] seemed to agree with the CIRA model. It must be remembered, however, that the calculation of grazing heights for satellites is difficult and may introduce systematic errors. In the case of our measurements, the grazing heights were determined from simultaneous intensity records of visible sunlight on the satellite, and the estimated height error should not exceed ±5 km, as indicated by error bars in Fig. 1. The observed difference, therefore, between the O_2 concentration of the CIRA 1965 model and our data seems to be significant.

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Size Distribution of Lunar Soil

When Duke *et al.*[1] measured the size of lunar soil returned by Apollo 11, they found a size distribution similar to that of glacial till and other terrestrial soil, except that the lunar soil is noticeably deficient in material finer than 15 μm. They feel that a partial explanation for this deficiency is that it is caused by melting and consequent consolidation into clumps of finer particles on the Moon. We wish to suggest another partial explanation, derived from wear theory—it is that the finer particles are missing in lunar soil because materials in a lunar environment have a higher surface energy.

Particles produced by wear during sliding have a diameter, d, given by a relationship which can be expressed most simply as

$$d = 60{,}000\ W_{ab}/p \qquad (1)$$

where W_{ab} is the surface energy of adhesion of the two rubbing bodies a and b, and p is the penetration hardness (Vickers or Knoop) of the body from which the particle is formed[2]. The theoretical basis for this relationship is an energy balance between the surface energy of a contact and the volume elastic energy of the material in the vicinity of a contact. The same equation gives the least size of particles formed by attrition during ball milling[3], and it is possible that it also governs lunar attritious processes.

For typical rock materials on the Moon, p is about 600 kg/mm², and for a pair of such materials *in vacuo* W_{ab} can be estimated to be 1,000 erg/cm². Thus d in equation (1) has a value of 10 μm, in good agreement with the observed value.

In a terrestrial dry air environment, W_{ab} is reduced by about a factor of two from the value I have given, while in a high humidity environment or in the presence of liquid water, further substantial reductions in W_{ab} take place. Corresponding reductions can be anticipated in the least sizes of particles formed on Earth by attritious processes.

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Coupling between Aerials used for measuring Ionospheric Drifts

An important consideration when designing an array of aerials to sample the diffraction pattern of a radio wave reflected from the ionosphere is to ensure that the results are not affected by mutual coupling. Signals reradiated from one aerial may be received by another, and this could affect the measured characteristics of the diffraction pattern. Any subsequent measurements (for example, of ionospheric drifts) would also be affected.

A simple check for the effects of such coupling is to look for differences in the cross correlation between records from different aerials. If coupling is important, the values of the cross correlation at zero shift between separated aerials may be expected to show consistent differences depending on their relative orientation. In particular, it may be expected that the correlation for aerials aligned parallel to each other may be different from the correlation for aerials which are mutually perpendicular, and that this effect should be greater for smaller separations.

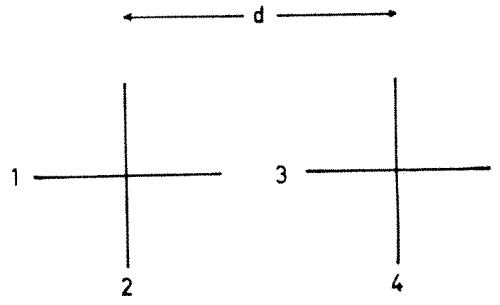


Fig. 1. The aerial configuration.

Such an experiment was carried out using the array of 178 dipole aerials at Buckland Park near Adelaide, South Australia[1]. Pairs of mutually orthogonal half wavelength dipoles (1·98 MHz) with their centres at the same pole were chosen from within the array as shown in Fig. 1. Separate receivers and a four channel pen recorder were used to record the amplitude fluctuations of circularly polarized radio waves reflected from the ionosphere at vertical incidence. From the four records obtained the cross correlation at zero shift for each of the six possible combinations was calculated. Two of these values represent the correlation between signals obtained by orthogonal dipoles at the same site (ρ_{12}, ρ_{34}), two represent the correlation between separated orthogonal dipoles (ρ_{14}, ρ_{23}), one the correlation between dipoles aligned end to end (ρ_{13}) and one represents the correlation between dipoles parallel to each other (ρ_{24}).

The effects are most likely to be seen when the correlation between the fading at adjacent dipoles is low. Partial reflexions from near 95 km were therefore used as these have been observed to give the smallest pattern scale among echoes returned from the ionosphere. In order to eliminate any possible effects due to the receivers or recording apparatus, the connexions between each dipole and the receiver/pen recorder combination were rotated between each record. The records were digitized using eighty levels.

Table 1. VALUES OF THE CROSS CORRELATION AT ZERO SHIFT BETWEEN FADING AT PAIRS OF AERIALS

	Local time (h)	ϱ_{12}	ϱ_{34}	ϱ_{14}	ϱ_{23}	ϱ_{24}	ϱ_{13}
d=91 m	1148	0·95	0·94	0·73	0·76	0·79	0·66
	1152	0·94	0·95	0·70	0·68	0·71	0·69
	1155	0·94	0·89	0·71	0·72	0·66	0·71
	1158	0·93	0·94	0·48	0·63	0·57	0·56
	1205	0·91	0·92	0·64	0·56	0·59	0·59
	1209	0·93	0·94	0·61	0·51	0·60	0·54
	1212	0·94	0·85	0·34	0·48	0·48	0·34
	1215	0·93	0·92	0·54	0·68	0·62	0·65
	1412	0·96	0·88	0·65	0·70	0·68	0·63
	1414	0·90	0·91	0·52	0·44	0·50	0·49
	1417	0·94	0·90	0·61	0·50	0·52	0·58
	1419	0·91	0·92	0·43	0·41	0·39	0·48
	Average	0·92		0·58		0·59	0·58
d=183 m	1219	0·92	0·94	0·15	0·07	0·14	0·09
	1224	0·93	0·92	0·20	0·24	0·23	0·18
	1231	0·90	0·95	0·19	0·18	0·19	0·16
	1239	0·93	0·95	0·40	0·45	0·39	0·44
	1249	0·90	0·84	0·10	0·08	0·13	—
	1252	0·94	0·92	0·17	0·23	0·22	0·21
	Average	0·92		0·21		0·22	0·22
d=274 m	1326	0·92	0·94	0·16	0·18	0·20	0·18
	1329	0·90	0·93	−0·17	−0·05	−0·13	−0·08
	1334	0·93	0·91	0·07	0·09	0·08	0·08
	1337	0·92	0·94	0·26	0·40	0·37	0·29
	1340	0·92	0·89	0·03	0·01	0·03	0·00
	1346	0·93	0·87	0·09	0·14	0·13	0·10
	1350	0·93	0·96	−0·08	−0·07	−0·07	−0·10
	Average	0·92		0·08		0·09	0·07

95 km echo. March 3, 1970.

The results for twenty-five records representing three different separations (d) between the crossed dipoles are given in Table 1. Each record is identified by a time, and each value of cross correlation is listed in the appropriate column. The variations with time do not represent

errors of measurement but real variations in pattern scale from one occasion to another. The values were averaged, as shown in Table 1, to look for any systematic differences between the pairs.

It can be seen that the absolute value of the cross correlation decreases with increasing separation as would be expected because of the random nature of the irregularities in the diffraction pattern. The difference between cross correlation values for dipoles the same distance apart but differently oriented with respect to one another is, however, small. This implies that coupling between any pair of separated dipoles has a negligible effect on the measured correlation even for the minimum separation of 91 m.

It has been suggested[2] that errors in the true velocity derived from full correlation analysis[3,4] may be a consequence of the depression of the measured cross correlation values from their true value especially when the correlation is high, as will be the case for closely spaced aerials. The above results suggest that this depression is not caused by aerial coupling. Experimental factors such as receiver differences or digitizing errors must therefore be responsible. This is confirmed in these experiments, which show that the cross correlation values at zero shift between orthogonal dipoles at the same location (ρ_{12} and ρ_{34}) are not unity, as might be expected, but have an average value of 0·92. The reasons for the experimental factors which cause this effect are at present being investigated.

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BIOLOGICAL SCIENCES

Phosphorylation of D-Arabinosyl Adenine by *Plasmodium berghei* and its Partial Protection of Mice against Malaria

9-β-D-arabinofuranosyladenine (ara-A) is a nucleoside antibiotic (Fig. 1) with a broad spectrum of activity against animal viruses[1–7] and little or no toxicity when administered parenterally to animals. The LD_{50} for an intraperitoneal injection is 4,677 mg/kg[7]. The rodent

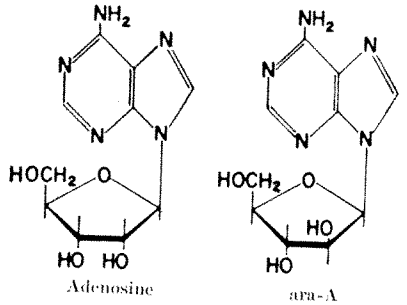


Fig. 1. Structure of 9-β-arabinofuranosyladenine (ara-A).

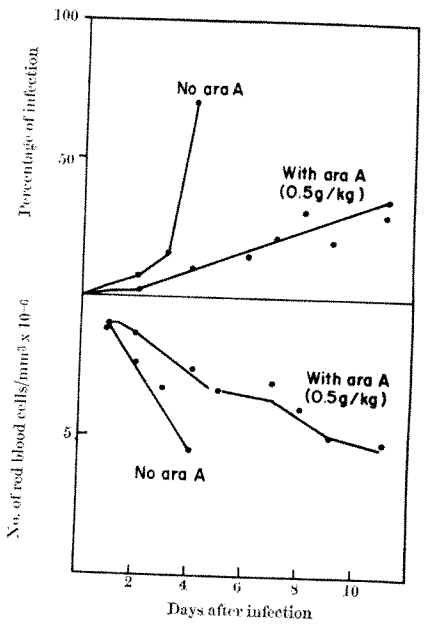


Fig. 2. Effect of ara-A on the course of infection of *P. berghei* in mice. *P. berghei* was maintained according to Wellde *et al.*[12]. Mice were injected intraperitoneally with 2×10^7 parasitized red blood cells per mouse. One group (ten mice per group) also received a daily injection of 0·5 ml. of saline containing 0·5 g/kg of ara-A. The mice which had not received the drug all died within 5 days after infection. Mice treated with drug remained alive from 10–14 days after infection. The number of red blood cells and of parasitized red blood cells was determined from blood removed from the tail.

malarial parasite, *Plasmodium berghei*, resembles viruses in that it is an intracellular parasite. Moreover, the host is protected by interferon[8]. There is evidence that *P. berghei* utilizes part of the host protein synthesizing system[9,10].

We have studied the effect of a daily dose of 0·5 g/kg of ara-A on infection of mice with *P. berghei* and found that the percentage of infected red blood cells was lower than in control animals (Fig. 2) which had been infected but not treated with the drug and that the development of anaemia was significantly slower in infected mice which had received ara-A. Moreover, drug treated animals remained alive twice as long as control animals.

Enzymes involved in nucleic acid biosynthesis are specific for the configuration of the 2′ pentose carbon of nucleotides. This is well documented for RNA and DNA polymerases. The effect of ara-A on *P. berghei* may reflect lack of ability of malarial enzymes to distinguish the "wrong" configuration. If malarial adenosine kinase "mistakenly" phosphorylates the drug, ara-A nucleotides will be formed. Ara-ATP is a competitive inhibitor of mammalian DNA polymerase *in vitro*[11].

^{3}H-ara-A was prepared[11] and 20 μCi (specific activity 4·81 mCi/mmole) together with 450 μCi of $^{32}P_i$ were injected in 0·1 ml. saline into each mouse three days after malarial infection (ten mice per group). Blood was collected 20 h later by heart puncture and livers were removed. Preliminary experiments indicated that uninfected blood could not phosphorylate ara-A. Free parasites were isolated in 10 ml. of distilled water containing 1 g of glass beads (200 μM in diameter) and homogenized in a Sorval 'Omni-mixer' for 5 min at speed control No. 5. 10 ml. of cold 10 per cent trichloroacetic acid (TCA) were added followed by 40 ml. of cold 5 per cent TCA. The mixture was centrifuged for 10 min at 15,000*g* at 4° C. Supernatant fluid was collected. The precipitate was washed with 20 ml. of 5 per cent of cold TCA. The combined TCA soluble extract was washed five times with three volumes of ether to remove TCA and applied on a 2·4 × 20 cm Dowex 1–8X column (200–400 mesh, chloride form). Preliminary experiments

showed two peaks in the elution with 5 mM HCl. The first represents nucleotide monophosphate, the second nucleotide di and triphosphate which are inseparable in these conditions. Nonphosphorylated ara-A was washed from the column with water and phosphorylated product was eluted with 5 mM HCl (Fig. 3). Only traces of ^{3}H-ara-A were present. Most of the nucleosides were phosphorylated to the di and triphosphates (second peak of ^{32}P marker). Further examination showed most of the material was ara-ATP. Phosphorylation of ^{3}H-ara-A by infected and control (non-infected) mouse liver was followed by the same procedure except that homogenization was carried out in 0·9 per cent saline without glass beads. In both controls (Fig. 4*A*) and infected livers (Fig. 4*B*) ^{3}H-ara-A was non-phosphorylated and eluted from the column with water. A very small amount of phosphorylated ara-A eluted with 5 mM HCl. A slight phosphorylation appeared in ara-AMP (first peak of the ^{32}P marker). No tritiated product appeared in the position representing nucleotide di and triphosphate (second peak of ^{32}P marker).

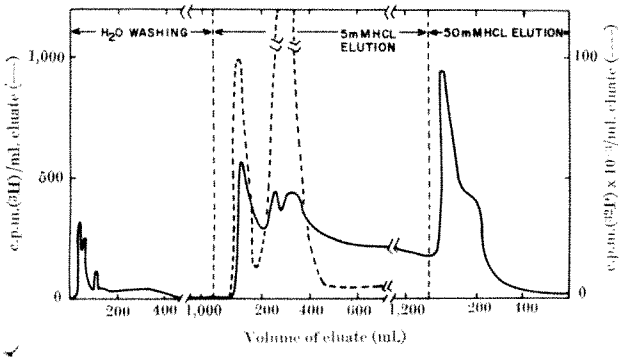


Fig. 3. Elution profile on a Dowex column of phosphorylated ara-A extracted from *P. berghei*. Mice infected with *P. berghei* were injected with ^{3}H-ara-A and ^{32}P$_{i}$. After 20 h incubation, free parasites were isolated and the acid soluble fraction obtained from the parasites was applied on a Dowex 1–8X column. Unphosphorylated ^{3}H-ara-A was washed off the column with water and the phosphorylated products were eluted with 5 mM HCl. The experimental details are described in the text. ———, ^{3}H/ml. eluate; - - - -, ^{32}P/ml. eluate.

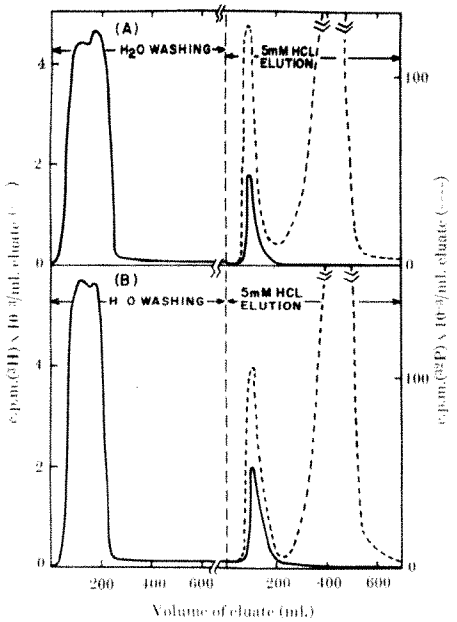


Fig. 4. Elution profile of phosphorylated ^{3}H-ara-A in the acid soluble fraction of mouse liver. Experimental conditions are described in the text. ———, ^{3}H/ml. eluate; - - - -, ^{32}P/ml. eluate. *A*, acid soluble fraction from uninfected mouse liver; *B*, acid soluble fraction from infected mouse liver.

Normal mouse liver accumulated 46 nmoles of ^{3}H-ara-A and its phosphorylated product per g fresh liver. Of this, only 5·3 nmoles (11·5 per cent) were ara-AMP. Infected mouse liver accumulated 100 nmoles of ^{3}H-ara-A and its derivative of which 18 nmoles were ara-AMP (17·8 per cent) per g fresh liver. *P. berghei*, however, accumulated 813 nmoles of ^{3}H-ara-A and its derivatives per g fresh tissue. 755 nmoles (93 per cent) were phosphorylated mostly to di and triphosphate.

P. berghei can efficiently accumulate and phosphorylate ara-A to ara-ADP and ara-ATP. We have evidence (unpublished) that *in vitro* ara-ATP is a competitive inhibitor of ATP for leucyl-tRNA synthetase obtained from a tryparosomatid. It is possible that inhibition of development of malaria by ara-A is directly related to the parasite's ability to phosphorylate D-arabinosyladenine to ara-ATP which then serves as a competitive inhibitor of ATP for essential enzyme systems. On the other hand, the mammalian host has little capacity to accumulate and phosphorylate ara-A. Moreover, phosphorylation is halted at the ara-AMP level.

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Effects of Lithium and of *p*H on Synaptosomal Metabolism of Noradrenaline

CURRENT hypotheses about mania and depression are that they correspond to greater or lesser availability of catecholamines at central synapses[1], or to changes in electrolyte and water distribution[2]. The mode of action of lithium salts, which seem to be effective at least in mania, has been related to both hypotheses[3]. There are several findings regarding catecholamine metabolism. They include a disappearance of brain noradrenaline after inhibition of tyrosine hydroxylase which is more rapid following Li^{+} treatment[4]; a shift in brain metabolism of noradrenaline from extracellular methylation (by catechol-O-methyl transferase), toward intraneuronal deamination (by monoamine oxidase)[5]; and a decreased rate of release of ^{3}H-noradrenaline during electrical stimulation of brain slices *in vitro*[6]. Evidence regarding effects of Li^{+} on active uptake of noradrenaline into neurones, a major means of inactivation of catecholamine released at syn-

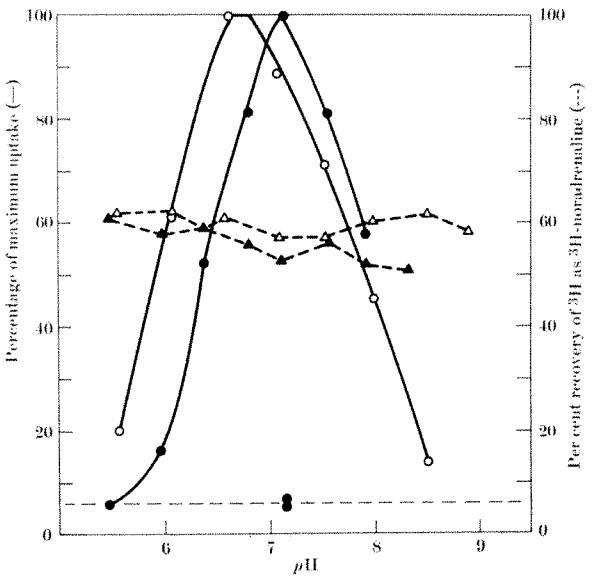


Fig. 1. Rat brain synaptosomal uptake of ^{3}H-noradrenaline as a function of *p*H in two physiological media. Two independent buffer systems were used: *tris*-(hydroxymethyl)-aminomethane-phosphate (○—○), or N-2-hydroxyethylpiperazine-N′-2-ethane-sulphonic acid (●—●) (California Biochem. Co.), with physiological concentrations of Na^+ (including NaOH used for titration), K^+, Mg^{2+}, and added glucose and ascorbate. Uptake blanks (● - - - ●) represent determination at 4° C, or at 37° C with boiled tissue. △ - - - △, Tris-phosphate recovery; ▲ - - - ▲, N-2-hydroxyethylpiperazine-N′-2-ethane-sulphonic acid recovery.

apses, is somewhat in conflict. One study[7] reported enhanced uptake *in vitro* of ^{3}H-noradrenaline into synaptosomes ("pinched-off nerve endings") prepared after the administration of large amounts of unneutralized Li_2CO_3. This finding has not been confirmed, and there is evidence that uptake of ^{3}H-noradrenaline into intact brain[5], or brain slices[6], may not be altered by prior treatment of rats with LiCl. Thus questions arise as to the effect of carbonate ion or *p*H on amine uptake. These findings, together, suggest that lithium may tend to keep noradrenaline in a pre-synaptic location, while increasing catabolism, particularly by oxidative deamination. The net effect would be decreased availability of this putative transmitter at central synapses, an interpretation consistent with the antimanic effects of lithium.

The effects of lithium on amine uptake, however, seemed to require further study. Rats were therefore given solutions of Li_2CO_3. Equal volumes of distilled water (which did not significantly alter uptake), or gastric intubation alone, were used in controls. All rats were given two to three divided daily doses by oro-gastric intubation for 3–5 days. Consistent $[Li^+]$ of 1 mequiv/l. of serum or 0·5 mequiv/kg of wet brain were found by flame photometry at a daily dose of 200–300 mg Li_2CO_3/kg body

Table 1. EFFECT OF LITHIUM *in vivo* ON RAT BRAIN SYNAPTOSOMAL UPTAKE OF ^{3}H-NORADRENALINE *in vitro*

Treatment	Mean per cent of control uptake*	s.e.	*N*	*P*
Li_2CO_3 300 mg/kg	108·3	±11·6	9 rats	NS
			18 assays	NS
Li_2CO_3/HCl, *p*H 7, 300 mg/kg	109·7	±5·7	9 rats	NS
			17 assays	< 0·02

Synaptosomal fractions[8] were transferred from sucrose gradients to tubes containing Krebs-bicarbonate medium[9] with $2–3 \times 10^{-8}$ M ^{3}H-noradrenaline (dl-NA-7-^{3}H, 9 c/mmole, New England Nuclear Corp.) incubated in air at 37°. Aliquots of the mixture were taken for protein determination[10], and tubes were centrifuged at 48,000*g* for 30 min. Perchloric acid preparations of supernatant medium and the synaptosomal pellet were chromatographed on alumina columns[11]. Total ^{3}H and ^{3}H-noradrenaline radioactivity were assayed[12]. "Uptake ratios", defined as: nCi ^{3}H-noradrenaline per gm synaptosomal protein ÷ nCi ^{3}H-noradrenaline per ml. incubation medium, were calculated. The mean uptake ratio for all untreated tissues was 918·2 (±62·2, s.e.m.). Experiments included controls with each experimental group; results are expressed here as per cent of control; statistics were done as *t*-tests of paired differences.

weight (6–8 mequiv Li^+/kg). Such doses of carbona solution (*p*H 11) were grossly toxic and often fatal. Tl treatment of rats with alkaline Li_2CO_3 solutions resulte (Table 1) in a small (8 per cent) insignificant increase ^{3}H-noradrenaline uptake by isolated brain synaptosome with marked individual variations. The addition of L ion *in vitro* at 4 mequiv/l. led to a significant decrease ^{3}H-noradrenaline uptake by synaptosomes from norm rats, and even greater decrease at 10 mequiv./l., where additional Na^+ (4 mequiv/l. above normal) had no signil cant effect on uptake *in vitro* (Table 2).

Table 2. EFFECT OF LITHIUM ON RAT BRAIN SYNAPTOSOMAL UPTAKE ^{3}H-NORADRENALINE *in vitro*

Ion	Concentration (mequiv/l.)	Mean per cent of control uptake	s.e.	*N*	*P*
Li^+	4	88·9	±3·9	8	< 0·05
	10	83·2	±1·5	6	< 0·00
Na^+	4	96·0	±5·2	8	NS

Either Li^+ as a neutralized bicarbonate–chloride salt, or NaCl, was add directly to the incubation medium, and *p*H readjusted, before uptake.

The effects of *p*H on uptake (Fig. 1) were striking, wit maximum uptake at approximately *p*H 6·8, and marke decreases toward minima approaching uptake "blanks" Recovery of total tissue tritium as ^{3}H-noradrenaline b column chromatography[11] remained constant at each *p*H suggesting that decreased uptake was not an artefact noradrenaline destruction.

Because of the gross toxicity of alkaline Li_2CO_3 ar the marked effect of *p*H on noradrenaline uptake *in vitr* rats were also treated with neutralized Li_2CO_3/H solutions, which were relatively well tolerated. Agai an apparent small (10 per cent) but less variable increa in synaptosomal uptake of ^{3}H-noradrenaline *in vitro* w noted. It was not significant at an *N* of nine rats, b attained a $P < 0·02$ at an *N* of 17 *in vitro* assays (Table 1

In other experiments, enzyme assays *in vitro* reveale no changes in activity[13] of monoamine oxidase prepare from beef renal cortex mitochondria[14] by additions Li^+, K^+ or Na^+ in the range of 0·3–8·3 mequiv/l., and changes in catechol-O-methyl transferase activity of soluble supernatant fraction of rat brain[15] added to medium with 4 mequiv Li^+/l. Furthermore, estimates rates of glucose oxidation by measurements of ^{14}C produced from labelled glucose[16] by mitochondria synaptosomal fractions of rat brain[8] showed an insigni cant small decrease in the presence of 4 mequiv Li^+/l.

The apparent enhancement of synaptosomal uptake noradrenaline *in vitro* by lithium, while small and questionable significance, is in the direction reporte previously by Colburn *et al.*[7]. The basis of this effect is n clear, although any mechanism proposed should inclu changes which persist through the synaptosomal prepar tion steps. It should therefore be noted that whi alkalotic effects of carbonate administration might expected to decrease uptake *in vivo*, the amount of alkali anion carried into the *in vitro* assay was not sufficient alter *p*H. Furthermore, most of the Li^+ present in bra after treatment is removed during the preparation synaptosomes. Estimates were made of $[Li^+]$ in synapt somes exposed to 4 mequiv Li^+/l. *in vitro*, or followi administration of 8 mequiv Li^+/kg per day for 3 day Suspended synaptosomes were centrifuged and pellet L contents were measured: in these studies, $[Li^+]$ w 2×10^{-4} mequiv/mg protein (about 3 mequiv/l. of syna tosomal water, assuming the synaptosomal pellet ma was 60 per cent water) after *in vitro* exposure to Li^+, b only 4×10^{-6} mequiv/mg protein (about 0·07 mequiv/ after *in vivo* treatment. It is conceivable that Li^+ pr duces alterations of intraneuronal $[Na^+]$ or $[K^+]$ *in viv* which survive synaptosomal preparation and which mig secondarily affect amine uptake[19–21]. The mechanis involved in altered uptake does not seem to include t alteration of activities of enzymes which catabolis noradrenaline or decreased energy supplies from gluco oxidation.

Another important question is whether current comparisons of lithium salt regimens in animals and in man are justified. It has been commonly assumed that animal doses should correspond to therapeutic human doses, producing serum [Li^+] in the range of 0·5–2·0 mequiv/l. In rats, however[4], daily doses (4–8 mequiv Li^+/kg) which produce serum [Li^+] around 1 mequiv/l. are 10 to 20 times larger than those required in patients[17] (0·3–0·5 mequiv/kg) and, as the carbonate salt, are highly toxic to rats. At even lower doses (1–4 mequiv Li^+/kg), lithium carbonate or chloride salts produce severe renal tubular and mitochondrial damage in the rat similar to that seen in heavy metal poisoning[18].

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Evidence that the Area Postrema mediates the Central Cardiovascular Response to Angiotensin II

Angiotensin can affect the cardiovascular system in several different ways. Its direct vasoconstrictor action is well recognized but it can also exert cardiovascular effects by stimulating the autonomic nervous system at a variety of sites including the adrenal medulla[1], the sympathetic ganglia[2], the post-ganglionic sympathetic nerve terminals[3] and the brain[4]. It has not been possible, so far, to quantitate the relative importance of these various effects of the hormone in either physiological or pathological circumstances.

We have been particularly interested in those autonomic effects of angiotensin which are mediated by the brain. Vertebral artery infusion of angiotensin in the anaesthetized greyhound causes withdrawal of vagal tone to the heart and results in significant increases of heart rate, cardiac output and blood pressure[5]. This specific response

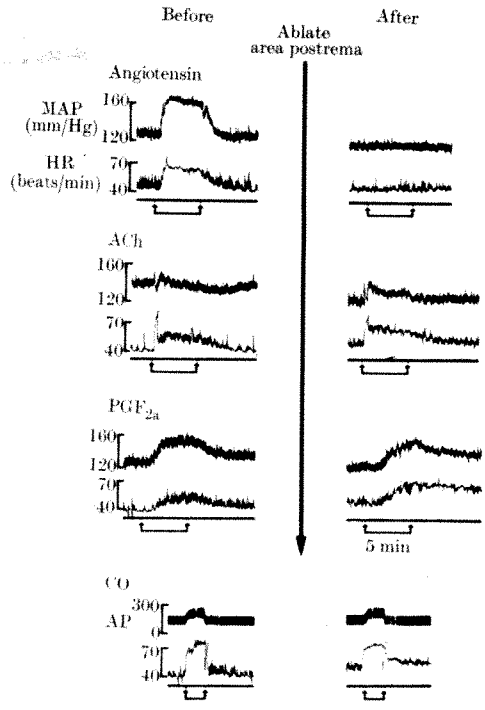


Fig. 1. The effect of ablation of the area postrema on the responses of mean arterial pressure (MAP) and heart rate (HR) to vertebral arterial infusions of angiotensin (1 ng/kg/min), acetylcholine (ACh) (10 μg/kg/min), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (8 ng/kg/min). Ablation of the area postrema completely abolished the response to angiotensin while the responses to the other procedures remained. CO, carotid occlusion.

can be demonstrated with vertebral artery infusion rates as low as 0·25 to 1·0 ng/kg/min which result in local blood concentrations ranging from 0·2 to 0·8 ng/ml. (ref. 6). We have shown that this response is due to an action of angiotensin in the caudal half of the medulla[7]. The area postrema lies in this region and is a known gap in the blood–brain barrier[8,9] which might allow angiotensin to reach the brain. Its histological appearance has led some workers to suggest that it is a chemoreceptor zone[10,11], responding to compounds in the bloodstream such as cardiac glycosides[12]. The area postrema lies in an accessible part of the caudal medulla, protruding into the fourth ventricle, and we have therefore studied whether its ablation alters the central effects of angiotensin.

We carried out experiments in nine greyhounds, anaesthetized with chloralose (100 mg/kg), after premedication with morphine (2 mg/kg) intravenously. Arterial pressure and heart rate were recorded by standard techniques using a Statham pressure transducer and Grass polygraph. Infusions into the vertebral artery were given through a polythene catheter through the side wall, without obstructing the flow. The opposite vertebral artery was clamped. All infusions of drugs were preceded and followed by control infusions of saline.

Bilateral ablation of the area postrema by thermocoagulation did not alter the baseline blood pressure or heart rate significantly, but abolished the response to intravertebral infusions of angiotensin. To verify that this was a specific effect we induced autonomically mediated cardiovascular effects by three other procedures: intravertebral infusions of acetylcholine[13] and prostaglandin $F_{2\alpha}$[14], and bilateral occlusion of the common carotid arteries. The autonomic responses to these other stimuli were not affected by ablation of the area postrema. Fig. 1 illustrates a sample experiment and Fig. 2 shows the pooled results of all the experiments.

These results show that the central effects of blood-born angiotensin in low concentrations are dependent on the

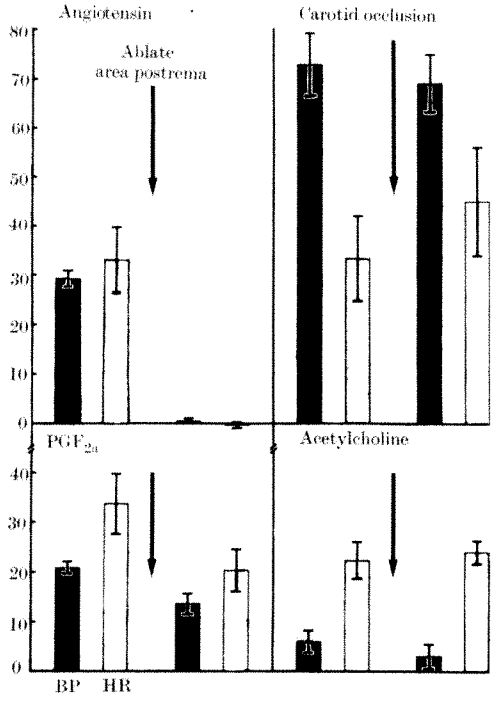


Fig. 2. Summary of the effects of ablating the area postrema in nine dogs. The histograms represent the mean responses of blood pressure (b.p., mm/Hg) and heart rate (h.r., beats/min). The bars represent one standard error on either side of the mean. Ablation of the area postrema abolished the response to intravertebral infusions of angiotensin but the responses to carotid occlusion, $PGF_{2\alpha}$ and acetylcholine remained.

integrity of the area postrema or immediately adjacent tissue. Because ablation of the area postrema neither changes the resting heart rate nor abolishes several other central autonomic effects, it seems likely that the area is relatively specific for angiotensin. Ablation of this area should provide a useful tool for studying the importance of the central effects of angiotensin in various physiological or pathological situations, for example in the response to severe haemorrhage or in chronic renal hypertension.

Note added in proof. Following submission of this work for publication, S. Katayama and H. Ueda have reported that local micro-injection of angiotensin into the region of the area postrema of the cat produces a pressor response (personal communication).

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Stimulation of Proliferation of 19S Antibody-forming Cells in the Spleens of Immunized Guinea-pigs after Exchange Transfusion

THERE is abundant evidence that the immune response, both primary (19S) and secondary (7S), is regulated by a feed-back mechanism involving serum antibody, particularly 7S antibody[1–4], but the exact mechanism remains unclear.

Rowley and Fitch[5] found that the primary immune response could be prevented if normal mouse spleen cells were mixed with specific antibody before transfer into irradiated and specifically immunized mice. This suggested that antibody had a direct suppressive effect on the antibody-forming cells. Möller[6], using the plaque technique to enumerate antibody-forming cells, demonstrated that spleen cells already making 7S antibody would give rise to increased numbers of 7S antibody-forming cells when transferred into a previously immunized irradiated animal. In other words, placing antibody-forming cells in an environment free of antibody resulted in their proliferation. *In vitro* studies by Pierce[7] led him to conclude that specific antibody inhibits the primary immune response by combining with antigen and interfering with the macrophage-dependent inductive phase.

Because passive administration of antibody, irradiation of host animals and *in vitro* studies introduce variables which are at times difficult to control or interpret, we have removed serum antibody from immunized animals by exchange transfusion in an attempt to evaluate the effect of endogenously produced antibody on the proliferation of 19S antibody-forming cells in the spleen. We conclude that serum antibody has a direct suppressive effect on the proliferation of 19S antibody-producing cells in the spleen.

Table 1. PRIMARY AND SECONDARY RESPONSE OF RATS AGAINST HEN ERYTHROCYTES

	Mean titre of agglutinins in saline	Mean titre of agglutinins in 'Dextran'
Control group (15 rats)		
Primary response	1/16 (1/4–1/128)*	1/512 (1/32–1/1,024)
Secondary response	1/32 (1/4–1/128)	1/1,024 (1/64–1/2,048)
α-Globulin treated group (10 rats)		
Primary response	— (0–1/2)	1/16 (1/2–1/64)
Secondary response	1/8 (1/2–1/32)	1/256 (1/32–1/512)

* Numbers in parentheses indicate lowest and highest titres obtained.

Hartley strain guinea-pigs (300–500 g) were immunized intravenously with 2×10^8 sheep red blood cells. In one group of animals, 6–22 days after immunization, 40 ml. of blood from unimmunized guinea-pigs was gradually exchanged for the immunized guinea-pigs' blood in 6–7 ml. increments over a period of 30 min or less. The titre of antibody in pre-exchange serum and serum immediately after exchange transfusion, as determined by haemagglutination of a 1 per cent suspension of sheep red blood cells in phosphate-buffered saline, *p*H 7·4, differed by one to sixteen-fold.

Two to four days after exchange transfusion, the animals were killed, their spleens removed and the number of 19S antibody-forming cells determined by the direct plaque technique[8]. Immunized but unexchanged guinea-pigs sacrificed at comparable intervals after immunization comprised the control group. The results are shown in Fig. 1. Each bar represents a single animal. The number of plaque-forming cells in the unexchanged animals reached a peak after 5 days and rapidly declined. Animals exchange-transfused 2–4 days before sacrifice had from three–ten times as many plaque-forming cells as comparable unexchanged animals.

The results of these studies indicate that removal of serum antibody by exchange transfusion from 6–22 days

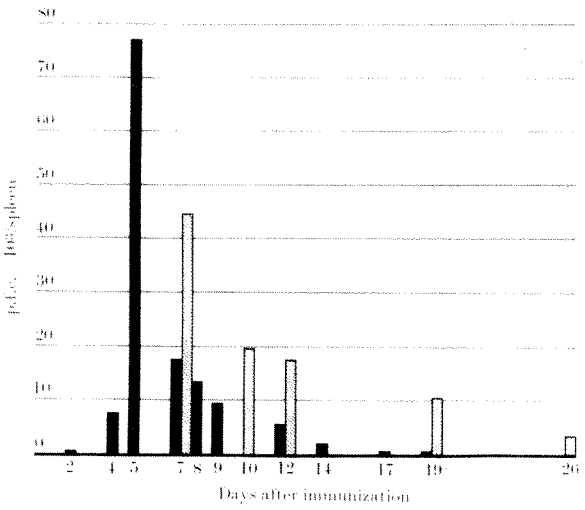


Fig. 1. Black columns, unexchanged; hatched columns, exchange transfusion.

after immunization causes an increase over the expected number of direct plaque-forming (19S antibody-producing) cells in the spleen and supports the concept of a direct feed-back control of the proliferation of 19S antibody-producing cells.

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Embryonic Mouse Peripheral Blood Colony-forming Units

HAEMOPOIETIC stem cells, characterized by their ability to differentiate and reproduce themselves, are believed to be precursors of all differentiated blood cells in the organism. During embryogenesis, they are presumably present in the yolk sac, liver, spleen and marrow—the successive sites of erythropoiesis. The stem cells may be derived from a common source or they may arise independently in each site during development. If they are not indigenous to the erythropoietic organ they may be either transported from one site to another through the peripheral blood[1] or incorporated into the developing organ in tissues shared by the previous erythroid site[2,3].

Cells with the characteristics of stem cells can be detected by the spleen colony technique[4]. Spleen colonies derived from stem cells either isolated from[5] or migrating in[6] the peripheral blood have been detected. Spleen colonies found after inoculation of embryonic peripheral blood into lethally irradiated CBA/H (ref. 7) and C57BL/6J mice suggest the possibility that stem cells use this route during development to migrate to successive sites of erythropoiesis.

All C57BL/6J mice used as donors and recipients were obtained from the Jackson Laboratory. Hormonally primed C57BL/6J females[8], mated to C57BL/6J males, were considered to be at zero day of pregnancy on the day when a copulation plug was found. Each donor (12, 14, 16, or 18 day embryo or newborn mouse) was placed in a Petri dish containing 1 ml. of a 1:1 mixture of ice-cold phosphate buffered saline[9] and 1 per cent heparin. They were decapitated and allowed to bleed freely. Blood cell number was determined by counting 0·1 ml. of blood diluted to 50 ml. with 0·9 per cent saline with a Coulter counter model B. The total number of blood cells in an embryo was determined by multiplying cell counts per ml. by the total number of millilitres of blood plus diluent. The volume of blood cell suspension remaining after counting was measured and injected intravenously less than 1 h after collection into lethally irradiated female recipients[10]. Survivors were killed 10 days later and the macroscopic colonies counted.

Table 1. NUMBER OF COLONY FORMING UNITS IN FOETAL AND NEWBORN BLOOD

Days gestation	Total CFU/embryo	Approximate No. of blood cell × 10^7 per embryo	Standard ± error	CFU/10^7 blood cells
12	4·56	0·6	0·01	7·60
14	1·49	1·4	0·06	1·06
16	1·66	11·6	0·47	0·14
18	2·32	15·7	0·60	0·15
Birth	6·65	20·4	0·60	0·33

Stem cells seem to be present in the circulating embryonic blood at all the stages of gestation examined (Table 1). Larger absolute numbers and larger proportions of stem cells were seen in blood of 12 day foetuses than in 14–18 day foetuses. At birth, more colony forming units (CFU) per animal have been detected than at any other stage. To determine the comparative number of stem cells in a unit volume of injected blood, the number of CFU in 10^7 cells was calculated. It seems that, although there are more CFU per embryo at birth, there are probably fewer CFU per unit volume of blood than at 12 days gestation in C57BL/6J mice.

The possibility that peripheral cells or cell products in the injected blood induced formation of endogenous colonies in the lethally irradiated host[11] has also been investigated. Foetal C57BL/6J liver cells, 14 days gestation, which include CFU[10], were diluted to 10^6 cells per ml. either with 0·9 per cent saline or with noncomplicating peripheral blood cells from adult WBB6F_1-W/W^v mice (known to have only 1/200 of the normal ability to produce spleen colonies[12]) in saline. The same number of CFU was detected in hosts whether or not the diluent accompanying foetal liver implants contained W/W^v erythrocytes.

The demonstration of erythroid stem cells in embryonic and newborn mouse blood suggests that during development stem cells can use this pathway to migrate from one haemopoietic site to another. Our experiments have not eliminated the possibility that some stem cells may originate intrinsically in each haemopoietic organ. Also yolk sac stem cells may become incorporated directly into the developing foetal liver during embryonic rotation[2,3].

Evidence has been presented that the yolk sac contains stem cells[3] which are multipotential and can populate all known adult haemopoietic organs in the lethally irradiated mouse[13]. Stem cells originating in the yolk sac may, in fact, be the original source of all haemopoietic stem cell progeny in the organism[13]. It is interesting that in the present study higher proportions of circulating stem cells were seen at 12 days, when the liver may have been adding stem cells[10], than at 14–18 days, when liver haemopoiesis was at its maximum level[14].

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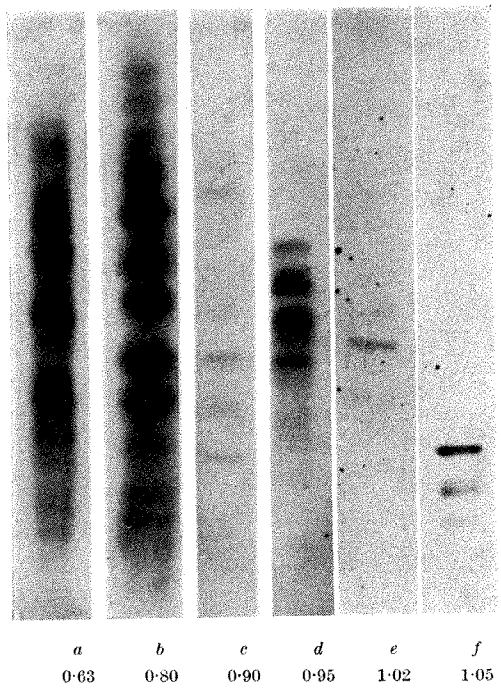


Fig. 2. Isoelectric spectra and heterogeneity indices of antibodies to DNP. Isoelectric focusing of sera was carried out using pH 5–8 ampholine carrier ampholytes in thin layer polyacrylamide gels[11], and anti-DNP components were visualized, after interaction with ^{131}I-DIHP-DNP-lysine, by autoradiography (A. R. W., in preparation). The details of immunizing regime and time of bleed for each rabbit were as follows: *a*, *c* and *d*, neonates primed with 0·1 mg, DNP_9-BGG with incomplete Freund's adjuvant; bleed taken 13, 9 and 5 days after boost 11; ages 54, 50 and 46 days respectively; *e*, a neonate primed with 0·1 mg DNP_9-BGG with incomplete Freund's adjuvant; bleed taken 8 days after boost 1; age 23 days: *b*, a rabbit primed with 1 mg DNP_9-BGG in saline on day 26; boosted with 2 mg DNP_9-BGG in saline on day 45; boosted with 2 mg DNP_9-BGG with complete Freund's adjuvant on day 97; bleed taken 10 days after boost 11; age 107 days: *f*, a murine anti-DNP antibody selected in CBA mice by cloning of antibody-forming cells[16]. The heterogeneity index was measured for each antiserum by a hapten-binding procedure[12]; each value represents the average of at least three determinations.

Molecular Restriction of Anti-hapten Antibody elicited in Neonatal Rabbits

THE origin of the diversity of antibody combining sites is one of the crucial questions of immunology. Many workers, hoping for evidence from structural studies of homogeneous antibodies, have attempted to elicit antibodies with restricted heterogeneity[1–6]. Another approach to the problem is to study the ontogeny of antibody diversity[7–10]. These and other approaches have been hindered by inadequate criteria for judging antibody heterogeneity. Techniques for the direct analysis of isoelectric heterogeneity of specific antibodies (A. R. W., in preparation) have facilitated the search for homogeneous antibodies and made it possible to study developmental aspects of diversity. These two approaches are combined in the studies we report here on the anti-hapten antibodies produced by neonatal rabbits. We have found that in the initial response to a heterogeneous presentation of hapten, neonatal rabbits frequently produce anti-hapten antibodies with restricted isoelectric spectra. The term "restricted" is used relative to the spectra of antibodies produced in adult animals. In certain neonates, the antibody spectrum resembled that characteristic of a single clone (monoclonal antibody).

New Zealand white rabbits were injected intramuscularly with DNP_9-BGG within 11 h of birth unless otherwise stated. The dose of antigen and the presentation (saline, incomplete or complete Freund's adjuvant) were varied. Animals were bled by heart puncture at 12 days. Subsequent subcutaneous injections of antigen in saline were followed by serial bleedings at 4–5 day intervals (Fig. 1). Aliquots (100 μl.) of each serum were analysed by isoelectric focusing in thin layer polyacrylamide gels[11] followed by detection of specific anti-DNP antibody components by the use of ^{131}I-α-N-(3,5-di-iodo-4-hydroxyphenacetyl)-ε-N-(2,4-dinitrophenyl)-L-lysine (^{131}I-DIHP-DNP-lysine) (A. R. W., in preparation).

A total of 42 rabbits has been examined so far and of these 81 per cent produced anti-DNP antibody either before or immediately after the second presentation of antigen. Antibodies with restricted isoelectric spectra (relative to adult antibodies) were found in 61·5 per cent of the responding neonates. 100 μg DNP_9-BGG in incomplete Freund's adjuvant most frequently elicited a restricted response. Typical isoelectric spectra are illustrated in Fig. 2; spectra were classed as heterogeneous (Figs. 2*a* and *b*), restricted (Figs. 2*c* and *d*) and mono-

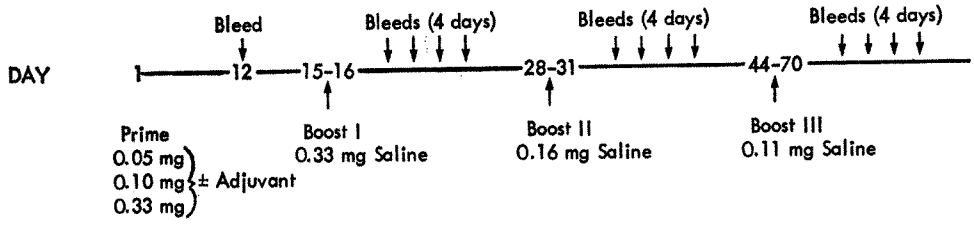


Fig. 1. Injection and bleeding schedule for neonatal rabbits. DNP_9-bovine γ-globulin (DNP_9-BGG) was injected intramuscularly in various amounts as shown, either as a solution in saline or emulsified with either complete or incomplete Freund's adjuvant. The age of the rabbit is given in days. Bleeds were taken at 4-day intervals after each boost injection of DNP_9-BGG.

clonal (Fig. 2*e*). The corresponding values of the heterogeneity index measured by the hapten binding procedure of Farr[12] are shown for comparison. The number of antibody bands seen in an isoelectric spectrum must be interpreted in terms of the multiple band patterns characteristic of monoclonal G-immunoglobulins; the spectrum of an IgG anti-DNP produced by a single antibody-forming cell-clone selected in CBA mice is shown in Fig. 2*f*. It has been shown for a G-myelom protein that the multiple band spectrum is a result of post-synthetic modifications of biosynthetically homogeneous protein (that is, the translation product of single mRNA sequences for light and heavy chains)[13]. There is an approximate correlation between the complexity of the isoelectric spectrum and the heterogeneity index of a given antiserum. This relationship does not necessarily hold in an exact way because the isoelectric spectrum yields both the number of different molecules and their proportional representation whereas the heterogeneity index is a complex function of these parameters with the spread of affinity constants.

The patterns of restriction observed in littermates have so far shown no interpretable similarities. Even from the small number of cases so far studied we can say that there is not a simple genetic control on the heterogeneity of antibodies produced early in life. From further study of littermates and litters from matings of the same parents (as well as back-cross) a pattern of control may emerge.

The persistent production of antibodies with the same simple isoelectric spectra by certain rabbits is of particular interest: it is important both from the standpoint of isolation of workable quantities of biosynthetically homogeneous antibody (the total antibody concentration in the sera of rabbits mounting a restricted response ranged from 50–250 μg/ml.) and in relation to the cellular control of antibody production. The course of immunization and the persistence of the restricted isoelectric spectrum of antibodies in one particular rabbit is shown in Fig. 3. The isoelectric spectra of antibodies after boosts 1 and 2 are strikingly similar. After boost 3 the spectrum shows a general resemblance to boost 2 antibody though there are several additional acidic lines. The heterogeneity index of hapten binding for boost 2 antibody (0·95) was consistent with the restricted isoelectric spectrum. But the heterogeneity index remained unchanged (0·95) for boost 3 antibody despite the more complex spectrum; this illustrates the extra sensitivity of isoelectric focusing as an assay for antibody heterogeneity.

The presence of similar antibody bands in each isoelectric spectrum (Fig. 3) indicates that antibody production by several individual clones has persisted up to 92 days during the injection schedule indicated. There is an increase in antibody levels after each boost so it is necessary to invoke recruitment of further specific precursor cells which upon antigenic stimulation yield clones of plasma cells continuing production of the particular molecular species of antibody. All rabbits studied eventually produced a heterogeneous antibody response to DNP-BGG, so precursor cells for clones producing other antibody species must be present or arise during the course of the experiment. The continued restriction on the heterogeneity of antibody production suggests a preferential generation of precursor cells for those clones originally proliferating. Production of antibody by the original clones may continue much longer than recorded here but our observations are limited by the onset of the heterogeneous response which would obscure the presence of the initially simple pattern.

Evidence from other sources has suggested that there may be long term continuity of similar antibody molecules. The continued production during a six-month immunization period of anti-*p*-azobenzoate antibodies with similar hapten binding properties has been deduced from light and heavy chain recombination studies[14].

Persistence of idiotypes over periods of up to 29 months has been reported by Oudin and Michel[15] though new idiotypes did arise during this period. An idiotype specificity does not, however, necessarily represent the product of a single clone.

The wide range of different restricted antibody patterns seen in litter mates must be viewed in the light of the significant number of heterogeneous responses in neonates. Taken together these two types of response support the notion that each neonate has extensive genetic potential for anti-DNP antibody production. This potential may be similar to that carried by adult rabbits which have invariably given a heterogeneous response to DNP-BGG

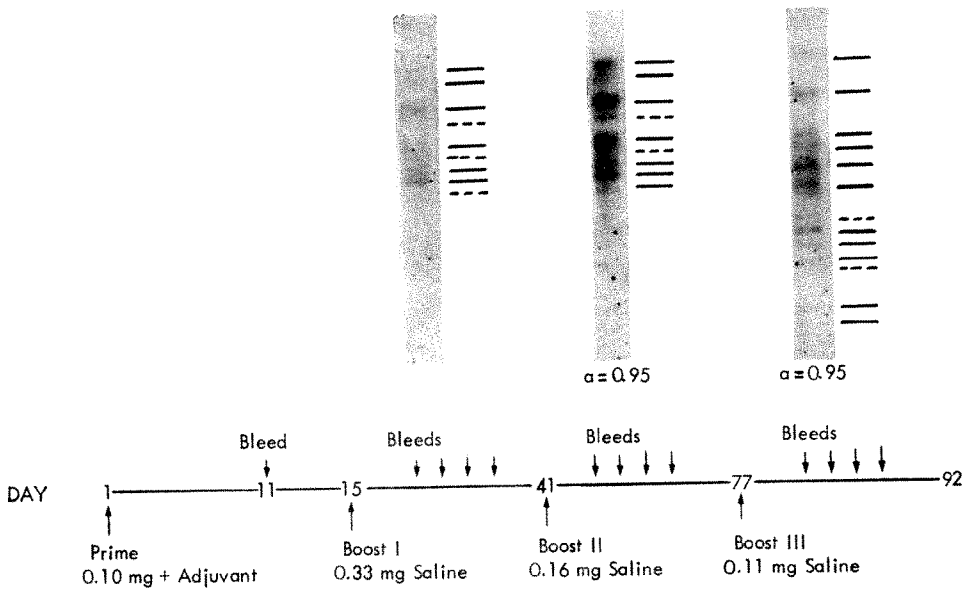


Fig. 3. The isoelectric spectra of anti-DNP antibodies produced in response to successive challenges with DNP_9-BGG. This neonatal rabbit was primed with 0·1 mg DNP_9-BGG and incomplete Freund's adjuvant and subsequently boosted with DNP_9-BGG in saline as indicated. Each serum sample was analysed by isoelectric focusing and antibodies to DNP-hapten visualized by autoradiography after development with ^{131}I-DIHP-DNP-lysine. The spectra and heterogeneity indices (*a*) shown refer to a single bleed in each case. Line drawings of each original autoradiograph spectrum are shown for clarity alongside the photographic reproductions.

even when production of antibody was low. Only 19 per cent of neonates did not respond to DNP-BGG presented a second time, though all surviving animals did eventually respond. It seems that individual neonates have differing capacities to express their extensive pre-existing genetic potential for antibody synthesis.

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Specific *in vitro* Cytotoxicity of Thymus-derived Lymphocytes sensitized to Alloantigens

THE immune response to a specific antigen is characterized by the development of antibody-producing cells and of sensitized lymphocytes active in cell-mediated immunity. Although there is good evidence that antibody-producing cells are bone marrow-derived cells[1-2], the role of thymus-derived cells is less well understood. Development of cell-mediated immunity is impaired in neonatally thymectomized animals[3], and it is therefore likely that sensitized lymphocytes are thymus-derived cells. Spleen cell populations of mice immunized with allogeneic cells contain lymphocytes able to destroy *in vitro* appropriate target cells bearing the sensitizing alloantigens[4-5]. Earlier studies demonstrating the *in vitro* cytotoxic activity of sensitized thymus cells[6] suggested that cytotoxic cells found in immune spleen cell populations were thymus-derived. As shown recently[7-8] mouse thymus-derived cells in the peripheral lymphoid organs carry a surface marker, the θ alloantigen[9], and are lysed when incubated in the presence of anti-θ serum and complement. This study was undertaken to test the effect of anti-θ serum on the cytotoxic activity of spleen cells sensitized to alloantigens. Its effect on alloantibody-producing cells present in the same spleen cell populations was tested in parallel.

Anti-θ C_3H serum was prepared as follows: 3 month-old AKR/J mice received three intraperitoneal injections of 100×10^6 C_3H thymus cells at 3 week intervals. *Haemophilus pertussis* vaccine (1×10^9 organisms/mouse) was added to the thymus cells as adjuvant for the first immunization. The animals were bled 7 days after the last injection and their sera pooled. The specificity of the anti-θ C_3H serum was checked by indirect immunofluorescence using thymus cells from various strains of mice (AKR, C_3H, CBA, C57BL/6, Balb/C and DBA/2): thymus cells from all but AKR mice showed a strong fluorescent staining after treatment in suspension[10] with anti-θ C_3H serum and fluorescinated rabbit immunoglobulin G (IgG) antibody to mouse IgG. Another specificity test for the anti-θ C_3H was to check its absence of effect on haemolytic plaque-forming cells (PFC) present in spleens of mice immunized with sheep red blood cells (SRBC)[11-12]. C57BL/6 mice were injected with 4×10^8 SRBC intraperitoneally and killed 4 days later. Spleen cell aliquots were incubated with anti-θ C_3H serum, or various other antisera, and fresh rabbit serum as the source of complement. After incubation for 30 min at 37° C the cells were washed and tested for PFC according to the technique of Cunningham and Szenberg[13]. As indicated in Table 1, treatment of spleen cells with anti-θ C_3H and complement had no effect on PFC, while DBA/2 anti-C57BL/6 antiserum completely prevented the formation of PFC.

Table 1. PFC FROM SPLEENS OF IMMUNIZED C57BL/6 MICE REMAINING AFTER INCUBATION WITH AKR ANTI-θ C_3H SERUM AND COMPLEMENT*

Serum used for incubation†	No. of PFC per aliquot remaining after incubation with serum
Normal C57BL/6	352
C57BL/6 anti-DBA/2	358
DBA/2 anti-C57BL/6	0
Anti-θ	343

* Spleen cells were assayed 4 days after immunization with SRBC.
† 10×10^6 spleen cells in 1·8 ml. were incubated with 0·1 ml. of serum and 0·1 ml. of rabbit complement for 30 min at 37° C.

Previous studies have shown that lymphocytes sensitized to alloantigens could be detected *in vitro* by measuring their cytotoxic activity against ^{51}Cr-labelled target cells carrying the sensitizing alloantigens[4]. The effect of anti-θ antiserum was therefore tested *in vitro* on spleen cells sensitized by transfer into heavily X-irradiated allogeneic recipients[6]. Fifty million C57BL/6 spleen cells were injected intravenously into DBA/2 mice irradiated with 800 rad. Four days later, cell suspensions were prepared from recipient spleens. Spleen cell aliquots (20×10^6 cells) were mixed with AKR anti-θ C_3H serum (final dilution 1:10) and complement (fresh rabbit serum at a final dilution of 1:20) in a total volume of 2·0 ml. After incubation for 30 min at 37° C, the cells were washed and incubated with ^{51}Cr-labelled DBA/2 target cells (P-815-X2 mastocytoma cells)[14] at lymphoid cell–target cell ratios of 50:1 and 10:1. The radioactivity released from target cells was measured after 6 h incubation. The percentage of specific lysis was calculated after correction for ^{51}Cr release from target cells incubated with normal spleen cells. Sensitized spleen cells exposed to normal AKR serum and complement were used as controls. The results of two representative experiments are shown in Table 2. Pretreatment of sensitized spleens cells with anti-θ C_3H serum and complement completely abolished the cytotoxic activity. These results could not be ex-

Table 2. *In vitro* CYTOTOXIC ACTIVITY OF C57BL/6 SENSITIZED SPLEEN CELLS AFTER INCUBATION WITH AKR ANTI θ C_3H SERUM AND COMPLEMENT (C)*

Serum used for incubation of sensitized spleen cells	Lymphoid cell–target cell ratio†	Per cent specific lysis of ^{51}Cr-labelled DBA/2 target cells ‡ Exp. 1	Exp. 2
Normal AKR + C	50 : 1	64	99
	10 : 1	29	64
Anti-θ + C	50 : 1	0	2
	10 : 1	0	2

* Cells obtained 4 days after transfer of 50×10^6 C57BL spleen cells into DBA/2 mice irradiateed with 800 rad.
† Based on cell counts performed before pretreatment of spleen cells.
‡ Target cells incubated with sensitized spleen cells for 6 h.

Table 3. EFFECT OF PRETREATMENT OF C57BL/6 IMMUNE SPLEEN CELLS WITH ANTI-θ C_3H SERUM AND COMPLEMENT ON *in vitro* CYTOTOXICITY AND ALLOANTIBODY PLAQUE FORMATION*

Serum used for pretreatment of immune spleen cells	Per cent specific lysis of ^{51}Cr-labelled target cells†	Antibody PFC per 10^6 immune spleen cells‡
Normal C57BL/6	74	70
Normal C57BL/6 + C	82	70
Anti-θ + C	0	72

* Cells obtained 5 days after transfer of 100×10^6 C57BL/6 spleen cells into heavily irradiated DBA/2 mice.

† DBA/2 target cells incubated in suspension with spleen cells for 6 h at a lymphoid cell-target cell ratio of 30 : 1.

‡ DBA/2 indicator cells incubated with spleen cells in 'agarose' for 1 h, complement added for another hour. Lymphoid cell–target cell ratio and PFC/10^6 spleen cells based on cell counts performed before pretreatment.

plained by simple reduction in spleen cell number. About 35 per cent of the spleen cells were killed by anti-θ serum and complement as tested by dye exclusion; at a lymphoid cell–target cell ratio of 50:1, however, anti-θ treated spleen cells failed to produce any significant activity, whereas control cells were still highly cytotoxic at a ratio of 10:1.

Similar experiments were performed using AKR and C_3H spleen cells sensitized to DBA/2 alloantigens. In this instance, pretreatment with anti-θ C_3H serum and complement had no effect on the cytotoxic activity of AKR sensitized spleen cells, whereas the activity of C_3H sensitized spleen cells was reduced by 80 per cent after such a treatment.

In another set of experiments, the effect of anti-θ serum was tested on immune spleen cells containing both sensitized lymphocytes and alloantibody PFC. Alloantibody PFC were detected with the help of a plaque assay system using as indicator cells the same target cells as employed in the cytotoxic assay system[6]. Immune spleen cells were obtained by transferring 100×10^6 C57BL/6 spleen cells into heavily irradiated DBA/2 mice. Five days later, recipient spleen cells were collected, treated with anti-θ serum and complement as indicated above and tested for the presence of sensitized lymphocytes and alloantibody PFC. Pretreatment with anti-θ serum completely abolished the cytotoxic activity, but had no effect on alloantibody PFC (Table 3).

These results clearly demonstrate that cytotoxic spleen cells, by contrast with PFC, carry the θ-alloantigen. Together with the results demonstrating *in vitro* cytotoxic activity of sensitized thymus cells[6], the data reported here indicate that *in vitro* target cell destruction by lymphocytes sensitized to alloantigens represents a specific activity of thymus-derived effector cells of cell-mediated immunity.

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Use of Transient Optical Rotation for Biopolymer Characterization

THE optical activity of macromolecules has long been recognized as a valuable indicator of certain molecular properties. Studies were initially made of the optical rotation of macromolecular solutions, that is, their ability to rotate the plane of polarization of incident plane-polarized light, at a single wavelength. Measurements were later extended to a variety of wavelengths so as to observe the dispersion of the optical rotation with wavelength, that is, optical rotatory dispersion (ORD).

The optical activity of macromolecules arises from the "short range" atomic asymmetry within the monomeric units and the "gross" asymmetry associated with the overall macromolecular conformation[1,2]. Optical rotation measurements are therefore sensitive to the three-dimensional, relative atomic array of the macromolecule and reveal changes in structure with variation in environmental conditions; for example, the helix-to-coil transition of α-helical polypeptides[3,4], the unfolding of proteins to reveal subunit structures[5], the denaturation of proteins[6], and the aggregation of large molecules[7,8]. Yet the optical rotation method has a great deficiency which is not shared by most other experimental techniques: it provides no measure of the physical dimensions of the solute molecules; it does not distinguish between long and short helices of the same type because they have the same specific rotation.

There is much interest in experimental systems which indicate the physical properties of polymer molecules through relaxation effects. Dielectric dispersion[9], electric birefringence[10], fluorescence depolarization[11], electric field scattering[12], viscoelastic relaxation[13], nuclear magnetic resonance[14], and some flow birefringence experiments[15] fall into this category. Each provides a number of specific molecular parameters, and they have in common the evaluation of a relaxation time (τ). This parameter is especially useful because it can be readily related to the size and shape of the molecule. Another advantage is that the methods are often rapid.

We report here our initial success with a new experimental method which yields both the structural information obtained from ORD experiments and information on the molecular geometry from evaluation of the relaxation time τ. It has the additional potential ability to give such electrical parameters as the permanent and induced dipole moments of the molecules. The latter give further insight into the local molecular structure and form a link between this and the overall molecular size and shape. The procedure and calculation can be very rapid.

Molecular optical activity generally differs along specific axes of a molecule. Thus, the specific rotation associated with the major axis of a helical molecule differs from that of a transverse or minor axis. In solution, the molecules assume random orientations so that the solution itself exhibits no anisotropy in optical rotation. This is the case for conventional measurements with existing commercial polarimeters where some average rotation is recorded. If the solute molecules are partially or completely aligned using an electric, magnetic, acoustic or flow field, for example, the optical rotation changes as the direction of the light beam becomes predominantly associated with a particular axis of the solute molecules. The magnitude of the rotation change is related to the degree of molecular orientation which is a function of the particular molecular property causing the orientation. For electric fields, which form the subject of this work, the molecular dipole moments are responsible. Tinoco and Hammerle[16] have derived equations relating the change in the observed optical activity to the intensity of applied, continuous direct current electric fields. Tinoco[17] has subsequently studied the response of poly-γ-benzyl glutamate solutions to direct current fields of the

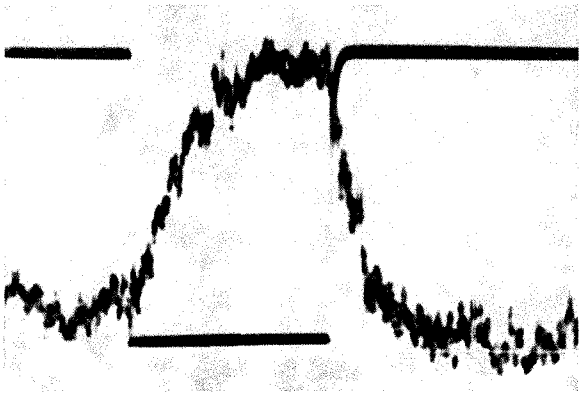


Fig. 1. Typical transient change in the optical rotation. Data for a 2 per cent solution of poly-benzyl glutamate in ethylene dichloride. $E = 1$ kV cm^{-1} and the pulse duration was 10 ms. An incident wavelength of 546 nm was used.

order of 4 kV cm^{-1}. Most of the interest in optical rotation concerns the study of biopolymers which are usually investigated in aqueous or conducting solvents. High voltage direct current fields have serious limitations because of electrophoresis, conductivity and heating effects. Further, the experiments do not enable calculation of molecular relaxation times. Improvements can be made by using alternating current fields or, better, short duration pulsed fields as reported here.

When the electric pulse is initiated the molecules become orientated and aligned but require a finite time to do so that depends on their size and shape. Once orientated, the system remains in this state, but when the field is removed, the molecules revert to a random distribution. The overall transient response in the optical rotation is thus an exponential build-up followed by an exponential decay. The build-up curve depends on two properties: the molecular geometry and the interaction of the applied field with the molecular permanent and induced dipole moments. The decay is, however, the result of brownian disorientation alone and depends only on the molecular geometry. By analogy with other electro-optic effects[18], the decay transient should obey the equation:

$$\Delta\theta = \Delta\theta_0 \exp(-2t/\tau) = \Delta\theta_0 \exp(-6Dt) \qquad (1)$$

where $\Delta\theta$ represents the change in the optical rotation from its residual (or zero field) value at any time t after the field is switched off. $\Delta\theta_0$ is the equilibrium of $\Delta\theta$ at $t = 0$. The rotary diffusion constant D is given by $D = 1/3\,\tau$.

Poly-γ-benzyl glutamate with a molecular weight of 300,000 was obtained from Koch-Light. It was dissolved in ethylene dichloride (a helix promoting solvent) to make a 2 per cent solution and was then filtered through a 5 μm 'Millipore' filter. A Bellingham and Stanley type 'Pepol 60' polarimeter was used to measure the optical rotation of the solution. Changes in the optical rotation can be observed if the field is applied either parallel or perpendicular to the direction of the light beam. Although the greatest effect is observed when the beam is perpendicular to the field[16,19], this condition also gives rise to induced birefringence which is difficult to separate from the optical rotation changes. Birefringence is not encouraged when the light is parallel to the field direction. This arrangement was therefore adopted in these measurements. Three basic modifications were made to the commercial apparatus. First, a special cell was constructed from a solid piece of polytetrafluorethylene. Conducting glass windows were spaced 1 cm apart, which enabled the field to be applied parallel to the light beam. Second, a rapid response and low noise photomultiplier with increased sensitivity was used. Third, a pulse generator was used to deliver pulsed fields of the order of 2 kV across the cell for durations of about 10 ms. The photomultiplier output was displayed on an oscilloscope and photographed. A xenon lamp and a suitable monochromator were used to produce the incident radiation of 546 nm. Full details of the apparatus and its mode of operation will be given elsewhere.

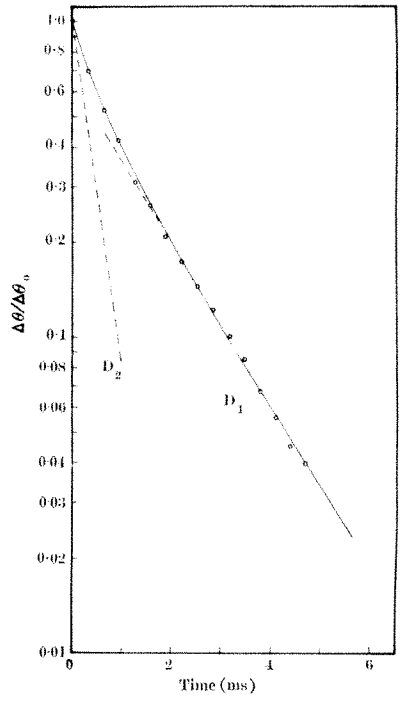


Fig. 2. Analysis of the transient decay. The natural logarithm of the ratio of the amplitude of the decay curve to its maximum value is plotted as the ordinate. The curve is a compound of two relaxation processes characterized by D_1 and D_2.

A typical pulse and transient response is shown in Fig. 1. Fig. 2 shows the analysis of the decay transient of Fig. 1 in terms of equation 1. The graph was not a straight line, indicating a spectrum of diffusion constants associated with the polydispersity of the solute. The transient optical rotation changes were therefore analysed on the basis of two average diffusion constants. The long-time data were fitted with a best line of slope $-6D_1$ to give D_1. The short-time data were then compensated for the contribution from D_1 to leave a second straight line of slope $-6D_2$. Rotary diffusion constants of 101 (± 8) s^{-1} and 400 (± 70) s^{-1} were indicated by this procedure. The analysis was checked using a rapid analogue simulator[20]. The length of the poly-γ-benzyl glutamate helices could be readily obtained from these values of D using Broersma's formula[21]. (The suitability of poly-γ-benzyl glutamate as a rod-like model for this study is discussed in ref. 22.)

To verify the transient optical rotation changes, transient electric birefringence measurements were made independently. The trace was similar to that of Fig. 1 except that it was much less noisy. It corresponded to a single relaxation process with $D = 95\ (\pm 7)$ s^{-1}, similar to the principal component of Fig. 2. It is thus possible that the optical rotation method is more sensitive to polydispersity.

The chief advantage of the transient optical rotation method is that molecular relaxation times can be obtained in addition to conventional optical rotatory dispersion data. Further, the magnitude of the changes when equilibrium has been attained ($\Delta\theta_0$ in equation 1) can be interpreted with Tinoco and Hammerle's equation[16]

to give molecular dipole moments (μ). The additional data (τ and μ) complement those from ORD measurements. Not only should one be able to predict the presence and sense of helical structures from measurements before the application of the electric field, but one should also be able to obtain the helix length from τ and discern a number of prospective helical structures per monomer length in terms of the dipole moment. Optical rotation is already one of the most sensitive indicators of molecular conformation changes. It functions arbitrarily in that the rotation itself is detached from any geometric molecular parameter. Determination of molecular relaxation times and dipole moments therefore should be especially valuable for "mapping" the arbitrary changes in optical activity in terms of specific physical parameters. The suspected molecular changes could be confirmed quantitatively, so providing further insight into the mechanisms and origins of these changes. This should also be true of the molecular aggregations reported from optical rotation studies. Knowledge of τ and the dipole moments should give a clear indication of the nature of the aggregate at various stages throughout its formation or disruption. More specifically, in the case of associating helices, side-by-side, head-to-tail and head-to-head associations should be easily differentiated. The degradation of proteins and their unfolding into subunits may be followed by using these parameters. Measurement of τ alone using the electric birefringence method enabled one of us[23] to demonstrate the unfolding of the subunit structure of bovine plasma albumin as the *p*H of the solvent was varied.



Fig. 3. Transient optical rotation trace using a laser source. Data as for Fig. 1 except that $E = 2$ kV cm^{-1}, and an incident wavelength of 633 nm was used.

Using 'Polaroid' film to record the oscilloscope display of the transient behaviour and the simulator[20] to analyse the exponential response, τ can be recorded within microseconds and evaluated within 1 min of applying the pulse to the solution. Such rapid recording and analysis should be valuable for the study of denaturation and aggregation kinetics. Even more attractive would be the possibility of following various reactions involving enzyme activity of proteins or coenzymes. Finally, the method is suitable whatever the molecular size, so long as the material is optically active. With smaller molecules, however, higher voltages are required for molecular orientation.

There are three apparent disadvantages of the method. First, the sensitivity is low compared with the electric birefringence method. Second, difficulties are encountered arising from strain in the cell windows because of the conducting coating. Third, the response is noisy (see Fig. 1), although it may be improved by using a laser as the light source. For the trace of Fig. 3 we used a System-Computors type G3 helium–neon gas laser of wavelength 633 nm, and a detecting photomultiplier which was not very sensitive to the red light. Although this wavelength is less favourable for optical rotation measurements, the transient is much better than that of Fig. 1. A laser operating at a lower wavelength together with a suitably matched detecting phototube should give a proportionately improved response.

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Relevance of Feedback Inhibition applied to the Biosynthesis of Hexosamines

Negative feedback inhibition has been demonstrated in the biosynthesis of hexosamines, where the end product, UDP-N-acetylglucosamine, potently inhibits the first enzyme of the sequence, L-glutamine D-fructose-6-phosphate aminotransferase (glucosamine-6-phosphate synthetase, EC 2.6.1.16)[1,2]. But to test if UDP-N-acetylglucosamine does indeed function as a physiological controller of the enzyme, two characteristics must be known: the cellular levels of UDP-N-acetylglucosamine and the response of the enzyme to the postulated modifier.

Expressed on the basis of cell water, the concentration of UDP-N-acetylglucosamine in neonatal rat skin was determined by Hardingham and Phelps[3] as 0·22 mM and has been variously quoted for rat liver[4–8] as 0·3–0·8 mM, the mean value being 0·55 mM. This concentration has been reported to remain virtually constant for 8 h (ref. 4).

L-Glutamine D-fructose-6-phosphate aminotransferase was purified 400 times from rat liver and from the skins of 1 day postpartum rats by the procedure of Winterburn[9], so utilizing the protective action of the substrate, fructose 6-phosphate. The activity of the enzyme was measured by assaying for formation of glucosamine 6-phosphate by a modification of the Morgan–Elson method[10] after incubation at *p*H 7·5 and 37° C in the presence of non-limiting concentrations of the substrates[9]. Because some regulatory enzymes become desensitized to ligands during purification, the ligand response characteristics

(substrates and modifiers) of the glucosamine 6-phosphate synthetase were checked at each stage of the purification and found to be constant. The properties of the purified enzyme seemed to approximate to those observed *in vivo*.

Studies of the effect of UDP-N-acetylglucosamine on the activity of the enzyme revealed that besides being highly inhibitory (87 per cent at 0·4 mM) the rate of formation of glucosamine 6-phosphate was virtually independent of the concentration of inhibitor at levels greater than 0·3 mM. Varying the concentrations of UDP-N-acetylglucosamine and the substrates showed this inhibition to be non-competitive for fructose 6-phosphate and uncompetitive for glutamine (using the nomenclature of Cleland[11]), the K_i values being collectively about 40 μM. In the presence of UDP-N-acetylglucosamine, glutamine displayed substrate inhibition characteristics. Thus three attributes —(*a*) the highly inhibitory non-varying cellular concentration of UDP-N-acetylglucosamine, (*b*) the substrate inhibition by glutamine in the presence of UDP-N-acetylglucosamine and (*c*) the K_m for fructose 6-phosphate (0·11 mM) situated within the fluctuating cellular level of this metabolite (0·04–0·14 mM) (ref. 12) suggest that here feedback inhibition constitutes an inefficient and inflexible way of controlling a biosynthetic pathway.

Table 1. EFFECT OF AMP, GLUCOSE 6-PHOSPHATE AND UTP ON THE ACTIVITY OF THE ENZYME IN THE PRESENCE AND ABSENCE OF UDP-N-ACETYLGLUCOSAMINE

Additions	Activity (μmole/mg protein/h)	Activity in presence of 0·05 mM UDP-N-acetylglucosamine	Percentage inhibition
	Experiment 1		
None	0·86	0·61	29
+0·5 mM AMP	0·80	0·51	36
+2·0 mM AMP	0·75	0·40	47
+1·25 mM UTP	0·92	0·80	13
+2·5 mM UTP	0·89	0·86	3
	Experiment 2		
None	0·65	0·43	34
+0·25 mM glucose 6-phosphate	0·63	0·36	43
+1·0 mM glucose 6-phosphate	0·63	0·35	45

Each incubation contained: 50 mM Tris, 1 mM EDTA, 100 mM KCl, 3 mM fructose 6-phosphate, 6 mM glutamine adjusted to *p*H 7·5 in a final volume of 0·6 ml.; other additions were made as indicated. In experiment 1 each incubation contained 60 μg protein; in experiment 2, 70 μg. Incubations were for 1 h at 37° C.

A search for possible cellular metabolites which might affect the further regulation of this pathway revealed that physiologically significant levels of AMP, glucose 6-phosphate and UTP[3,12] modified the activity of the enzyme, but only in the presence of UDP-N-acetylglucosamine. As shown in Table 1, AMP and glucose 6-phosphate potentiated the inhibitory action of UDP-N-acetylglucosamine while UTP behaved as an activator. Further investigation showed that AMP (Fig. 1) acted by decreasing the apparent K_i for UDP-N-acetylglucosamine—leading to increased inhibition (glucose 6-phosphate behaved similarly to AMP); while UTP (Fig. 2) increased the apparent K_i—leading to a relief of inhibition. The effects evoked by AMP and glucose 6-phosphate were especially interesting because these metabolites have been implicated in the regulation of carbohydrate metabolism.

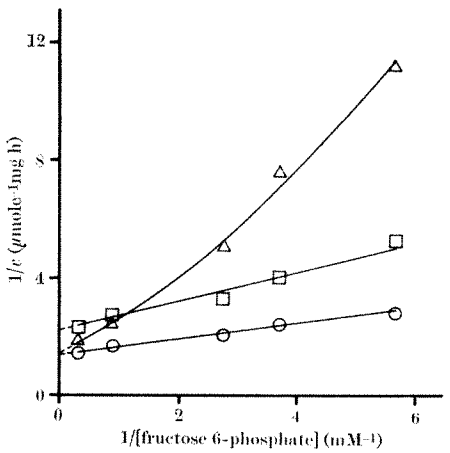


Fig. 1. Each 1 h incubation contained: 50 mM Tris, 1 mM EDTA, 100 mM KCl, 6 mM glutamine and 25 μg enzyme adjusted to *p*H 7·5 in a final volume of 0·6 ml. The additions were: ○, 1 mM AMP; □, 0·025 mM UDP-N-acetylglucosamine; △, 0·025 mM UDP-N-acetylglucosamine+1 mM AMP.

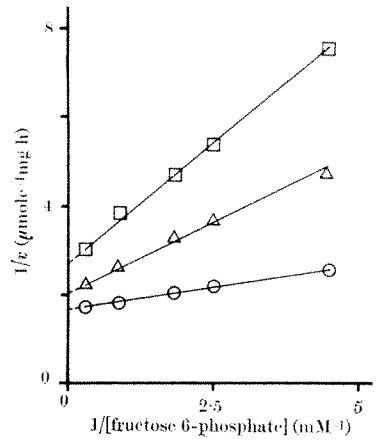


Fig. 2. Each 1 h incubation contained 50 mM Tris, 1 mM EDTA, 100 mM KCl, 6 mM glutamine and 40 μg enzyme adjusted to *p*H 7·5 in a final volume of 0·6 ml. The additions were: ○, 1·5 mM UTP; □, 0·05 mM UDP-N-acetylglucosamine; △, 0·05 mM UDP-N-acetylglucosamine + 1·5 mM UTP.

In the cell, therefore, assuming no marked compartmentation in the distribution of these effectors, the enzyme should be combined with its inhibitor giving rise to the prediction that in the intact cell the full potential activity will not be realized, and that the "normal" activity is that displayed by a heavily inhibited enzyme. Hardingham and Phelps[3] calculated that in neonatal rat skin the flux through the hexosamine pathway was 0·09 μmole/g wet weight/h. Careful extraction of glucosamine 6-phosphate synthetase from this tissue revealed that this enzyme has a potential maximal activity of 1·0 μmole/g wet weight/h (ref. 9). In rat liver, Spiro[13] found the hexosamine flux to be 0·1 μmole/g wet weight/h whereas the calculated maximal activity of the synthetase in this tissue is 2 μmole/g wet weight/h (ref. 9). In these different tissues, therefore, there was an order of magnitude discrepancy between the potential and realized rates, suggesting that the prediction regarding the heavily inhibited enzyme may be basically correct.

We propose that the primary control on the flux through the hexosamine pathway is at the level of the glucosamine 6-phosphate synthetase. This control is not by a fluctuation in the UDP-N-acetylglucosamine concentration but is through an alteration in its binding constant evoked by the other modifiers. A secondary control in this pathway by N-acetylglucosamine 6-phosphate inhibiting either the acetyltransferase or glucosamine 6-phosphate synthetase as suggested by Kent[14] is at present under investigation. Possibly UDP-N-acetylglucosamine should be regarded as an "antifactor" instead of its present classification as a feedback inhibitor.

This type of control mechanism, a straightforward extension of the present theories on the mode of action of modifiers, although here advanced for the case of hexosamine biosynthesis, may be far more prevalent in other metabolic pathways than at present envisaged.

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Protein Degradation and Synthesis in Amino-acid Deficiencies

IT is well known that most body proteins are continually degraded and resynthesized. The protein content of various organs must represent the net balance between synthesis and degradation over some period of time. Nutritional or other factors which influence the protein content of tissues might act on either protein synthesis or degradation or both.

Most effort has been directed toward studies of the rates of protein synthesis using isotopically labelled amino-acids. The difficulties in interpreting such studies are well known[1–3]. These include the assumption that the amino-acids are uniformly distributed in an "amino-acid pool" (in fact, there are apparently multiple pools[4,5] which one may not be able to sample), and that a "steady state" exists. Many of the situations in which one wants to investigate the rates of protein synthesis or degradation are precisely those in which a "steady state" does not exist: for example, protein deficiency. But if amino-acids can be incorporated into proteins which are then broken down, and re-enter the "pool" to be reused, some of the usual assumptions seem doubtful, especially if this takes place on a local, perhaps even intracellular, basis.

In the technique devised by Goldberg[6–8] animals were injected with a labelled amino-acid and after the tissue proteins were labelled the animals were given an excess of the amino-acid by injection as well as a high protein diet. This technique would minimize the re-use of the labelled amino-acids and loss of label from the protein would then be a measure of protein degradation. Protein synthesis could be estimated from the total protein content of the tissue after correction for degradation.

We have used a similar technique to investigate the effects of specific amino-acid deficiencies on tissue protein degradation and synthesis. The studies were done with young adult female rats weighing approximately 200 g. They were fed a purified diet which contained a mixture of amino-acids as the sole source of protein. After one week on the diet they were injected with approximately 3·5 μCi of a ^{14}C labelled amino-acid and continued on the diet for an additional week. This allowed time for the tissue proteins to become labelled and for the activity of the amino-acid pool to fall to rather low levels. One group of animals was then killed for tissue analysis and the remaining animals fed the appropriate diets.

Data obtained from animals which had received a previous injection of L-^{14}C-lysine (New England Nuclear) and were then fed diets in which the lysine level varied from zero to 0·45 per cent over a six week period are shown in Table 1. The activity in the various tissues decreased with increasing levels of dietary lysine up to 0·3 per cent but the diet containing 0·45 per cent did not produce a further decrease. We interpret this to mean that levels of dietary lysine above 0·3 per cent are sufficient to dilute the lysine pool so that reincorporation of radiolysine is practically eliminated. A similar experiment done with animals injected with L-^{14}C valine and then fed several levels of valine produced similar results. A level of 0·22 per cent valine in the diet appeared to prevent reincorporation of labelled valine into the tissues.

Table 1. TISSUE RADIOACTIVITY

Tissue	Dietary levels of lysine 0	0·15	0·30	0·45
	Per cent			
Plasma, d.p.m./mg N	1,052	830	594	616
Muscle, d.p.m./mg N	997	818	547	565
Liver, d.p.m./mg N	852	679	484	443
Kidney, d.p.m./mg N	808	585	396	386
Small intestine, d.p.m./mg N	692	523	285	313

Radioactivity of various tissues from animals injected with L-^{14}C-lysine and then fed diets containing various amounts of lysine for 6 weeks.

Table 2. LIVER PROTEIN DEGRADATION AND SYNTHESIS

Dietary valine	Total liver nitrogen	Total liver counts	Difference Nitrogen	Difference Counts	Total degradation	Total synthesis
Per cent	mg	d.p.m. × 10^{-3}	mg	d.p.m. × 10^{-3}	mg N	mg N
Control, 0·07 (zero day)	224·0	109·3	—	—	—	—
0·00 (6 weeks)	171·0	20·8	−53·0 (24·0%)	−88·5 (80·0%)	181·5	128·0
0·07 (6 weeks)	200·0	21·6	−24·0 (10·7%)	−87·8 (80·0%)	179·9	155·8
0·15 (6 weeks)	231·0	23·0	+6·5 (2·9%)	−86·4 (79·0%)	177·0	183·6

Animals labelled with ^{14}C-lysine; 6 weeks on diet.

In the next experiment the animals were injected with labelled lysine as before and then fed a diet containing 0·45 per cent lysine but with either 0, 0·07 or 0·15 per cent valine. Data on the liver protein are shown in Table 2. In the animals killed at the beginning of the experiment (zero day) the average liver contained 224 mg of total nitrogen and 109,300 c.p.m. After six weeks on the experimental diets, approximately 88,000 c.p.m. had disappeared (80 per cent of the original counts) irrespective of the level of dietary valine. We therefore calculate that during this time approximately 80 per cent of the original liver protein or 180 mg of nitrogen had been degraded. The change in total liver protein, however, was substantially less and this difference is a measure of the total liver protein synthesis during the six week period. The total liver protein nitrogen synthesized is shown in the last column. The synthesis of new liver protein was decreased by the complete valine deficiency but was still substantial. At the highest level of dietary valine, which allowed some growth in the animals, synthesis slightly exceeded the total protein degradation.

A similar experiment was done in which the animals were injected with labelled valine and then fed the diet containing 0·22 per cent valine but with various levels of dietary lysine. The data from the two experiments on the rates of protein degradation were similar. We calculate a mean liver protein degradation in six weeks of $79·6 \pm 1·4$ per cent compared with $80·1 \pm 1·2$ per cent in the previous experiments. The total degradation of kidney protein in the two experiments was 72·2 per cent and 69·2 per cent and of the total plasma proteins (calculated from an assumed plasma volume of 4·1 per cent of the body weight) was 80·4 per cent and 71·5 per cent. In each tissue examined, there was no evidence that the rate of tissue protein degradation was influenced by the level of valine or lysine in the diet, whereas the rate of synthesis was directly related to the level of these amino-acids supplied.

We therefore conclude that deficiencies of either lysine or valine and presumably other essential amino-acids do not stimulate the breakdown of tissue proteins and that the changes in protein content that do occur are due to different rates of protein synthesis.

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Changes in the Properties of Reconstituted Collagen brought about by Mechanical Treatment

GROSS has shown that the decreasing ability of a collagen gel to pass back into solution when the *p*H or the temperature is lowered represents an ageing effect in *in vitro* collagen gels[1]. These alterations in physical properties may be a result of the formation of covalent cross linkages between oxidized lysine residues in one tropocollagen molecule and free lysine ε-amino groups in adjacent ones[2]. We have shown that similar changes in physical properties and alterations in the enzyme susceptibility of the precipitated collagen are caused by the application of cyclical mechanical stress to the collagen during the period of precipitation.

Polymeric collagen was obtained from three year old bovine achilles tendon by exhaustive extraction with 5 M NaCl to remove all the soluble proteins[3] and prolonged incubation with crude α-amylase. After dispersion in acetic acid and reprecipitation with NaOH, a solution was prepared in 0·2 M acetic acid containing 75 mg protein/100 ml., measured by the method of Lowry *et al.*[4] using gelatine as standard. This material conformed to the standards of purity suggested by Grant and Jackson[5] by having less than 1 mole of hexosamine and 6 moles of hexose per ninety-four residues of hydroxyproline. This solution was slowly neutralized by exposure to an atmosphere of moist ammonia at 20° C for 16 h. The precipitated gel, consisting of fine fibrils up to 2 mm in length, was shaken with acetic acid to determine the reversibility of the "ageing" process. After shaking for 5 h with 5 ml. of 0·2 M acetic acid, 46·6 ± 8·4 per cent of the gel prepared from 0·2 ml. of the collagen solution redissolved. Other samples of precipitated collagen were subjected to cyclical alterations in mechanical stress. These were produced by a specially designed piece of apparatus in which the collagen is stretched by precipitation in a nylon mesh, the area of which is extended by 33·6 per cent every 8 min by a piston. Collagen (0·2 ml. of 0·075 per cent solution) was applied to the centre of the nylon; the piston was set in motion, and the vessel containing the piston and mesh was filled with a mixture of nitrogen and moist ammonia. The rate of passage of the ammonia was regulated so that precipitation of the collagen fibres, as indicated by the appearance of opacity in the liquid in the nylon mesh, appeared after 3 h. A similar amount of collagen solution supported on an unstretched mesh was exposed to the same alkaline atmosphere in the body of the vessel at the same time.

Sixteen hours after the initial application of the collagen solution, disks of fabric 2·5 cm in diameter were cut from the stretched and unstretched samples. In one series of experiments the two separate pieces of fabric with their entrained collagen precipitates were shaken for 5 h in 5 ml. of 0·2 M acetic acid. In the other series they were divided into two equal portions; one was incubated in 5 ml. 0·2 M sodium acetate buffer (*p*H 7·8) containing 0·01 M calcium chloride and 100 μg of *Clostridium* collagenase, and the other was incubated in 5 ml. 0·15 M borate buffer (*p*H 8·4) containing 100 μg pancreatic elastase[6]. Both preparations were incubated at 37° C for 3 h and, after filtration, the solubilized protein was measured with a biuret reagent[7].

In contrast to the unstretched sample, only 15·0 ± 2·3 per cent of the stretched precipitate redissolved in acetic acid, a difference which is significant at the $P = 0{\cdot}005$ level. A 34 per cent stretch decreased the collagenase susceptibility of the precipitated fibres (measured in arbitrary units) from 56 to 42, whereas the elastase susceptibility increased from 8 to 17, an increase of 112 per cent. This change in relative susceptibility is significant below the $P = 0{\cdot}01$ level. It therefore seems that mechanical stress has two effects on neutralized collagen fibre: the fibrous gel is rendered less extractable with acetic acid and the susceptibility to digestion by elastase is increased whilst that by collagenase is decreased.

Keech[8] demonstrated (by electron microscopy) that the appearance of collagen gels changes considerably when it is stirred vigorously shortly after precipitation. Even the less drastic treatment used here may also affect the physical state of collagen. A reduction in solubility might indicate that an increasing number of cross links are formed. This could be attributed to an alignment of the molecules that brings the reactive centres in adjacent peptide chains sufficiently close to one another for reaction to take place. On the other hand, the changes which are observed in the relative enzymic susceptibility seem to necessitate exactly the opposite situation, namely one of denaturation and disorientation: Hall *et al.*[9] and Banga and Balo[10] have demonstrated that denatured collagen is more susceptible to elastase than native collagen. This contradiction can be rationalized, however, if one assumes that the stretching alters the spatial relationships of carboxyl and hydroxyl groups in the collagen molecule in a fashion that renders them capable of attracting and binding elastase, similar to that which occurs in elastin[11].

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Increased Recovery of Anaesthetized Hypothermic Rats induced by Intracarotid Infusion

WE have shown previously that if rats were cooled to cardiac arrest without assisted ventilation, held thus for 30 min, and rewarmed with artificial ventilation, only 9 per cent of them recovered[1,2]. This incidence could be increased to 45 per cent if their abdomens were pumped

before rewarming[3,4]. In these experiments, if the abdominal pumping had failed to restart the heart, spontaneous respiration did not subsequently recover. We now report new measures which further increased the incidence both of the hearts restarting and the recovery of spontaneous respiration.

Thirty rats were anaesthetized without assisted ventilation, cooled until their hearts stopped, and left cooled in cardiac arrest for 30 min; their hearts were then excised and perfused with Eagle's solution on a Langendorff apparatus at 37° C. All resumed beating. A further series of seven rats were similarly cooled to cardiac arrest, and rewarmed for 15 min with artificial ventilation. When it was seen that their hearts had failed to restart, they were excised, and six of them restarted on perfusion. It was therefore concluded that during cooling changes in the chemistry of the coronary arterial blood occurred, which prevented the hearts from restarting, but that the cardiac muscle still had the potential to contract. Thus if the coronary arterial blood could be replaced *in vivo*, more rats might recover.

Centripetal injection of 2 ml. of dilute methylene blue in isotonic saline into the right jugular veins or right carotid arteries of each of six rats demonstrated that the coronary arteries were perfused by the latter route only. Two ml. of Eagle's solution were therefore injected intra-arterially in thirty-one cooled rats; twenty-six of them (84 per cent) restarted spontaneous respiration after abdominal pumping, and rewarming with artificial ventilation. In similar conditions, isotonic saline injected into the carotid arteries of seventy-one rats caused fifty-six of them (79 per cent) to respire. In both cases, the incidence of recovery was significantly greater than with abdominal pumping alone ($P < 0.001$). But injection of saline into the jugular veins of thirty-six rats followed by abdominal pumping caused eighteen of them (50 per cent) to recover, which was not significantly different from the incidence of recovery after abdominal pumping alone.

Thus whereas in previous experiments only 9 per cent of the cooled unventilated rats recovered spontaneous respiration on rewarming, the further measures of centripetal intracarotid injection of Eagle's solution or isotonic saline, as well as abdominal pumping, enabled 79 per cent to 84 per cent to recover. These findings may be relevant to resuscitation after cardiac arrest in man.

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Mechanism of Toxicity of Injected CS Gas

IN 1928 Corson and Stoughton[1] prepared a number of benzylidene malononitriles, including 2-chlorobenzylidene malononitrile (CS). Although these authors were primarily concerned with the isolation and chemistry of the condensation products of malononitrile and various aldehydes, sternutatory and irritant properties on the part of some of the compounds were reported. CS is now the active material in a wide range of devices used against civil disturbances and in war (see, for example, refs. 2–6). Benzylidene malononitriles are reactive substances, and have been shown to form adducts with amines[7–10], *n*-butanethiol[10–12], Grignard reagents[13,14], hydrazoic acid[15], hydrogen cyanide[1,15–17], salts of certain transition elements[18,19], and trialkylphosphines[20–22]. Reactions also occur with hypochlorite[23–24], water[1,9,17,25,26], oxidizing[1] and reducing[7,27,28] agents. Certain aspects of the physiological effects[2,7,8,29–32] and toxic properties[23,33–38] of CS have also been studied. We here describe a brief investigation into the mechanism of toxicity of CS. Because elevated levels of thiocyanate have been found in the blood of human patients treated with 4-nitrobenzylidene malononitrile[39], and because malononitrile is one of the products when CS is hydrolysed in aqueous ethanol[9], the toxicities of cyanide and malononitrile have also received attention.

Various benzylidine malononitriles, including CS, were synthesized by way of the base-catalysed condensation of malononitrile with the appropriate aldehyde[1]. CS itself was administered exclusively in solution in arachis oil (5–20 mg/ml.); other derivatives were given in either oil (0·19 mmoles/kg body weight) or dimethyl sulphoxide. Aqueous solutions of malononitrile (4–10 mg/ml.), potassium cyanide (0·5 mg CN/ml.) and sodium thiosulphate (50 mg pentahydrate/ml.) were prepared just before use. The compounds were administered to adult male or female mice (LAC strain) by intraperitoneal injection.

LD_{50} values were derived graphically[40,41] (Table 1). The figure for malononitrile is in good agreement with the previously reported value of $12{\cdot}75 \pm 0{\cdot}39$ mg/kg[42], by the same route of administration. Both the symptoms and the onset of poisoning by CS and malononitrile were very similar at dosages of comparable molarity. Different degrees of intoxication were noticed within 5–15 min of the injection of sublethal amounts of either compound, depending on the dose. The animals panted vigorously, and were soon gasping for breath. When lethal amounts were injected, the manifestations were more severe, and culminated in a loss of consciousness. The onset of panting and gasping was more rapid, but both phases could still be clearly distinguished. The rate of gasping decreased until death ensued, generally 15–60 min after injection. Convulsions were observed in a minority of animals.

Symptoms of cyanide poisoning showed some similarities with those of CS and malononitrile, provided that the dose did not exceed 4–4·5 mg/kg; above these levels death occurred rapidly. At dosages of 2·5–3·5 mg/kg the animals collapsed within 1–2 min of injection, and were briefly convulsed. Although the panting phase was difficult to discern, gasping was readily apparent. In those animals which recovered, the rate of gasping gradually increased; a phase of violent shivering very often set in, which persisted for up to 10 min. Animals which failed to recover usually died within 6–25 min of injection.

Table 1. TOXICITIES OF CS, MALONONITRILE AND CYANIDE

Agent	LD_{50} ± standard error, mg/kg body wt.	LD_{50} ± standard error, mmoles/kg	Number of animals used
CS	35 ±1·2	0·186 ± 0·006	70
Malononitrile	12·9 ± 0·6	0·196 ± 0·009	72
Cyanide (as CN)	3·0 ± 0·09	0·115 ± 0·004	70
2-Chloro-β-cyanocinnamamide*	120 ±7·4	0·58 ±0·036	57

* Given as a solution in dimethyl sulphoxide. Vehicle dose never exceeded 6 ml./kg.

The toxicity of malononitrile has been ascribed to a gradual conversion to cyanide in the tissues[43,44]. The closeness of the LD_{50} values for malononitrile and CS when expressed on a molar basis (Table 1) therefore suggested that the poisonous effect of CS might also be caused by the liberation of cyanide. This possibility was tested by injecting sodium thiosulphate[42–45] (0·3 ml.) within 6–8 min of administering up to five times the LD_{50} of malononitrile or CS. Recovery was rapid in the case of malononitrile; gasping soon gave way to hyperventilation, which was sometimes accompanied by shivering and mild tetany,

The animals began to walk within 5 min of receiving thiosulphate, and survived without any permanent ill-effects. In the short term thiosulphate produced identical results in mice which had received CS, but the animals began to die 20 h after injection. Most deaths were recorded within the first 48 h, but survivals of up to 5 days were observed. Treatment with thiosulphate enabled about half the animals to survive 70–140 mg/kg of CS, but only one mouse out of a total of ten survived a dosage of 175 mg/kg. Moribund animals often exhibited a degree of hypothermia which was readily apparent to the touch (compare ref. 43).

Post-mortem examinations were performed on animals within a few minutes of death following the administration of CS, with or without thiosulphate. Macroscopically, the internal organs showed no abnormality apart from congestion, which was especially apparent when survival had been prolonged with thiosulphate. Histological examination of sections of liver, kidney, adrenals, spleen, heart, lung, and brain stained with haematoxylin and eosin from fifteen animals revealed no differences between creatures dying within 90 min of CS administration and four mice which were killed 1 h after injection of vehicle alone. At the microscopical level there appeared to be no noteworthy changes, other than venous congestion, in the organs of animals whose survival had been prolonged by the administration of thiosulphate. Neither necrosis nor significant cellular degeneration was observed in test or control mice.

Other benzylidene malononitriles were tested on mice to see if toxic effects were produced, and whether or not these were reversible by thiosulphate. In the short term benzylidene malononitrile itself produced symptoms precisely similar to those caused by CS; at the LD_{50} for CS (0·19 mmole/kg body weight) five animals died out of a total of eight. At three times this level (0·57 mmole/kg) all five animals in another group died. Five others which received thiosulphate, however, made rapid and permanent recoveries. On the other hand, 4-hydroxybenzylidene malononitrile (0·19 mmole/kg) induced panting but not gasping in four animals without causing fatalities; twice this dose killed two animals out of four. At a higher level still (0·57 mmole/kg) there were no survivors from two groups totalling ten animals. The effect was insensitive to thiosulphate, regardless of the order of administration. Deaths occurred within minutes of injection, and the corpses went into rigor. When 3-nitrobenzylidene malononitrile (0·19 and 0·57 mmole/kg) was injected into ten animals, all died, mostly between 6 and 22 h of injection. Gasping was observed in most cases; at the higher dose level thiosulphate exerted no remedial action. In a further group of six animals, 4-nitrobenzylidene malononitrile (0·57 mmole/kg) produced negligible effects in the short term, but four animals died 2–6 days following administration. The action was again insensitive to thiosulphate. Dimethyl sulphoxide was employed as vehicle at a level of 3·5 ml./kg for doses in excess of 0·19 mmole/kg.

Patai and Rappoport[9] have implicated 1-(2-chlorophenyl)-1′-hydroxy-2,2′-dicyanoethane as an intermediate in the hydrolysis of CS to malononitrile and 2-chlorobenzaldehyde. Since the same mechanism of breakdown might operate to some extent in living tissues, an attempt was made (G. R. N. J., Zauli, C., and Diamond, P. S., in preparation) to prepare this compound as Horner and Klüpfel[20] prepared 1-phenyl-1′-hydroxy-2,2′-dicyanoethane from benzylidene malononitrile. The actual product obtained, however, seemed to be 2-chloro-β-cyanocinnamamide (G. R. N. J., Zauli, C., and Diamond, P. S., in preparation). This material, although toxic, was not only much less poisonous than CS (Table 1), but also produced quite different symptoms, rendering the animals inert but not unconscious. A small number of mice died within a few hours of injection; most deaths occurred 20–40 h later. Only one animal, given 160 mg/kg, encountered the respiratory difficulties described above, and died 10 min after injection.

The effect of benzylidene malononitriles on the body is seen to depend critically both on the nature and on the position of substituents in the aromatic nucleus. The general effects of 4-hydroxybenzylidene malononitrile and the insensitivity to thiosulphate of its lethal action are not at variance with its ability to uncouple oxidative phosphorylation[46]. CS, however, has two distinct loci of toxicity, of short-term and long-term duration. The short-term locus, which is the more toxic of the two and which is also exhibited by benzylidene malononitrile, is akin to the action of malononitrile. This effect involves the liberation of cyanide within the body, and is reversed by thiosulphate. The resemblance of the symptoms to those produced by malononitrile and benzylidene malononitrile suggests that rates of cyanide formation from the three compounds are similar, and underlines the view that benzylidene malononitriles are reactive compounds. In order to explain its sternutatory properties, Holmes[7,8] has suggested a multistep scheme for the breakdown of CS. A convincing explanation of the whole range of effects must take differential racial sensitivity[31] into account. According to this hypothesis, CS could react with strongly basic amino groups, such as found in lysine residues, to give benzylamine adducts. These would undergo biologically catalysed oxidation to give imines or their tautomers before breaking down to form the $—CH(CN)_2$ ion and 2-chlorobenzoylamino derivatives. The carbanion would then be rapidly protonated to give malononitrile[47]. A further possibility is that decomposition might occur in the same way as in aqueous ethanol[9], but despite the stoicheiometric molar basis of toxicity on the part of CS, malononitrile (Table 1) and benzylidene malononitrile, the slowness of CS hydrolysis[9] and the similarity between the rates of poisoning of the three compounds suggest that cyanide ions may be cleaved from the original benzylidene derivatives at least to some extent without intermediate breakdown to malononitrile and the corresponding aldehyde.

Unlike benzylidene malononitrile and malononitrile itself, CS exhibits a long-term locus of toxicity, the mechanism of which has not been elucidated. Among the factors which actually cause death might be included general irritation[29], shock, or stress, although damage to the adrenals was not observed in the present work (see ref. 35). Comparisons with or extrapolations to the effects of CS aerosols on people are extremely difficult to make, but accidental ingestion of the material might cause poisoning in a way similar to malononitrile[48]. One immediate point of difference concerns the cytotoxic effect of CS in the respiratory tract of animals, where solid particles produced necrosis at sites of impact[35]. This necrosis may be a direct consequence of high local concentrations of the material or of its breakdown products, a situation which does not obtain in the present work.

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Mechanism and Prevention of Inert Gas Narcosis and Anaesthesia

BECAUSE of the raised partial pressure of nitrogen, compressed air induces symptoms of narcosis similar to those of the volatile anaesthetics[1,2]. Inert gases of the noble gas series, except possibly helium and neon[3,4], also cause narcosis and anaesthesia. Many mechanisms have been suggested for this narcosis[1,2,5–10] but those involving polarizability and molecular size seem most probable.

The Meyer–Overton theory infers preferential adsorption of the inert gas to the lipid constituent of cells, whereas alternatives involve the aqueous phase with the formation of clathrates[8] or ordered water[9] known as "icebergs". The use of fluorinated compounds, however, has provided evidence for the superiority of the correlation of anaesthetic potency to lipid solubility[11,12].

Most of the previously suggested mechanisms affecting membranes suggest an ion block as the cause of the narcosis[6,13,14]. The Meyer–Overton hypothesis would predict, on the contrary, an increased cation permeability through lipid membranes compared with the less likely hydrate theories, which predict decreased mobility[15].

In support of an increased permeability theory, Clements and Wilson[16] noted: "Inert gases, sufficient to bring about a standard effect in a biological system, act on a lipoprotein–water interface to produce a standard decrease of 0·39 dyne/cm in the interfacial tension". This results in expansion of the membrane and affects the dielectric constant at the interface. Their work supported lipid involvement and also implied the possibility of permeability changes. Later, Bangham *et al.*[17] reported that such changes in interfacial tension by volatile anaesthetics do increase ion permeability and concluded that "narcosis represents a transient reversible increase in membrane permeability to cations, higher concentrations resulting in a degree of permeability which the energy driven pumps in biological membranes cannot keep up with".

More recently, Bennett *et al.*[3] showed that increased pressures of nitrogen and argon, and also oxygen and carbon dioxide, similarly will adsorb to lipid membranes and affect the interfacial tension in accordance with the rule of Clements and Wilson[16]. Helium did not penetrate the membrane, even at 100 absolute atmospheres, showing that it is probably not narcotic. It is significant that no narcosis was detected in two men exposed recently to over 46 absolute atmospheres helium[4].

That such adsorption of inert gases to lipid membranes does cause an increase in intracellular ions is supported by *in vivo* studies showing a decrease of Na^+ and Cl^- in the cerebral spinal fluid of cats exposed to increased pressures of nitrogen and argon but not helium[18]; a decrease in plasma Na^+ and increase in erythrocyte Na^+ in men at 7 absolute atmospheres compressed air[19] and retention of urinary Na^+ and Ca^+ in divers exposed to 5·5 to 10 absolute atmospheres compressed air but not helium[20]. Similar electrolyte changes have also been reported during general anaesthesia and alcoholic intoxication[21,22].

In contrast, Halsey, Smith and Wood[23] found no *in vitro* evidence of an effect on active cation transport by ether or chloroform in erythrocytes until a concentration was reached which disrupted the membrane. At low concentrations, however, some evidence of increased cation transport was observed. Because the inert gases are only comparatively weak narcotics an effect on cation permeability seems more likely. Further, Mikikits *et al.*[24] have reported a general lack of correlation between inhibition of neuronal activity and red cell stabilization.

It is relevant also to consider the manner of the adsorption responsible for any resulting change in permeability. Benson and King[25] have postulated that this could well be electrostatic, involving preferential adsorption at charged sites on nerve cell membranes.

Some simple long-chain cationic detergents prevent liver necrosis in the rat resulting from carbon tetrachloride poisoning by preventing the associated intracellular accumulation of water, NaCl and calcium and loss of potassium and cellular proteins[26]. Such compounds are believed to be surface active and form complexes with lipids at lipid–water interfaces, especially if the former are negatively charged[27,28]. Anionic detergents, however, prevent neither such permeability changes nor liver necrosis. The results of these drug studies suggest that stabilization of the lipid membrane is achieved by the presence of either non-polar portions of amphipathic molecules (that is, with a hydrophobic portion and a polar group), or by the strategic positioning of positively charged groups at the water–lipid interface of the cell membrane.

If, as suggested here, the mechanism of inert gas narcosis is a preferential electrostatic adsorption to charged sites on neurone lipid membranes causing an increased ion permeability, cationic detergents should also prevent such narcosis. Experiments were therefore performed to

Table 1. EFFECT OF SURFACE ACTIVE ANIONIC OR CATIONIC DRUGS ON INERT GAS NARCOSIS IN RATS AT 0·9 O_2/6·41 N_2 ABSOLUTE ATMOSPHERE

Drug	Dose	Mean percentage change in evoked response	No. of rats	Serum β-glucuronidase units, in rat liver 4 h after CCl_4 (ref. 26)	Significance
1. No drug, no pressure	—	+4±4·66	10	98	—
2. No drug at pressure	—	−29·8±3·87	10	—	(1–2) $P<0·001$
		Anionic drugs			
3. Sodium hexadecyl sulphate	70 μmoles/kg	−34±3·69	10	78	(2–3) Not significant (1–3) $P<0·001$
4. Sodium dodecyl sulphate	80 μmoles/kg	−20±4·08	10	82	(2–4) Not significant (1–4) $P<0·001$
		Cationic drugs			
5. Stearylamine	55 μmoles/kg	−7±3·81	10	25	(1–5 Not significant (2–5) $P<0·001$
6. Cetyl trimethyl ammonium bromide	14 μmoles/kg	−6.5±4.14	10	22	(2–6) $P<0.001$ (1–7) Not significant
		Anti-inflammatory drug			
7. Acetyl salicylic acid	50 mg/kg	−1±3·68	10	—	(2–7 $P<0·001$ (1–7) Not significant

study the effects of cationic and anionic detergents on inert gas narcosis in rats. In addition, because low concentrations of non-steroidal anti-inflammatory drugs protect erythrocytes against lysis, acetylsalicylic acid also was investigated. Earlier studies have established that aspirin, among a number of other drugs, does ameliorate narcosis in the rat[29,30].

Seventy male Wistar rats were used in the present investigation. The narcotic effect of exposure to a pressure of 0·9 O_2/6·41 N_2 absolute atmospheres for 45 min was obtained by measurement of the mean spike height of the a_2 (25–45 ms) − b_2 (15–60 ms) wave of five averaged cortical potentials, recorded from two scalp electrodes and an earth electrode on the tail and evoked by flash stimuli presented through a 1 inch window in the pressure chamber situated immediately in front of the face of the rat. A flash was presented every 1·5 s with a sweep time of 320 ms and the effect of 64 flashes averaged. Comparison of results at atmospheric pressure with measurements at pressure were expressed as the mean percentage change in evoked response[31]. The drugs were administered intraperitoneally 30 min before compression.

The results (Table 1) indicate that the anionic compounds had no significant effect on the 29·8 per cent mean reduction of the evoked response as a result of the raised nitrogen partial pressure. Thus narcosis was still present. The cationic drugs and acetylsalicylic acid were, however, significantly effective ($P<0·001$) in preventing the decrease in evoked response. These compounds are therefore acting in a manner similar to that with carbon tetrachloride poisoning[26]. The relationship of this action to permeability changes, such as the leakage of β-glucuronidase into the sera (Table 1), indicate that protection in the liver and probably also in the present study in the brain is attained by restoration of the cell membrane to normality with a consequent reduction in the mobility of ions across the membrane.

This accords with a mechanism of narcosis and anaesthesia involving electrostatic adsorption to the lipids of neurone membranes which causes increased permeability to ions and their redistribution between the intracellular–extracellular fluids in accordance with their concentration gradients. Such changes in ion distribution interfere with the ion movements necessary for propagation of potentials and failure of transmission at the synapse occurs involving a maintained depolarization.

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Mauve Factor re-identified as 2,4-Dimethyl-3-ethylpyrrole and its Sedative Effect on the CNS

Irvine[1] and Hoffer and Mahon[2] reported on the occurrence of a compound giving a characteristic mauve colour with Ehrlich's reagent on paper. The significance of this metabolite is that it occurs with greater frequency in psychotic individuals. This finding has been confirmed by several investigators[3–5]. Ellman *et al.*[6], however, reported that a metabolite apparently identical to the one in question may be derived from phenothiazines and occurs in the urine of patients on phenothiazine therapy. Recently, Irvine[7] has identified the mauve factor as 2,4-dimethyl-3-ethylpyrrole on the basis of chromatographic and mass spectral studies. This report is concerned with the identity of the mauve factor, a critique of various isolation procedures used by different investigators and its pharmacological action on the central

Table 1. COMPARISON OF RESULTS FROM CHLOROFORM, ETHER AND CHARCOAL PROCEDURES

Subject	Drugs	Dose	Ferric chloride test for urinary phenothiazine derivatives	Irvine's charcoal procedure	Mauve factor spot Hoffer's ether extraction procedure	Ellman's $CHCl_3$ extraction procedure
1. A. S.	'Thorazine' 'Stelazine'	150 mg T.I.D. 10 mg B.I.D.	++++	−	N.D.	+
2. C. H.	'Thorazine' 'Stelazine'	100 mg T.I.D. 5 mg T.I.D.	++	−	−	−
3. V. P.	'Thorazine' 'Stelazine'	200 mg T.I.D. 10 mg B.I.D.	++++	−	+	+
4. T. B.	'Thorazine' 'Stelazine'	100 mg T.I.D. 5 mg	++++	+	+	+
5. W. T.	'Thorazine'	150 mg T.I.D.	++++	−	+	+
6. E. G.	'Thorazine'	300 mg B.I.D.	+++	−	+	+
7. J. K.	'Trilifon'	80 mg T.I.D.	+	−	−	−
8. T. P.	'Trilifon'	30 mg B.I.D.	+	−	−	−
9. E. S.	None		−	+	+	+
10. T. A.	None		−	+	+	+

N.D. = Not determined.

nervous system (CNS). The possible CNS effect is important because several laboratories have postulated a metabolic product in the schizophrenic which may be stimulant and hallucinogenic.

The mauve factor fractionations were carried out by the charcoal adsorption procedure of Irvine[1] as modified by Sohler[3], the ether extraction procedure of Hoffer and Mahon[2] and the chloroform extraction procedure of Ellman *et al.*[6]. Urines were tested for the presence of phenothiazine derivatives by the ferric chloride procedure of Forrest *et al.*[8]. 2,4-Dimethyl-3-ethylpyrrole was obtained from Aldrich Chemical Company. In experimental procedure, the trained test rabbits, chronically implanted with skull electrodes, were unrestrained and unanaesthetized, prepared with indwelling ear vein catheters for remote drug injection, and mildly sedated with sodium pentobarbital (3 mg/kg, intravenously) to provide a controlled baseline level of reference. Six rabbits were used at each dose level. 2,4-Dimethyl-3-ethylpyrrole (in 'Tween', methyl-cellulose suspension) was administered at dose levels of 1, 5, 10 and 20 mg/kg. Cortical electrical activity, as reflected in the electroencephalogram (EEG), was evaluated using a technique of quantitative EEG amplitude analysis[9]. We confirmed that the chloroform extraction procedure described by Ellman does extract from urine of patients on phenothiazines a phenothiazine derivative having the same R_f in the isopropanol–ammonia–water system as bufotenine and the mauve factor. The compound is not found, however, in fractions prepared by the charcoal procedure of Irvine. Several patients on phenothiazines were run using both procedures. With the chloroform procedure these patients would have been found to be mauve positive. By the charcoal procedure they were negative (Table 1).

The first six patients were on therapeutic doses of phenothiazines. Of this group, five of the six were positive using Ellman's procedure, while five of the six were negative using Irvine's procedure. Also in this table are two patients who are not on any medication but who are mauve positive with all three procedures. Of the patients on phenothiazine, four of the six are positive using the Hoffer and Mahon ether extraction procedure. These four are also positive by Ellman's procedure, indicating that with the ether extraction procedure, phenothiazine derivatives would be detected and reported erroneously as the mauve factor. The results obtained with the various extraction procedures indicate that the isolation procedure used must have a high degree of specificity, for phenothiazine derivatives and even bufotenin reported in the urine of some schizophrenic patients have similar chromatographic properties to the mauve factor in the principal solvent systems used. The mauve factor seems to differ significantly from the phenothiazine derivative in that the colour produced with Ehrlich's reagent fades on standing while this colour only appears on heating in the case of the mauve factor. With Ehrlich's reagent, the phenothiazine derivative produces a fairly stable colour almost immediately. The phenothiazine derivative, unlike the mauve factor, reacts strongly with Dragendorf's reagent. Although these tests are not absolute proof of a difference because the differences in the concentration of reactants could account for the negative tests, it is highly probable that the mauve factor found by Irvine's procedure is not a phenothiazine derivative.

The identification of the mauve factor with 2,4-dimethyl-3-ethylpyrrole and related compounds by Irvine has been confirmed in this laboratory on the basis of paper and thin-layer chromatography. Table 2 presents the results of these studies with regard to mauve factor isolated from urine and synthetic 2,4-dimethyl-3-ethylpyrrole. This not only has identical chromatographic behaviour, but reacts typically with Ehrlich's reagent, giving a mauve spot which appears slowly on heating and (at the concentrations usually examined in urine) fades after a short time. Mauve factor samples isolated from urine by the charcoal procedure require further purification by thin-layer chromatography before satisfactory comparisons can be made by paper chromatography in the 20 per cent KCl and 8 per cent NaCl systems. The fractions obtained in the charcoal procedure were chromatographed as a streak on Mallinckrodt, silica gel G, in ethyl acetate–isopropanol–ammonia (9 : 7 : 4). The band corresponding to the mauve factor was cut out, eluted with methanol, concentrated *in vacuo* and spotted on paper. This material was also examined spectrophotometrically and reacted with Ehrlich's reagent and the product examined spectrophotometrically. In all cases the behaviour of the mauve fraction from urine was identical with that of 2,4-dimethyl-3-ethylpyrrole. Recoveries using the charcoal procedure are very good, of the order of 90–100 per cent, and this with studies of the intensity of the Ehrlich reaction indicates that in mauve positive subjects the daily urinary excretion of 2,4-dimethyl-3-ethylpyrrole would be of 0·2–1 mg/24 h.

Table 2. CHROMATOGRAPHIC BEHAVIOUR OF MAUVE FACTOR FROM URINE AND 2,4-DIMETHYL-3-ETHYLPYRROLE

System	Urinary mauve factor	R_f 2,4-dimethyl-3-ethylpyrrole	Bufotenine
TLC, silica gel, butanol–methanol (50 : 50)	0·86, 0·98	0·86, 0·94	0
TLC, silica gel, hexane–benzene (40 : 60)	0	0	0
TLC, silica gel, ethyl acetate–isopropanol–ammonia (9 : 7 : 4)	0·97	0·97	0·80
Whatman 3 MM, isopropanol–ammonia–water (20 : 1 : 2)	0·91	0·90	0·91
Whatman 3 MM, 8 per cent NaCl, 1 per cent acetic acid	0·89	0·89	0·52
Whatman 3 MM, 20 per cent KCl	0·85	0·85	0·49

TLC = thin-layer chromatography.

Table 3. SEDATIVE EFFECT OF 2,4-DIMETHYL-3-ETHYLPYRROLE

Intravenous dose mg/kg	Per cent increase in sedation over intravenous saline treated rabbits
1	33
5	51
10	79
20	88

which is easily oxidized. As well as the spot represented by 2,4-dimethyl-3-ethylpyrrole several faster moving related components are often observed. These have also been observed in mauve positive urines.

The association of the mauve factor with psychotic states has led to a testing of 2,4-dimethyl-3-ethylpyrrole for CNS stimulatory activity in rabbits. In brief, the CNS response to the mauve factor compound was essentially a progressive increase in the baseline sedation in proportion to increasing dose. Judging from this sedative effect the mauve factor can be eliminated as one of the possible stimulant metabolites producing over-arousal in the schizophrenic patient.

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Triosephosphate Isomerase Gene not Localized on the Short Arm of Chromosome 5 in Man

THE assignment of the gene for triosephosphate isomerase (TPI, EC 5.3.1.1) on the short arm of chromosome 5 (ref. 1) needs confirmation. Sparkes *et al.*[1] reported diminished activities of TPI in the heterozygous range in a patient with a partial deletion of the short arm of chromosome 5 (cat cry syndrome). Both her parents, however, had normal values of this enzyme and were considered homozygous normal for the TPI gene. The authors concluded that the patient may have been hemizygous for TPI owing to loss of one TPI locus with the deleted chromosomal region. We now report data on five patients with the same chromosome deletion and normal activities of TPI.

The five patients were a twelve month old boy and four girls aged 2, 3½, 6, and 9 years, respectively. All patients had the characteristic clinical features of the cat cry syndrome, including the high pitched plaintive cry, microcephaly, the typical round face, ocular hypertelorism and motor-mental retardation. Acoustical measurements in the first three patients confirmed the presence of the typical physical parameters of the cry in this syndrome[2].

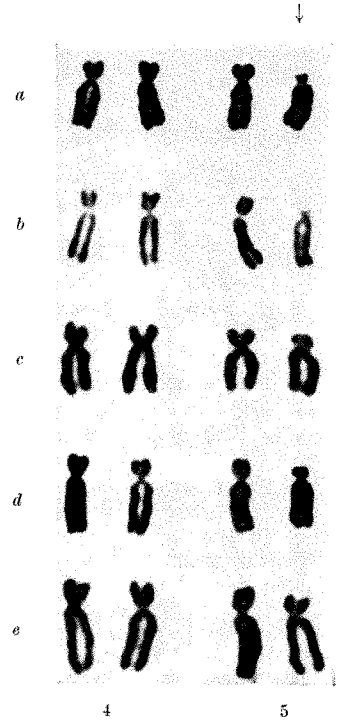


Fig. 1. *B*-group chromosomes from one cell of each patient. The deletion varies between individuals. The member of pair 5 indicated by arrow is affected. (*a* = A. K., *b* = B. S., *c* = A. P., *d* = C. H., *e* = S. G.).

Chromosomal preparations were derived from lymphocytes cultured for 72 h using a modified microtechnique[3]. A partial deletion of the short arm of a *B*-group chromosome was present in all cells of the five patients (at least twenty-five metaphases were examined by microscopy in each case). The deletions differed in size and the apparent loss of chromosomal material ranged from about 80 to 20 per cent of the short arm as shown in Fig. 1. Autoradiographic studies established the identity of the chromosome affected as number 5 in the first three patients. The characteristic phenotype and the similarities of these patients leave no doubt, however, that their deletion involves chromosome 5. The karyotypes of the parents of four patients were normal, but the mother and the maternal grandfather of patient 4 were carriers of a balanced reciprocal translocation between the part of the short arm that is deleted in the child and an autosome not yet identified, presumably a member of group *C*.

The activity of red cell triosephosphate isomerase was determined by a modification of the method of Beisenherz[4]. The results, indicated in Table 1, show that all patients and their parents had normal activities of this enzyme. Other red cell enzymes had normal activities (glutathione reductase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, hexokinase, phosphoglucomutase, glucosephosphate isomerase, 6-phosphofructose kinase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, monophosphoglycerate mutase, enolase, pyruvate kinase, lactate dehydrogenase). This indicates that no overall elevation of erythrocyte enzyme activity was present which could have accounted for a spurious normal level of TPI activity. We did not perform starch gel electrophoresis because no distinct electrophoretic differences exist between the enzyme of normal homozygotes and that of persons heterozygous for TPI deficiency[5]. Other gene markers that failed to show the usual inheritance or non-paternity were: Gc, Gm, Hp, red cell acid phosphatase, phospho-

2,4-Dimethyl-3-ethylpyrrole is a highly reactive compound

Table 1. ACTIVITIES OF TPI IN FIVE PATIENTS WITH PARTIAL DELETION OF THE SHORT ARM OF CHROMOSOME 5 AND THEIR PARENTS

Subjects	Age	Mean TPI activity*
Patient 1 (A. K., HG 6933)	1	1,845
Mother	25·5	2,003
Father	29·5	2,189
Control		2,273
Patient 2 (B. S., HG 6849)	2	2,080
Mother	28·5	—
Father	31	2,040
Control		—
Patient 3 (A. P., HG 69126)	9	2,060
Mother	49	1,850
Father	53	2,590
Control		2,550
Patient 4 (C. H., HG 69215)	3·5	1,840
Mother	21	1,920
Father	25	1,990
Control		2,060
Patient 5 (S. G., HG 7091)	6	1,840
Mother	32·5	1,930
Father	38	—
Control		1,790

* Normal range: 2,155 ± 532 μmoles/10^{11} cells.

glucomutase, adenylate kinase, adenosine deaminase and C'3 complement (analysed by electrophoresis), and ABO, CcDEe, Cw, MNSs, Fy, P, K and Cellano (analysed by serological methods). Sparkes *et al.*[1] argued that failure to confirm their findings would not necessarily rule out the localization of the gene for TPI on the short arm of chromosome 5, because the length of the deleted segment differs between patients. But the normal activities of TPI in our five patients occurred in deletions of different sizes, and in at least one patient the deletion was larger than described by Sparkes *et al.* Clinically it seems to make little difference whether the deletion is large or not. Our results indicate that the localization of the gene for triosephosphate isomerase on the short arm of chromosome 5 is unlikely.

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Is Inactivation of the *X* Chromosome in the Female Mule Non-random ?

HAMERTON *et al.*[1] have presented evidence which they interpret as a demonstration of preferential late replication of the donkey *X* chromosome (paternal *X*) in the female mule complement. This is in disagreement with cytological evidence[2] for random *X*-inactivation[3] in the female mule complement. This apparent contradiction prompted us to repeat the latter study and we report here our observations.

According to the available information, the *X* chromosome, which replicates its DNA later than all other chromosomes in a mammalian female complement, is that which becomes genetically inactivated during embryogenesis. The hypothesis of random inactivation of the maternal (X^M) or paternal (X^P) *X* chromosome could therefore be tested in a mammalian species whose two *X* chromosomes in a female complement are morphologically distinguishable. The female mule complement is unique in that its X^M (from the horse) and X^P (from the donkey *X*) can be distinguished morphologically[4].

Fibroblast cultures were established from the skin biopsy of a female mule. Two samples of peripheral blood were obtained from a second animal at two different times for lymphocyte culture, but both cultures failed to grow. Tritiated thymidine (^{3}H-TdR, specific activity 10 Ci/mmole, New England Nuclear Corp.) was added to exponentially growing fibroblast cultures in a final concentration of 1 μCi/ml. of culture medium. Colchicine was added 1 h later. Four hours after the addition of ^{3}H-TdR the cells were collected from the medium which still contained isotope. Only well spread metaphase figures with all sixty-three chromosomes were photographed and used for this experiment (317 in all), because one medium sized acrocentric chromosome of the mule complement completes replication only slightly earlier than the late *X*, and it could easily be mistaken for the late X^P chromosome in an incomplete figure in which both *X* chromosomes or the late X^M had been lost. Kodak NTB3 emulsion was applied to each slide, and they were exposed for seven days. Metaphase figures photographed before the application of emulsion were rephotographed at the focal plane of the overlying silver grains. Only metaphase figures with a single heavily labelled *X* chromosome (that is, an *X* chromosome with at least half the total number of grains as all other chromosomes of the complement combined) were used (119 in all) for this study (Fig. 1). The relative frequencies of metaphase figures containing a late replicating X^P or X^M were 57·9 and 42·1 per cent respectively.

These results are in accord with the previous results obtained with cultured lymphocytes[2], and confirm unequivocally that either the X^P or X^M can be the late replicator in different cells of the same animal. The relative frequencies of figures with late replicating *X* or X^M obtained from this experiment varied slightly from the previous results[2] (X^P, 51·5 per cent; X^M, 48·5 per cent) but varied significantly from the frequencies reported by Hamerton *et al.*[1] (X^P, 88–100 per cent; X^M, 0–12 per cent). Although 100 per cent of the fibroblast cells from one animal in that study were found to contain a late X^P (only eight figures were examined), only 88 per cent of the lymphocytes of the same animal had a late X^P chromosome. Furthermore, the *X* linked glucose-6-phosphate dehydrogenase (G-6-PD) isoenzyme patterns of both lymphocytes and fibroblast cells observed in this study showed that the paternal G-6-PD was at least "weakly represented". These observations suggest that cells with a late X^P are more abundant than those with a late X^M in the tissues studied, but do not support the conclusion[1] that inactivation of *X* in the female mule is non-random. It should be pointed out that according to the single active-*X* hypothesis[3] one of the two *X* chromosomes in a mammalian female complement becomes genetically inactivated early in development, possibly at implantation. The cells examined in these experiments[1,2] were those at the end of the line of differentiation, far removed from their cells of origin in which random inactivation of one *X* chromosome occurred. The frequency of distribution of cells with inactivated X^P and X^M in any given tissue would depend on the distribution and selection of these two cell types during differentiation of such tissue. The fact that the relative frequency of figures

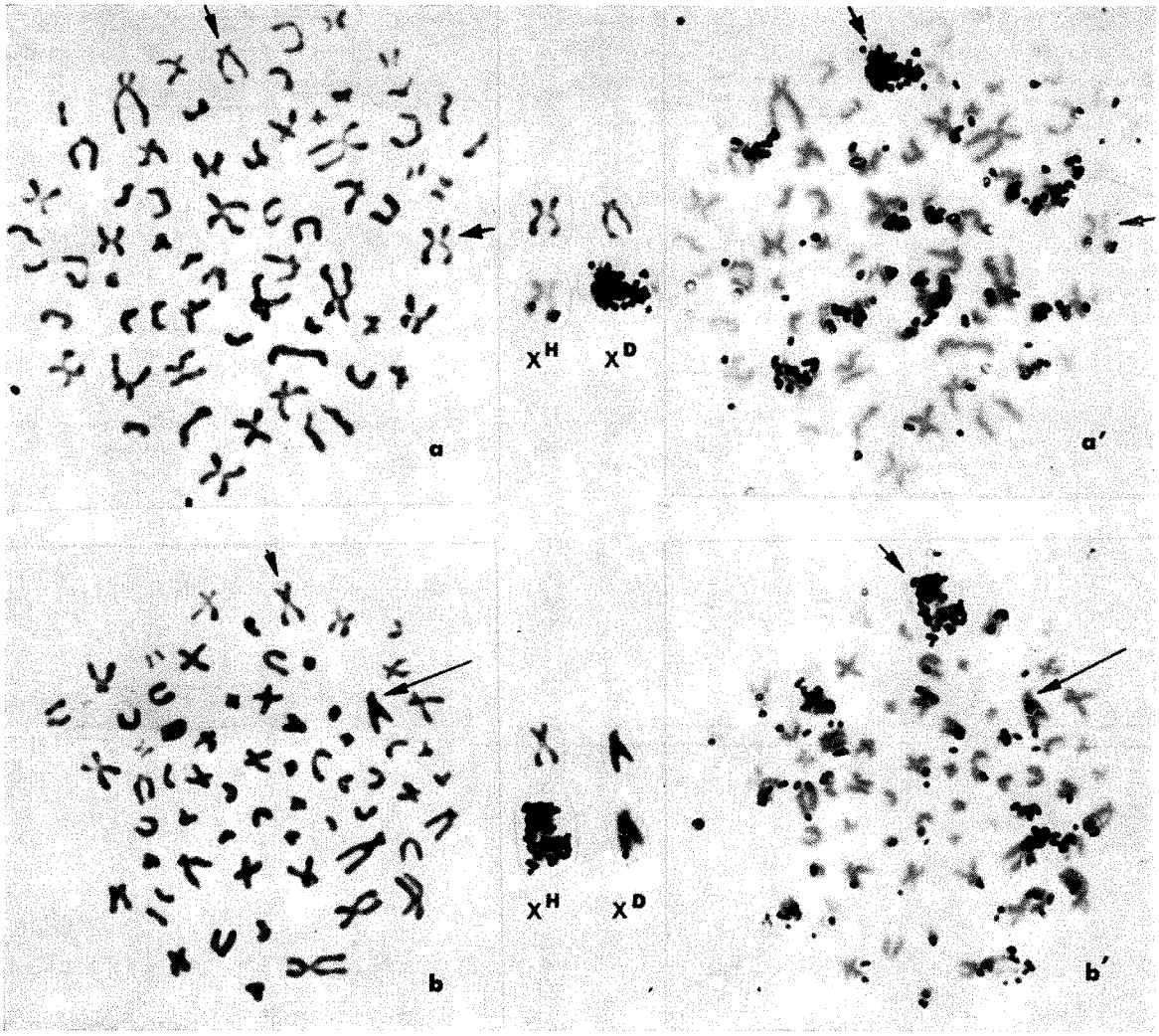


Fig. 1. Representative metaphase figures (*a* and *b*) and the corresponding autoradiographs (*a'* and *b'*). The X^P is the late replicating X chromosome in the upper figure but X^M is late in the lower figure.

with late replicating X^P or X^M varies between different tissues of the same animal and also between animals supports our conclusion.

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Non-random Inactivation of the *X* Chromosome in the Female Mule

WE were interested that Mukherjee *et al.*[1] have a further mule in which the donkey X chromosome (X^P, X^D of our report)[2] is late replicating in 50 per cent of the cells. This is in agreement with the report by Mukherjee and Sinha[3] and at variance with our findings[2]. We were extremely careful not to speculate on the significance of our observations[2], and although we stressed that our findings suggested preferential activity of the paternal X we refrained from speculating about the origin of this phenomenon in "either inactivation or subsequent cell selection". We agree that cell selection between inactivation and the time when we looked at the cells could account for our results. If this is so it is extremely interesting that in our three animals strong selective pressures seemed to favour activity of the $X^M(X^D)$ as opposed to the $X^P(X^H)$. We believe that our alternative hypothesis[2] could equally account for our results. It is interesting that in the animal studied by Mukherjee *et*

al.[1] 57·9 per cent of cells had a late replicating X^P, while 42·1 per cent had a late replicating X^M. Although this distribution is significantly different from ours and not significantly different from 50 per cent, it reveals a similar trend. It is a pity that they[1] do not report results on glucose-6-phosphate dehydrogenase isoenzyme patterns for this animal.

It is important to consider whether cytological support for the Lyon hypothesis can come from random inactivation of the two morphologically distinct X chromosomes of the mule as judged by autoradiography. We believe that it may not be justified automatically to extend the concept of random inactivation of either the X^M or X^P to interspecific hybrids. In situations when the two X chromosomes are not strictly homologous, for example, Searle's translocation in the mouse and certain structurally altered X chromosomes in man, the random nature of X inactivation breaks down. Even structurally normal X chromosomes may display non-random activity in some adult tissues[4]. This is apparently dependent on which of the two alleles they are carrying[4]. Ohno[5] has shown that in certain interspecific hybrids the hybrid condition can pose considerable problems in gene regulation. In the female mule, however, there is no evidence that the two X chromosomes are strictly homologous, nor can this be postulated confidently. If this is correct, their patterns of late replication might not be expected to be random. For this reason the mule cannot be used to provide a simple cytological test for the postulate of random inactivation, even disregarding objections which could be raised concerning uneven post-inactivation cell distribution, cell selection and balance. The mule, however, provides useful material for the study of the relationship between late replication of one or other X chromosomes and gene inactivation. This was the principal object of our study.

In brief, the relationship between the autoradiographic data on the mule X chromosomes and the Lyon hypothesis are represented in algebraic form as

$$\frac{A_1Z_1L_1}{A_2Z_2L_2} = p$$

where L is the probability that one of the two homologous X chromosomes is inactivated in a female cell; A is the probability of progeny resulting from a female mule cell with one or other of the two X chromosomes active; Z is the probability that one or other of the two female mule X chromosomes is inactivated early in embryogenesis; 1 and 2 specify one of the two X chromosomes of normal female mammals, and p is a ratio of cells showing the donkey and horse X chromosome differentially labelled in the late S phase.

The values of A and Z are not known, and so the equation cannot be solved for L and it is impossible to test whether $L_1 = L_2 \sim 0{\cdot}5$ as suggested by the Lyon hypothesis.

But, if $L_1 = L_2 \sim 0{\cdot}5$ is taken for granted, inferences can be made about the products A_1Z_1 and A_2Z_2. We hope that data will become available about the value of A and Z. At present, if $P = 0{\cdot}5$ as found in two female mules[1,3], then $A_1Z_1 = A_2Z_2$. This suggests that the donkey and horse X chromosomes are equivalent with regard to the mechanism responsible for X chromosome repression or derepression, and with regard to the selective value that the activity of one or other of these two chromosomes confers on the cells. On the other hand, our data for three mules[2] and unpublished results for five further hybrids suggest that the two parental X chromosomes differ in one, the other or both of these respects, for the cells with a late labelling X^M are in a minority in all hybrids which we have studied.

We do not know the reason for the difference between our findings and those of Mukherjee *et al.*[1,3]. If it does not rest on differences of technical or analytical procedures it could have a biological meaning, perhaps in terms of "strengths" (or even homology) of the X chromosomes in two geographically distinct populations of mules.

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Production of Heterologous Cytotoxic Antibodies *in vitro*

THE possibility of inducing an immunological response *in vitro* to viral[1], erythrocyte[2,3] and tissue[4-6] antigens has recently been the subject of much research. Ginsburg and Sachs[4] have shown the destructive activity of rat spleen cells stimulated *in vitro* with mouse embryo cells and proved the immunological nature of this phenomenon, but they found no activity in the tissue culture medium of these spleen cells cocultivated with mouse embryo cells. Interestingly, in these experiments the medium was harvested in the presence of mouse tissue which could have absorbed antibodies specific to mouse antigens; and, the culture medium was tested without adding complement, a substance shown to be necessary for the detection of cytotoxicity of antibodies induced *in vivo*.

We here report an investigation on a mouse–hamster system. The maturation of the immunological response was accomplished in the absence of stimulating antigen. The activity of the medium was tested by a modification of the colony inhibition (CI) technique of Hellström and Sjögren[7], using fresh guinea-pig serum as a source of complement.

The spleen from one adult female C57BL mouse was cut in small pieces and placed on the top of a 48-h-old monolayer of SV40-transformed hamster cells (designated F5-1) growing in a 10 cm Petri dish. For each experiment three or four Petri dishes were prepared. Eagle's minimum essential medium supplemented with 10 per cent foetal calf serum was used as a cultivation medium. After 1–2 days of incubation at 37° C in 5 per cent CO_2 atmosphere, the floating pieces of spleen tissue, together with spleen cells, were removed, sedimented by centrifugation, resuspended in fresh medium and put on a 24 h old feeder layer of syngeneic C57BL fibroblasts. After incubation for 4–5 days in similar conditions, the fluid was removed and the cells were sedimented by centrifugation for 30 min at 2,000 r.p.m. The activity of this cell-free medium was tested by CI assay. Medium from spleens cultivated in the same way, but in the absence of antigenic stimulation (F5–1), served as control. To 0·1 ml. of cell suspension containing 500 to 1,000 target cells was added 0·1 ml. of experimental medium, and the reaction mixture (F5–1) was incubated for 1 h at 37° C in CO_2 atmosphere, after which 0·2 ml. of undiluted guinea-pig serum was added and the incubation was continued for 30 min. The same experimental medium (12 ml.) was then added and 4 ml. distributed to each of three plastic Petri dishes and incubated for 5–7 days at 37° C in 5 per cent CO_2. The dishes

Table 1. THE COLONY INHIBITION ACTIVITY OF MEDIUM FROM MOUSE SPLEEN TISSUE STIMULATED *in vitro* BY HAMSTER CELLS

Medium used	Target cells	No. colonies (av.) Expt 1	Expt 2
Anti F5–1	F5–1	0, 0, 0 (0)	0, 3, 3 (2)
Control	F5–1	87, 86, 105 (93)	67, 93, 94 (85)
Anti F5–1	BHK	0, 0, 0 (0)	0, 0, 0 (0)
Control	BHK	148, 104, 109 (120)	97, 117, 110 (108)

were then fixed and stained with Giemsa and the colonies were counted.

The results, presented in Table 1, show that the medium from spleen tissue stimulated by F5–1 cells inhibited not only the growth of F5–1 cells, but also the formation of colonies of baby hamster kidney (BHK–21) cells; it had no effect on the ability of methylcholanthrene-induced mouse sarcoma cells to form colonies. These data indicate that the activity of the medium is directed indiscriminately against antigens of either F5–1 or BHK hamster cells.

Table 2. THE SPECIFICITY OF THE IMMUNE MEDIUM FROM MOUSE SPLEEN TISSUE STIMULATED *in vitro* BY HAMSTER CELLS (F5–1)

Medium used	Target cells	No. colonies (av.)
Anti F5–1	F5–1	1, 0, 3 (1)
Anti F5–1, absorbed by F5–1	F5–1	65, 91, 93 (73)
Anti F5–1, absorbed by BHK	F5–1	85, 55, 62 (67)
Control	F5–1	87, 81, 105 (91)

Table 3. COMPLEMENT DEPENDENCE OF THE COLONY INHIBITION ACTIVITY OF A MEDIUM FROM MOUSE SPLEEN TISSUE STIMULATED *in vitro* BY HAMSTER CELLS (F5–1)

Medium used	Target cells	No. colonies (av.)
Anti F5–1 + C^1	F5–1	3, 0, 4 (2)
Control + C^1	F5–1	69, 83, 71 (74)
Anti F5–1 (inactivated)	F5–1	59, 71, 83 (70)
Anti F5–1 (inactivated) + C^1	F5–1	7, 7, 11 (8)

For further analysis the active medium was absorbed with approximately 100 mg of either F5–1 or BHK cells for 1 h at 4° C, and the supernatant fluids were assayed by the CI test. The results (Table 2) demonstrated that both F5–1 and BHK cells were able to absorb the component in the medium responsible for colony inhibition. These data reinforce the conclusion that the activity of this medium is directed against an antigen common to hamster cells. Table 3 shows the results of an experiment designed to determine the complement dependency of this reaction. It is clear from these data that heat-inactivated medium showed no activity in the CI test. The inhibitory activity of this medium could, however, be fully restored by addition of fresh guinea-pig serum.

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Effect of Mineral Adjuvant on Lymphocyte Cooperation in the Secondary Antibody Response to a Hapten–Protein Conjugate

THE determinants of the hapten and the protein carrier cooperate in the initiation of the primary antibody response to hapten–protein conjugates. Benacerraf and his colleagues[1] demonstrated that deoxynucleoprotein–polylysine alone does not elicit a primary response in certain strains of guinea-pigs, but DNP–polylysine complexed to bovine serum albumin elicits a vigorous response to the determinants of the albumin and DNP moieties. Similar findings were reported by Rajewsky and Rottländer[2] in rabbits using isoenzymes of porcine lactate dehydrogenase, and by Schierman and McBride[3] in chickens using chicken erythrocyte isoantigens. Further studies[4,5] demonstrated that the determinants of the hapten and carrier also cooperate in the initiation of the secondary antibody response of rabbits to hapten–protein conjugates; and synergy is manifested between cells primed to the carrier and cells primed to the hapten in the adoptive immune response of X-irradiated mice to NIP–protein conjugates[6], so establishing a cellular basis for cooperation between carrier and hapten in the secondary response.

Hitherto, hapten–protein conjugate in adjuvant has been used for the primary immunizations and the soluble conjugate for the secondary challenge. The object of this experiment was to determine if cell cooperation in the adoptive immune response to a hapten–protein conjugate is altered by adsorption of the conjugate to the surface of a particulate mineral adjuvant, alum, for the secondary challenge. In particular, we tested the ability of thoracic duct cells from donors immunized to diphtheria toxoid (DT) or to DNP–bovine serum albumin (DNP–BSA) to cooperate in the restoration of the antibody response of irradiated rats to DNP–DT adsorbed to alum or to DNP–DT in aqueous solution. DNP–DT was used as the conjugate because thoracic duct cells from normal (unimmunized) donors restore a negligible antibody response to the carrier and hapten portions of the alum adsorbed complex[7].

Inbred Lewis rats (Microbiological Associates) weighing 150–200 g and immunized to DT, DNP–DT or DNP–BSA were used as thoracic duct cell donors. Coupling of DNP to DT (supplied by the Commonwealth of Massachusetts Department of Health) or BSA (Nutritional Biochemicals) was accomplished with sodium 2,4,dinitrobenzene sulphonate (technical grade, Eastman)[8]. The number of DNP groups bound per molecule of protein was determined spectrophotometrically[9]. The coupling reaction was stopped by repeated dialysis of the reaction mixture against distilled water. The composition of the conjugates was DNP_{11}–DT and DNP_{23}–BSA. Adsorption of the DNP–DT and DT to precipitated alum was performed according to the method of Williams and Chase[10]. Approximately 10 mg of alum was used to adsorb 1 mg of protein.

Immunization to DNP–DT or DT was achieved by the subcutaneous and intraperitoneal injection of 0·5 ml. alum suspension to give a total dose of 0·1 mg of protein. Rats were immunized to DNP–BSA in complete Freund's adjuvant (Difco Laboratories) by the injection of 0·1 ml. of emulsion into each hind foot pad to give a total dose of 0·4 mg protein. The thoracic duct of immunized donors was cannulated by a modification of the method of Bollman *et al.*[11] 4–8 weeks after the injection of antigen. Recipients of thoracic duct cells were challenged with a single intraperitoneal injection of 0·5 mg DNP–DT in 0·5 ml. phosphate buffered saline or with a subcutaneous and intraperitoneal injection of 0·1 mg alum adsorbed DNP–DT as described.

Antibody titres were determined by a modification of the Farr technique[12]. Five-fold serial dilutions of antiserum were made in 10 × 75 mm test tubes in 0·4 ml. of 10 per cent normal rat serum. Thereafter, 0·1 ml. of 1×10^{-8} M [^{3}H]DNP–ethylaminocaproic acid ([^{3}H]DNP–EACA; supplied by Dr R. Asofsky, National Institute of Allergy and Infectious Diseases) was added to each tube. [^{3}H]DNP (New England Nuclear Corp.) was coupled to EACA by the method of Green *et al.*[13]. The test tubes were shaken and allowed to stand for at least 1 h at room temperature. Globulins were precipitated by the

addition of 0·5 ml. saturated ammonium sulphate solution to each tube. After 1–2 h the precipitates were spun down and 0·1 ml. of the supernatant was counted in a liquid scintillation spectrometer. Antibody titres were taken as that dilution of antiserum which bound 33 per cent of the labelled antigen. Groups of six animals were used to obtain mean antibody titres.

Table 1. ADOPTIVE ANTIBODY RESPONSE* TO DNP FOLLOWING A SINGLE INTRAPERITONEAL INJECTION OF 0·5 mg DNP–DT IN AQUEOUS SOLUTION

No. thoracic duct cells transferred to irradiated host (antigen used to immunize donor)	Days after injection of DNP–DT 7	14
50×10^6 (DT)	$<0\cdot1$	$0\cdot29\pm0\cdot21$
50×10^6 (DNP–BSA)	$0\cdot10\pm0\cdot12$	$1\cdot06\pm0\cdot45$
25×10^6 (DT)+25×10^6 (DNP–BSA)	$1\cdot22\pm0\cdot05$	$2\cdot48\pm0\cdot30$
50×10^6 (DNP–DT)	$3\cdot21\pm0\cdot15$	$3\cdot22\pm0\cdot13$

* Expressed as $\log_{10}$ mean antibody titre ±s.e. which bound 33 per cent of antigen ([^{3}H]DNP–EACA).

Table 1 shows the antibody response to DNP–DT in Lewis rats given 500 rad, whole body X-irradiation, and an intravenous injection of thoracic duct cells 2 h later. DNP–DT in aqueous solution was injected intraperitoneally 24 h after irradiation. A vigorous antibody response (titre > 3 $\log_{10}$ units at 7 days) was restored by 50×10^6 thoracic duct cells from donors immunized to DNP complexed to the homologous carrier (DNP–DT). The responses restored by an equal number of thoracic duct cells from donors immunized to the free homologous carrier (DT) or to DNP complexed to a heterologous carrier (DNP–BSA) were barely detectable at 7 days. The latter cells restored a moderate response by 14 days. The response restored by a combination of cells from donors immunized to DT or to DNP–BSA was much greater than the sum of the responses restored by each cell type independently. It is of interest that the response restored by the combination of cells was not as great as that restored by cells from donors immunized to DNP complexed to the homologous carrier (DNP–DT) alone.

Similar experiments in which the irradiated hosts were challenged with alum adsorbed DNP–DT instead of DNP–DT in aqueous solution showed that thoracic duct cells from donors immunized to DNP–DT restore a vigorous response again, and cells from donors immunized to DT restore a feeble response (Table 2). By day 9, however, the response restored by cells from donors immunized to DNP–BSA was similar to that restored by cells from donors immunized to DNP–DT. Further, 50×10^6 DNP–BSA primed cells produced a greater response than a combination of 25×10^6 DNP–BSA and 25×10^6 DT primed cells. These findings indicate that DNP–BSA primed cells are considerably more effective in restoring the response to DNP–DT adsorbed to alum than to DNP–DT in aqueous solution.

Table 2. ADOPTIVE ANTIBODY RESPONSE* TO DNP FOLLOWING A SINGLE INTRAPERITONEAL AND SUBCUTANEOUS INJECTION OF 0·1 mg ALUM ADSORBED DNP–DT

No. of thoracic duct cells transferred to irradiated host (antigen used to immunize donor)	Days after injection of DNP–DT 7	9	14
50×10^6 (DNP–DT)	$1\cdot66\pm0\cdot14$	—	$2\cdot39\pm0\cdot12$
10×10^6 (DNP–DT)	—	$0\cdot81\pm0\cdot38$	$0\cdot98\pm0\cdot25$
50×10^6 (DNP–BSA)	$0\cdot92\pm0\cdot23$	$2\cdot24\pm0\cdot30$	$2\cdot25\pm0\cdot23$
10×10^6 (DNP–BSA)	$0\cdot18\pm0\cdot14$	$0\cdot29\pm0\cdot13$	$0\cdot59\pm0\cdot26$
25×10^6 (DT)+25×10^6 (DNP–BSA)	—	$1\cdot63\pm0\cdot31$	$1\cdot67\pm0\cdot28$
50×10^6 (DT)	—	$<0\cdot1$	$0\cdot35\pm0\cdot22$
50×10^6 (DNP–BSA)†	$<0\cdot1$	$0\cdot46\pm0\cdot19$	$0\cdot58\pm0\cdot29$

* Expressed as $\log_{10}$ mean antibody titre ±s.e. which bound 33 per cent of antigen ([^{3}H]DNP–EACA).

† Recipients received an intraperitoneal and subcutaneous injection of 0·1 mg alum adsorbed DT and an intraperitoneal injection of 0·5 mg DNP–DT in aqueous solution.

A possible explanation of these findings is that alum adsorbed DNP–DT rapidly transforms some of the thoracic duct cells from donors immunized to DNP–BSA into carrier (DT) primed cells. Thus carrier primed cells may be present in sufficient numbers to augment the response to DNP–DT, even when they are not injected into the irradiated host. To investigate this point, irradiated hosts were given 50×10^6 thoracic duct cells from donors immunized to DNP–BSA and were challenged with an intraperitoneal and subcutaneous injection of 0·1 mg alum adsorbed DT and an intraperitoneal injection of 0·5 mg DNP–DT in aqueous solution 6 h later. Table 2 shows that the response elicited by the combined challenge was minimal, indicating that the adsorption of DNP–DT to alum does not result in the production of large numbers of carrier primed cells.

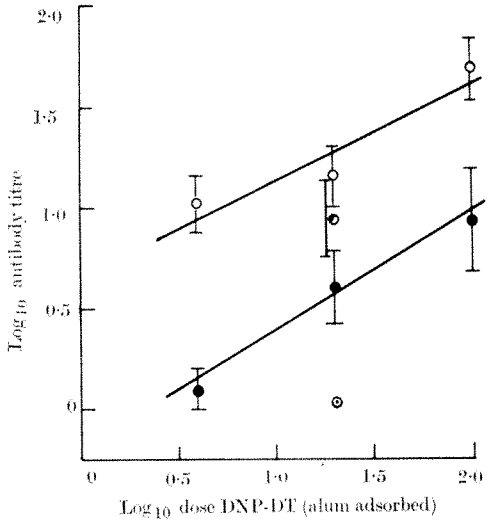


Fig. 1. Adoptive antibody response of irradiated hosts reconstituted with thoracic duct cells. Each point represents the $\log_{10}$ mean antibody titre ±standard error of the mean which bound 33 per cent of antigen ([^{3}H]DNP–EACA). The dose of alum adsorbed DNP–DT is expressed as the $\log_{10}$ μg protein injected. Antibody titres were measured 7 days after the injection of antigen. ●, Mean response restored by 50×10^6 cells primed to DNP–BSA; ○, mean, response restored by 50×10^6 cells primed to DNP–DT; ○, mean response restored by 50×10^6 cells primed to DT; ◐, mean response restored by a combination of 50×10^6 cells primed to DNP–BSA and 50×10^6 cells primed to DT.

To determine if the relative restorative action of the cell types noted in Table 2 is dependent on the dose of DNP–DT, several experiments were performed using lower doses of antigen for the secondary challenge. Fig. 1 compares the antibody response restored by DNP–DT and DNP–BSA primed cells at day 7 for three doses of the alum adsorbed antigen. The double logarithmic plots for both cell types are parallel. This shows that the relative restorative action of the two cell types is constant over the range of antigen doses tested. Further, the difference between the titres restored by the two cell types (about 0·75 $\log_{10}$ units) is considerably smaller than that noted when DNP–DT in aqueous solution is used as antigen (> 3 $\log_{10}$ units).

In further experiments, DNP–BSA and DT primed cells were tested for their ability to cooperate in the restoration of the secondary antibody response to alum adsorbed DNP–DT. Fig. 1 shows that the mean antibody titre restored by a combination of 50×10^6 DNP–BSA and 50×10^6 DT primed cells is greater than the sum of the responses restored by 50×10^6 DNP–BSA or 50×10^6 DT primed cells alone. But the difference between the means is not statistically significant ($P>0\cdot05$) according to the Student t test. On the other hand, the antibody response restored by a combination of DNP–BSA and DT primed cells was significantly greater ($P<0\cdot001$) than the sum of the responses restored by either cell type alone when DNP–DT in aqueous solution is used as antigen.

It is not clear how the addition of carrier primed cells or the adsorption of antigen to alum assists hapten primed cells to initiate the antibody response to the hapten–protein conjugate. The carrier primed cells may bind the conjugate to the cell surface producing antigen coated particles which are similar to the antigen coated alum particles and such antigen coated surfaces

may facilitate the presentation of haptenic determinants to the hapten primed cells, so helping these cells to initiate the antibody response. The carrier primed cells may function much like an adjuvant, and play a direct part in the "processing" of antigen and a more indirect part in immune recognition of the haptenic determinants.

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Allogeneic Inhibition in Thymectomized Animals lacking Homograft Reactivity

THE phenomenon of allogeneic inhibition, a term coined by Hellström[1], was first observed by Snell *et al.*[2,3]. These investigators noted that certain mouse lymphoma tumours grew less well in semi-syngeneic F_1 hybrid mice than in the original parental strain. Hellström[1,4] demonstrated that carcinoma and sarcoma growth was also inhibited in semi-syngeneic hosts. He thought it unlikely that the phenomenon was caused by a graft versus host reaction leading to "allergic death" of the tumour cells; or that allogeneic inhibition was caused by a homograft reaction directed against hypothetical recessive isoantigens of the tumour[4], because all histocompatibility antigens of the mouse are codominantly expressed and F_1 hybrid mice are therefore genetically tolerant to parental strain grafts[5,6].

The mechanism of allogeneic inhibition remains obscure. Hellström *et al.*[7] and Möller[8] proposed that the *in vitro* cytotoxic effect of normal F_1 hybrid lymphoid cells on parental type target cells, and growth inhibition *in vivo*, was caused by target cell confrontation with foreign isoantigens. Alternatively, the F_1 hosts may react more efficiently against the tumour-specific antigens on the tumour cells than the parental hosts (hybrid hyperreactivity)[9–11]. It has also been suggested that the phenomenon of "hybrid resistance"[12] is related to allogeneic inhibition. It was found that the growth of haematopoietic cells from mice carrying the $H\text{-}2^b$ allele were inhibited in semisyngeneic F_1 hybrid mice. Allogeneic inhibition is not confined to $H\text{-}2^b$ cells, however, and therefore need not have a similar basis.

We have investigated whether the immunological status of the host can influence the growth of a parental tumour in a semisyngeneic F_1 hybrid host. Mice of the following inbred mouse strains, 2–4 months old, were used: A/Sn ($H\text{-}2^a$) and the F_1 hybrids (A × CBA)F_1 ($H\text{-}2^a$) ($H\text{-}2^k$) and (A × DBA) F_1 ($H\text{-}2^a$) ($H\text{-}2^d$). A Moloney virus induced ascites lymphoma indigenous to the A strain (YAC) was used throughout the experiments. Ascites fluid containing YAC cells was passaged in the A strain.

Quantitative estimates of tumour growth were carried out according to the technique developed by Lindholm *et al.* (unpublished), which measures the rate of tumour growth by the incorporation of ^{3}H-thymidine. Tumour cells injected into the experimental animals were collected from ascites fluid in pasteur pipettes, washed twice in balanced salts solution and counted in a Bürker chamber. 10^4 trypan-blue unstained cells in 1 ml. were injected intraperitoneally into each mouse. Seven days later the mice were injected with ^{3}H-thymidine (specific activity 5,000 mCi/mmole). After 2 h the mice were killed by cervical spine dislocation. Two ml. of sterile tissue culture medium (Parker '199') was injected intraperitoneally and the fluid was withdrawn. The number of tumour cells was counted under phase contrast microscopy. Smears were prepared from the fluid and were fixed in cold 95 per cent ethanol. Autoradiographs were made according to Caro and van Tubergen[13], using Ilford nuclear research emulsion type K-5. The autoradiographs were exposed for 10 days in the dark at room temperature, developed, fixed and stained through the emulsion with the methyl-green pyronin stain[14]. The percentage of tumour cells labelled with ^{3}H-thymidine was estimated from counts of 1,000 cells.

Thymectomy was performed at the age of 4–5 weeks. Ten days later they were subjected to 600 rad whole body dose of X-irradiation generated in a Siemens X-ray machine at 15 mA and 200 kV filtered by 0·5 mm Cu. Immediately thereafter $15 \times 10^6 - 20 \times 10^6$ syngeneic bone marrow cells were injected intravenously in the tail vein. Skin grafts were transplanted essentially according to the method of Billingham and Medawar[15] with slight modifications[16]. The "tolerant" state of animals so treated (adult thymectomy, X-irradiation and reconstitution with bone marrow cells) was tested with H-2 incompatible skin grafts (C57BL-($H\text{-}2^b$)). Only animals carrying healthy skin grafts at 1–2 months after grafting were used in the experiments.

The growth of 10^4 YAC ascites lymphoma cells was measured in the six following groups of animals: (1) normal syngeneic A/Sn mice, and semi-syngeneic F_1 hybrid mice; (2) syngeneic and semi-syngeneic F_1 mice that were thymectomized at the age of 4–5 weeks, at least 2 weeks before inoculation with tumour cells; and (3) syngeneic and semi-syngeneic F_1 mice that were thymectomized at 4–5 weeks, thereafter lethally irradiated and reconstituted with $10–15 \times 10^6$ syngeneic bone marrow cells immediately after irradiation. All experiments were not performed at the same time but animals belonging to the same experiment (see Table 1) were injected with the same tumour suspension. Seven days after injection of tumour cells the total number of tumour cells was estimated and the percentage of cells actively synthesizing DNA was measured by incorporation of ^{3}H-thymidine following a single intraperitoneal injection as described.

Experiments 1, 2 and 3 show that the number of tumour cells present on the seventh day of growth is not lower in A mice subjected to thymectomy or thymectomy followed by irradiation and restoration with syngeneic bone marrow cells than in untreated A mice (Table 1). The percentage tumour cells in DNA synthesis is also not reduced in treated A mice as compared with untreated A mice. On the other hand, both the number of tumour cells present on the seventh day of growth and the percentage of tumour cells in DNA synthesis are lower in all groups of F_1 animals than in A mice (experiments 3–6). Further, there is not much difference between tumour behaviour in untreated F_1 hybrids and in F_1 hybrids which have been thymectomized, or thymectomized, X-irradiated and bone marrow protected. It has been shown (L. L., and Britton, S., unpublished) that during

Table 1. GROWTH OF TUMOUR CELLS IN HOSTS

Experiment	Strain	No. of animals	Treatment	No. of cells $\times 10^6$	Per cent labelled cells
1	A/Sn	5	None	2·8±1·0	43±8
	A/Sn	3	Thx + X-ray + BM cells	2·3±1·8	47±9
2	A/Sn	6	None	77±18	50±6
	A/Sn	3	Thx + X-ray + BM cells	90±21	51±8
3	A/Sn	8	None	3·3±1·4	66±4
	A/Sn	7	Thx	4·6±2·0	67±3
	A/Sn	4	Thx + X-ray + BM cells	13·3±7·4	66±5
	(A × CBA)F_1	7	None	0·4±0·1	8±2
	(A × CBA)F_1	8	Thx	0·6±0·1	5±1
	(A × CBA)F_1	5	Thx + X-ray + BM cells	1·3±0·7	23±9
4	A/Sn	6	None	2·7±0·5	61±5
	(A × CBA)F_1	7	None	0·9±0·1	8±1
	(A × CBA)F_1	4	Thx + X-ray + BM cells	1·1±0·5	17±11
5	A/Sn	6	None	6·8±1·4	54±4
	(A × CBA)F_1	6	None	1·4±0·2	5±1
	(A × CBA)F_1	6	Thx + X-ray + BM cells	1·1±0·2	5±2
6	A/Sn	5	None	23±9·0	50±7
	(A × DBA)F_1	6	None	1·9±0·5	5±1
	(A × DBA)F_1	9	Thx + X-ray + BM cells	2·3±0·5	11±6

* Thx stands for thymectomy; BM = bone marrow.

Growth of 10^4 YAC ascites tumour cells injected intraperitoneally into groups of 2–4 months old syngeneic A/Sn and semisyngeneic F_1 hybrid mice. The recipients were either untreated, thymectomized as adults or thymectomized as adults, lethally irradiated and reconstituted with syngeneic bone marrow cells. On day 7 the total number of YAC tumour cells as well as the percentage of cells actively synthesizing DNA measured by ^{3}H-thymidine uptake was assayed.

allogeneic inhibition fewer YAC tumour cells are synthesizing DNA than during growth in syngeneic hosts. The results given in Table 1 therefore indicate that allogeneic inhibition of the YAC tumour is a phenomenon which is expressed also in animals incompetent to carry out cellular immune reactions.

To explain the reduced growth of parental grafts in F_1 hybrids, it has been postulated that a heterosis effect in the F_1 hybrids increases the ability of the hosts to react against the tumour-specific antigens present in the tumour. Huemer[9] recently presented data in support of this hypothesis, but these arguments lack direct experimental proof. Our findings would rule out this hypothesis, for the phenomenon could be reproduced in animals which by thymectomy and X-irradiation had been deprived of homograft reactivity to the extent that they accepted *H*-2 incompatible skin grafts. We therefore conclude that these findings substantiate the hypothesis that growth inhibition of parental strain grafts in F_1 hybrids is caused by a reaction which is independent of cellular immune reactivity of the host. Inhibition of tumour growth probably depends on tumour cell contact with foreign isoantigens present in the host tissues[7,8]. Our preliminary results suggest that growth inhibition of parental tumour grafts in F_1 hybrids depends on close contact with host cells, for no inhibition is observed with tumours enclosed in cell-impermeable diffusion chambers.

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Agarose Derivatives for Purification of Protein by Affinity Chromatography

AFFINITY chromatography[1–5], which in principle is related to the use of immunoadsorbants for the purification of antibodies[6], exploits the reversible and specific interactions of ligands with macromolecules. Purification is affected by chromatographing the protein to be purified on a column containing an insoluble matrix to which the specific ligand is covalently attached. Beaded agarose ('Sepharose') is a useful insoluble support which has recently been used successfully in the selective purification of enzymes[1–5,7,9], antibodies and antigens[10–15], chemically synthesized peptides[16,17], thyroxine binding serum proteins[18], and in the study of the interaction of insulin with cell membranes[19]. In these studies the ligands or peptides were attached through their amino groups after activation of 'Sepharose' with cyanogen bromide[20,21].

Successful purification by affinity chromatography depends in many cases on placing the ligand far enough from the matrix backbone to minimize steric restrictions in the interaction between the ligand and the macromolecule to be purified[2]. This is particularly important for protein–ligand complexes of relatively weak affinity ($K_i > 10^{-4}$ M). An effective approach in these cases is to attach first a hydrocarbon extension "arm" to the solid support; the ligand is then attached to this arm.

Chemical derivatives of agarose make it possible to attach a wider range of ligands and proteins to the support, and so increase the general versatility of affinity chromatography, especially in cases where (*a*) hydrocarbon chains of varying length (arms) are to be interposed between the matrix and the ligand, (*b*) amino groups are not present on the ligand, and (*c*) it is desirable to remove the intact protein–ligand complex by specific chemical cleavage of the ligand–matrix bond. We now report methods for preparing derivatives (Fig. 1); further details will be described elsewhere[2].

Ligands containing primary aliphatic or aromatic amino groups can be coupled directly to 'Sepharose' by the cyanogen bromide procedures previously described (Fig. 1; 1)[1,2]. ω-Aminoalkyl-'Sepharose' can be prepared by reacting diamine derivatives, $NH_2(CH_2)_xNH_2$, with cyanogen bromide-activated 'Sepharose' (Fig. 1; 2). Very little crosslinking occurs if a large excess of diamine is used, so that it is easy to insert extensions of considerable length, depending on the nature of $(CH_2)_x$. The amino-'Sepharose' derivatives thus prepared are the basis of a variety of subsequent procedures.

Bromoacetyl-'Sepharose' is prepared from amino-'Sepharose' by reaction in aqueous media at neutral *p*H with O-bromoacetyl-N-hydroxysuccinimide (Fig. 1; 3). Alkylated 'Sepharose' derivatives can then be prepared by reaction with proteins, peptides, or ligands containing amino, phenolic, or imidazole substituents (Fig. 1; 4). Ligands containing carboxylic groups can be coupled directly to amino-'Sepharose' with water soluble carbodiimides (Fig. 1; 5). Amino-'Sepharose' can be treated in aqueous media, at *p*H 6, with succinic anhydride to form carboxylic-'Sepharose' derivatives (Fig. 1; 6). Primary aliphatic or aromatic amines can in turn be coupled to these with water soluble carbodiimides (Fig. 1; 7). Diazonium-'Sepharose' derivatives are prepared by treat-

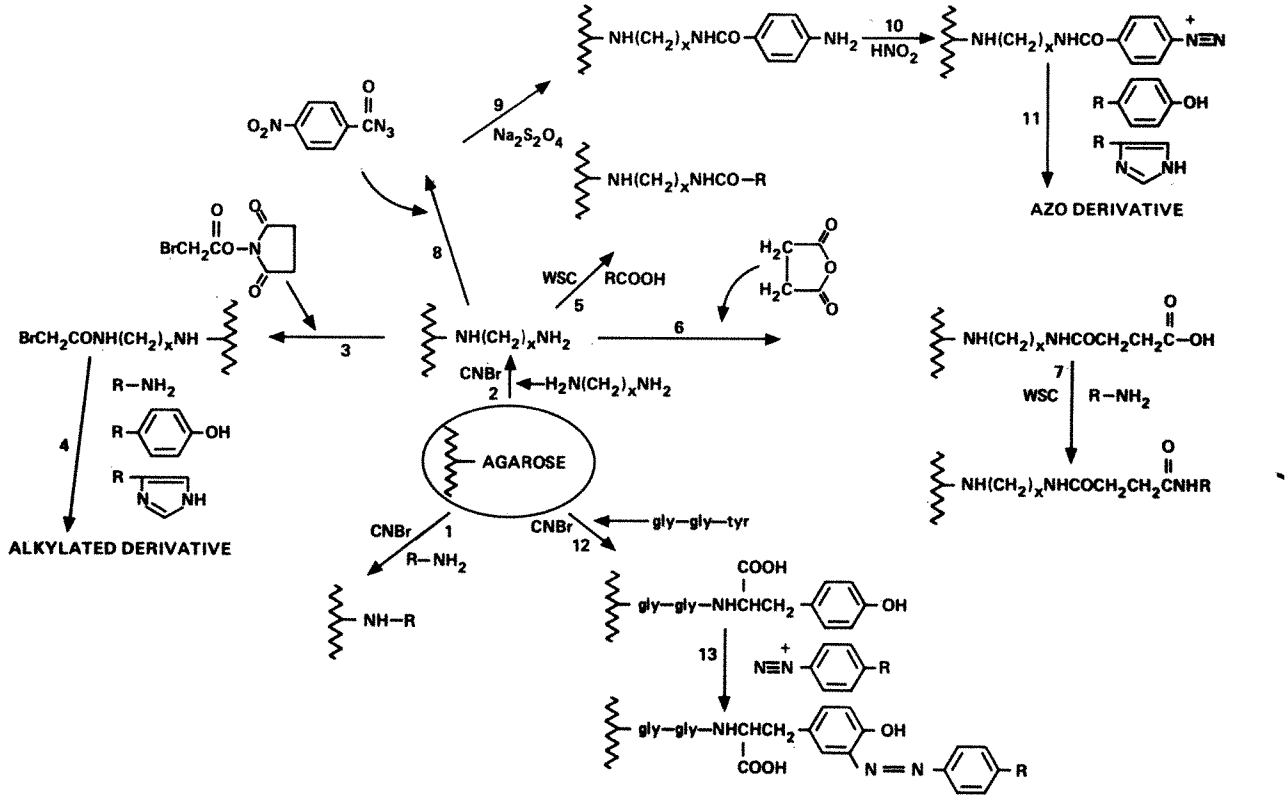


Fig. 1. Procedures for preparing derivatives of 'Sepharose 4B'.

ing amino-'Sepharose' with *p*-nitrobenzoyl azide in 50 per cent dimethylformamide (Fig. 1; 8), reduction to the aminophenyl derivative with sodium dithionite (Fig. 1; 9), and diazotization with nitrous acid (Fig. 1; 10). Ligands having phenolic or imidazole groups react rapidly with diazonium-'Sepharose' (Fig. 1; 11). Ligands that contain aromatic amino groups, which can be diazotized, can be coupled in azo linkage to a 'Sepharose' derivative which contains a covalently linked carboxy-terminal tyrosine peptide (Fig. 1; 12, 13). A special advantage of azo-linked ligands is the susceptibility to cleavage of the ligand-'Sepharose' bond by reduction with 0·1 M sodium dithionite at *p*H 8. The ligand–protein complex can thus be removed intact from a solid support which contains a selectively adsorbed protein. This form of elution may be necessary in cases where other forms of elution, such as changing the *p*H or ionic strength, or adding protein denaturants, cause irreversible inactivation of the protein.

Sulphydryl–agarose derivatives can be prepared by treating amino-'Sepharose' with N-acetylhomocysteine thiolactone at 4° C and *p*H 9·7[2]. Ligands containing free carboxyl groups can in turn be coupled to such thiol-agarose derivatives by reaction with water soluble carbodiimides. The thiol ester linkage produced can be specifically cleaved by a short exposure to *p*H 11·5, or with neutral hydroxylamine.

Many of these derivatives have been used in the purification of staphylococcal nuclease, amyloid protein, oestradiol binding serum protein, β-galactosidase, and antibodies (P. C., unpublished). In general, affinity adsorbants prepared by these procedures are superior to those prepared by attaching ligands or proteins directly to the agarose backbone by the cyanogen bromide procedure used until now. Bacterial β-galactosidase, for example, does not bind to adsorbants prepared by attaching *p*-aminophenyl-β-D-thiogalactopyranoside directly to 'Sepharose'. By contrast, strong adsorption of the enzyme occurs to adsorbants prepared by attaching the inhibitor to 'Sepharose' derivatives which contain long (20 Å) hydrocarbon "arms" (Fig. 1; 7) (Steers, jun., E., Pollard, H., and P. C., in preparation). Even where specific enzyme adsorption does occur with adsorbants prepared by direct attachment of the ligand, the total protein binding capacity is greater with adsorbants prepared from agarose derivatives which contain long hydrocarbon chains[2].

These agarose derivatives also provide alternative ways of insolubilizing peptides and proteins, which may be especially useful in the study of interactions of proteins or polypeptides with intact cells or particulate cell structures.

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Cross Partition and Isoelectric Points of Proteins

AQUEOUS mixtures of dextran and polyethylene glycol give rise to liquid two-phase systems suitable for the partition of proteins[1–5]. The partition behaviour of proteins depends to a great extent on the ionic composition of the phase system and on the charge of the protein. Negatively charged materials, for example, have higher partition coefficients in sodium sulphate than in sodium chloride while the reverse holds true for positively charged materials[5,6]. It was found that when a protein was partitioned at different *p*Hs in dextran–polyethylene glycol phase systems containing two different salt compositions two curves were obtained when partition coefficients were plotted against respective *p*Hs[5]. These curves crossed close to the isoelectric point of the protein. We have now carried out such experiments on many different proteins and found the relationship between cross point and isoelectric point to be general.

The partition coefficients of four proteins at different *p*Hs in either Na_2SO_4 or NaCl are shown in Fig. 1. For each protein two curves are obtained, one for each salt, which cross. In all cases proteins have higher K values in NaCl than in Na_2SO_4 to the left of the cross point while the reverse is true to its right. The cross point is very close to the protein's isoelectric points not only for the proteins shown in Fig. 1 but also for those described in Table 1.

Ribonuclease and trypsin are exceptions. In the case of ribonuclease a line (between *p*H 7·2 and 9·7) rather than a point joins the NaCl–Na_2SO_4 partition curves. Interestingly, the literature reports widely divergent isoelectric points for ribonuclease which fall into this *p*H range. All other proteins tested show a good correspondence between isoelectric and cross points. We propose therefore that the determination of a cross point can be used to estimate the isoelectric point of a protein (Table 1).

Cross points similar to those reported here can also be obtained with other pairs of salts; for example, KCl–Li_2SO_4. The *p*H of the cross point may then shift slightly. Also, higher concentrations of salts may be used. The cross points, when NaCl–Na_2SO_4 are used, shift only slightly with ionic strength. Isoelectric points can therefore be determined for proteins which are soluble or stable only at high salt concentration and for which electrophoresis is therefore difficult.

The partition coefficient at the corresponding cross-point is only slightly dependent on the ionic composition. It may be considered to be a characteristic constant of a protein.

There is ample evidence that the partition of proteins,

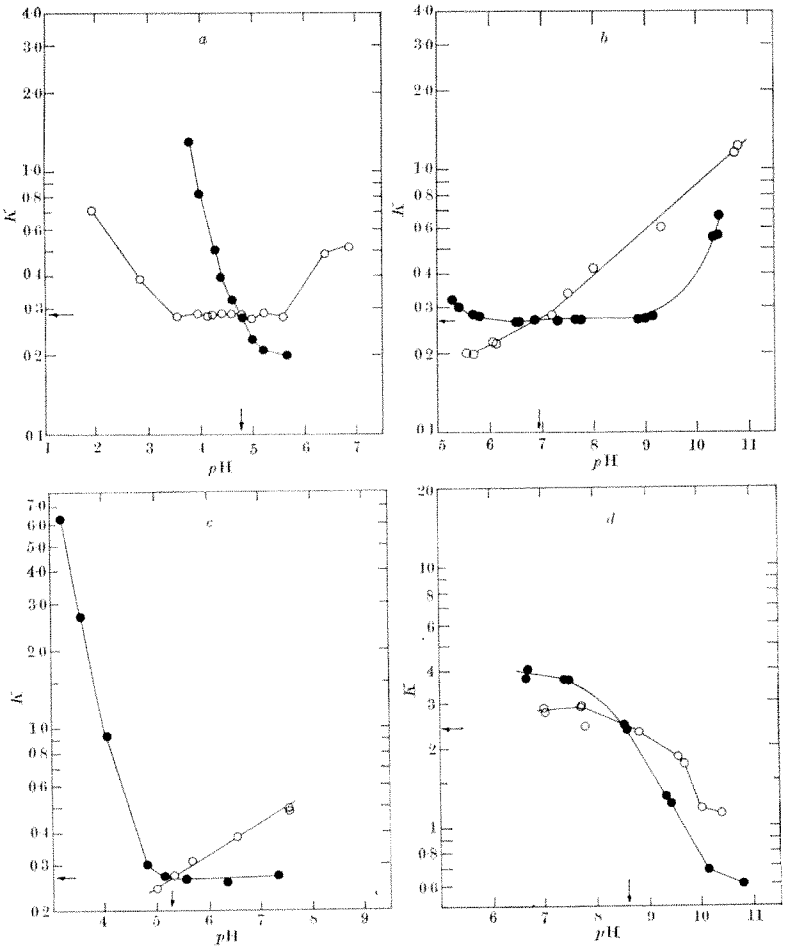


Fig. 1. Partition coefficient of proteins in two-polymer phases as a function of *p*H and two different salt compositions: (*a*) serum albumin, (*b*) haemoglobin, (*c*) transferrin, (*d*) papain. Phase system is composed of 7 per cent (w/w) dextran, 4·4 per cent (w/w) polyethylene glycol, 0·01 M buffer and either 0·1 M NaCl (●) or 0·05 M Na_2SO_4 (○). Dextran was obtained from Pharmacia, Uppsala, as 'Dextran 500'. Batch Nos. 3202, 4024 and 5996 were used interchangeably. Polyethylene glycol (PEG, 'Carbowax 6000') was obtained from Union Carbide. The procedure for preparation, partition and assay of proteins in the phases has been described[3]. Partition coefficients (K) are defined as protein absorbance at 280 nm (or 540 nm for haemproteins) in the top phase/protein absorbance in the bottom phase. Serum albumin, human, was obtained from Boehringer, Mannheim, papain was obtained from Sigma, and transferrin was a gift from Dr J.-O. Jeppson[24], Department of Medical Chemistry, Umeå.

Table 1. CROSS PARTITION POINTS AND ISO-ELECTRIC POINTS OF VARIOUS PROTEINS (*p*H UNITS)

Proteins	Cross point	Isoelectric point	References
Albumin			
Beef serum	4·7	4·71	7
Human serum	4·75	4·71	7
Hen egg white (ovalbumin)	4·75	4·6	8
α-Amylase			
Bacterial	4·7	—	—
Aspergillus oryzae	—	4·2	9
α-Chymotrypsin	9·0	{8·1 {8·6	7 10
Cytochrome *c*			
Horse	9·85	{9·8 {10·1 {10·65	11 12 13
Sheep	9·85	—	—
Haemoglobin (oxy)			
Human A	7·0	{6·87 {7·07	14 11
F	7·1	7·0	15
C	7·5	7·3	16
S	7·2	7·09	14
Horse	6·95	{6·92 {6·8	17
Pig	6·75	—	—
Dog	6·75	—	—
Lamb	6·9	—	—
Rabbit (oxy)	6·95	—	—
(met)	7·1	—	—
Insulin (beef)	5·4	5·35	11
Lysozyme			
Hen	11·0	10·5–11·0	18
Turkey	10·8	—	—
Myoglobin			
Beef	7·2	—	—
Horse	7·2	6·99	11
Papain	8·6	8·75	19
Ribonuclease A	7·2–9·7	{7·8 {8·2–9·6	20 21
Transferrin (human)	5·15	5·2	22
Trypsin	9·4	10·8	23

as well as that of other polyelectrolytes, is to a great extent dependent on their charge. Thus the partition of positively charged proteins (that is, below their isoelectric points) and of negatively charged proteins (above their isoelectric points) is salt-dependent in opposite ways. In NaCl the positively charged protein has a higher partition than the negatively charged protein while in Na_2SO_4 the reverse is true. It follows that the phases behave as if the bottom (dextran-rich) phase is more negative electrically than the top (polyethylene glycol-rich) phase in Na_2SO_4 than it is in NaCl. This phenomenon may be related to the significant, though small, unequal partition of ions in these phase systems (G. Johansson, in preparation).

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Effect of Free Fatty Acids on ADP-induced Platelet Aggregation

HUMAN platelets, like most mammalian cells, take up and metabolize long-chain free fatty acids (FFA)[1–3]. Unbound long-chain fatty acids are known to cause platelet aggregation which is associated with the release of adenine nucleotides and serotonin[4–6]. The purpose of this investigation was to determine whether exposure of platelets to albumin-bound FFA, in concentrations that are elevated but which can occur in physiological conditions, might affect platelet function. We have studied the influence of the FFA/albumin molar ratio ($\bar{\nu}$) on the response of platelets to adenosine diphosphate (ADP), a compound which may have an important role in haemostasis and thrombosis.

The effects of incubation of platelets with increasing concentrations of albumin-bound palmitic acid are shown in Fig. 1. As the $\bar{\nu}$ was raised, the degree and rate of aggregation induced by a standard amount of ADP increased. Aggregation is represented by an upward deflexion of the curve, and the size of aggregates is reflected by the length of the oscillations. The amount of ADP was constant in each experiment and was selected so that a definite but submaximal response occurred with the albumin solution containing no added FFA. In contrast to the results with palmitate (16 : 0), the unsaturated FFA, oleic (18 : 1) and linoleic (18 : 2) caused

Table 1. UPTAKE OF ^{3}H-PALMITATE BY HUMAN PLATELETS

FFA/albumin ratio (ν)	Total lipid	Uptake (nequiv/10^9 platelets) FFA	Phospholipid	Other
2	4·0 ±0·1	2·2 ±0·2	1·4 ±0·1	0·4 ±0·005
5	15·4* ±0·4	12·0* ±0·1	2·4* ±0·2	1·0* ±0·1

Results represent mean values ± s.e., obtained from two experiments and a total of six determinations. Incubation period was 5 min. Blood from healthy, non-fasting, human donors was drawn into Formula A–ACD in transfer packs (Fenwal Laboratories, Morton Grove, Illinois). The blood was transferred to siliconized conical tubes, and was centrifuged at 1,300 r.p.m. for 15 min at 20° C. The platelet-rich plasma (PRP) was transferred to siliconized conical tubes, and 1 ml. of 0·077 M EDTA was added for each 10 ml. of PRP. The PRP was then centrifuged at 2,500 r.p.m. for 15 min at 4° C. The platelet pellet was resuspended in a solution of 2×10^{-3} M human albumin in Tris-buffered saline containing $2{\cdot}4\times10^{-3}$ M glucose, and $1{\cdot}54\times10^{-3}$ M EDTA and then centrifuged at 1,200 r.p.m. Crystalline human albumin was obtained from Pentex Inc., Kankakee, Illinois, USA. The platelet pellet was resuspended in Tris-buffered saline containing 3×10^{-3} M glucose. Fatty acids were added to 1×10^{-4} M solutions of albumin in Tris–NaCl–KCl buffer (*p*H 7·4) (0·0154 M Tris–0·113 M NaCl–0·0057 M KCl) by the method of Spector and Hoak[7]. The FFA concentration of the resulting solution was determined by a modification of the method of Noble[8]. Unlabelled fatty acids were purchased from the Hormel Institute, Austin, Minnesota, and ^{3}H-palmitic acid was obtained from Amersham-Searle, Chicago, Illinois. Platelet aggregation was determined at 37° C using a turbidimetric method[9]. In each experiment, 0·75 ml. of the FFA-albumin mixture was incubated with 0·25 ml. of platelet suspension (containing 0·8 to 1×10^8 platelets/cm^3) for 3 min before adding 0·1 ml. of human fibrinogen[10] solution (1·5 mg/ml.). Two minutes later calcium was added (final concentration $2{\cdot}2\times10^{-3}$ M), and then ADP (1×10^{-6} to 5×10^{-4} M, final concentration) was added. A fatty acid–poor albumin solution of the same molarity was used as the control. In the uptake studies 2 ml. of ^{3}H-palmitate–albumin solution was incubated with 1 ml. of the platelet suspension containing 10^8 cells at 37° C with air as the gas phase[11]. These media contained $7{\cdot}5\times10^{-3}$ M glucose.

* Values are significantly different ($P<0{\cdot}01$) from corresponding values at $\bar{\nu}$ of 2.

little or no enhancement of aggregation even when the $\bar{\nu}$ was 6. Results obtained with stearic acid were similar to those with palmitic acid. When FFA of shorter chain length were used, less enhancement of aggregation was found. Myristic acid (14 : 0) produced a moderate enhancement of the ADP response, but lauric acid (12 : 0) had little or no effect.

The uptake of FFA by the platelets at different $\bar{\nu}$ values is shown in Table 1. At a $\bar{\nu}$ of 2, 55 per cent of the platelet lipid radioactivity was in FFA and 45 per cent was in ester forms. When the $\bar{\nu}$ was increased to 5, the incorporation of ^{3}H-palmitate into all platelet lipids increased significantly, but the greatest increase, over a five-fold rise, was in the FFA fraction. Thus the major effect of increasing the extracellular FFA concentration was to produce a marked increase in the amount of labelled palmitate incorporated into the platelet as FFA.

Only small differences have been noted in the amounts of various long-chain saturated or unsaturated fatty acids incorporated into platelet phospholipids, triglycerides or CO_2[11]. In contrast, much more saturated fatty acid was taken up by the platelet as FFA than was unsaturated fatty acid[11]. The results of our aggregation experiments indicate that fatty acids which are taken up in unesterified form to a greater extent by the platelet also produce greater enhancement of ADP-induced aggregation. In earlier studies performed with the unbound salts of fatty acids long-chain saturated FFA also produced greater platelet aggregation than did unsaturated or short-chain FFA[6].

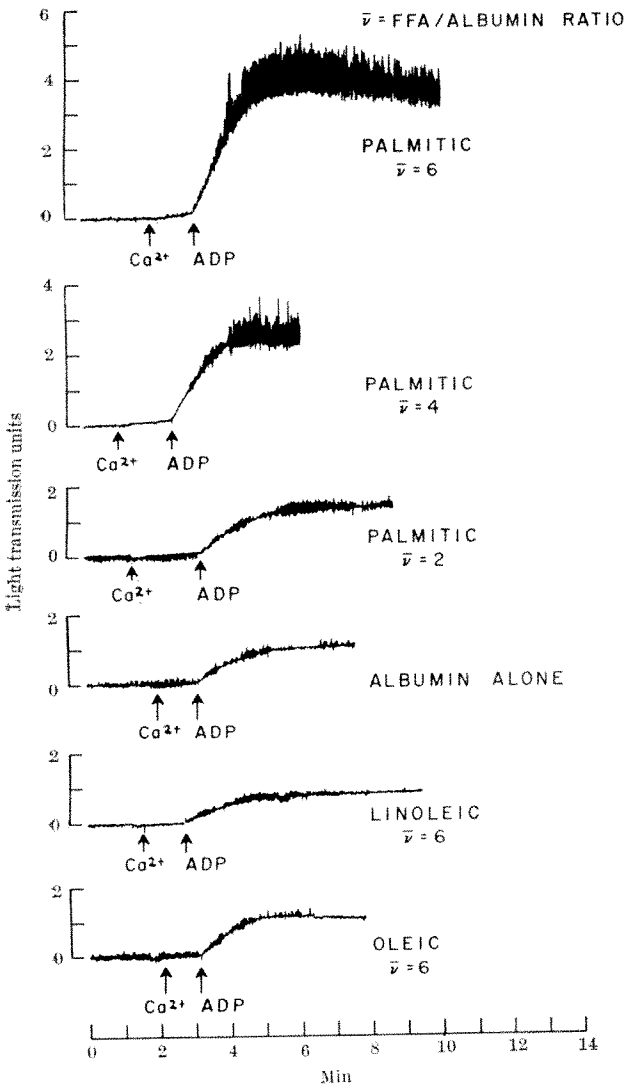


Fig. 1. Effect of albumin-bound palmitic (C16 : 0), oleic (C18 : 1) and linoleic (C18 : 2) acids on platelet aggregation induced by adenosine diphosphate (final ADP concentration added was 1×10^{-6} M). Final Ca^{2+} concentration was $2{\cdot}2 \times 10^{-3}$ M.

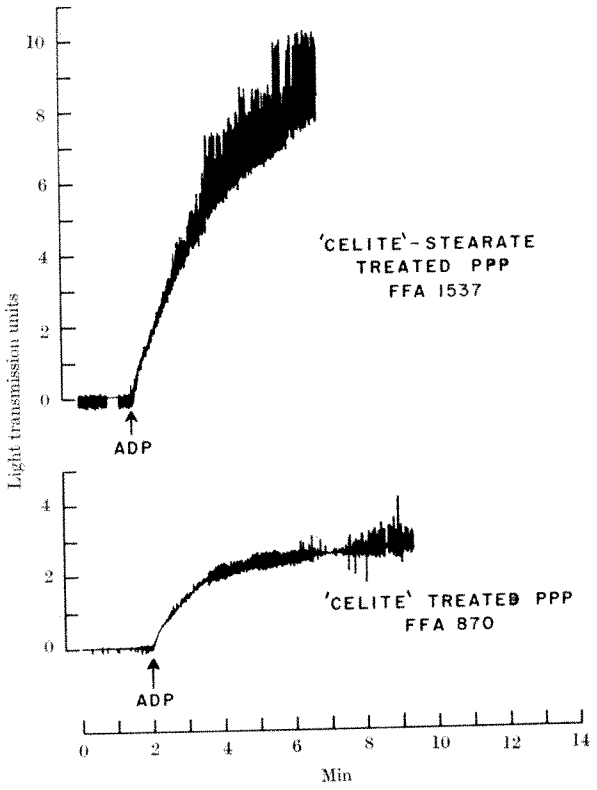


Fig. 2. Effect of high concentration of FFA in the plasma on aggregation by ADP. Donor platelets were exposed to platelet-poor donor plasma which had its FFA concentration increased by incubation with Celite' particles coated with stearic acid. After the 'Celite' was removed, the platelets were resuspended in the plasma before ADP was added. Final ADP concentration added in each experiment was $2{\cdot}5 \times 10^{-6}$ M.

In additional experiments, a mixture of FFA–albumin solutions was used with the FFA composition comparable with that found in plasma (myristic 5 per cent, palmitic 25 per cent, stearic 15 per cent, oleic 40 per cent and linoleic 15 per cent). No enhancement of aggregation was seen at a $\bar{\nu}$ of 2, but definite effects similar to those found with long-chain saturated FFA were observed when the molar ratio of total FFA to albumin was 4 or 6.

In other experiments, donor platelets were first separated from the plasma and the FFA content of the donor plasma was raised by incubation with 'Celite' particles coated with stearic acid. The 'Celite' was removed, and the platelets were then resuspended in the plasma before ADP was added. As a control, ADP was added to donor platelets resuspended in platelet-poor donor plasma that had been incubated with uncoated 'Celite'. Plasma FFA concentrations were determined on both plasma samples. Enhanced aggregation by ADP was observed only when platelets were suspended in plasma containing a high concentration of FFA (Fig. 2).

We interpret these results to indicate that exposure to a high concentration of fatty acid may alter the reactivity of the platelet and thereby enhance its responsiveness to ADP on to other agents which influence platelet function *in vivo*. The principal effect of an increase in the FFA/albumin molar ratio was to increase the FFA content of the platelet. A dynamic equilibrium state may exist between the binding of FFA by albumin and the uptake of FFA by platelets and other cellular elements. Acute lipid mobilization causing high plasma FFA concentrations or disease states in which the albumin

content or FFA binding capacity are reduced might produce a transient state in which excessive amounts of FFA associate with platelets. In this condition the platelet may develop a propensity toward aggregation which could lead to the formation of thrombi.

The enhanced aggregation of platelets related to the uptake of FFA may have relevance to variation in the response of platelets from different donors or at different time intervals in the same donor to aggregating agents; to the development of thrombocytopenia in some patients treated with heparin due to activation of plasma lipolytic activity; and to a link between stress and an increased susceptibility to thrombosis.

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Genetic Content of Adult Somatic Cells tested by Nuclear Transplantation from Cultured Cells

It has yet to be proved that somatic cells of an adult animal possess genes other than those necessary for their own growth and differentiation. A particularly stringent test of the genetic content of the nucleus of a somatic cell is provided by transplanting it to an enucleated egg. The development of the egg tests the capacity of the genes in the transplanted nucleus to elicit normal early development and differentiation. Although this method has been applied to certain cells of embryos, larvae[1,2], and newly metamorphosed froglets[3], it has not been possible, until now, to transfer nuclei successfully from the cells of normal adult animals.

The experiments described here use cell cultures (Fig. 1) to obtain rapidly growing cell populations from adult organs of the frog *Xenopus laevis*. Nuclei from cultured cells are transplanted into enucleated eggs by the method described previously[4]. The results show that cells cultured from several different adult organs contain a wide range of genes which are not normally expressed in the original tissues. The development of enucleated eggs which received a transplanted nucleus is shown in Table 1. As in our previous experiments with nuclei from cultured tadpole cells[5], most eggs which received nuclei from adult organs failed to cleave or cleaved abortively to form blastomeres of irregular sizes. Most of the nuclear transplant eggs which contained a region of apparently normal cells also contained an uncleaved or abortively cleaved region. In previous experiments[5], it was observed that blastulae which are imperfect in any way usually die before the early gastrula or late blastula stages. This is true of the partial blastulae prepared from adult organ nuclei, and it is not informative to consider the development of first-transfer embryos beyond the late blastula stage.

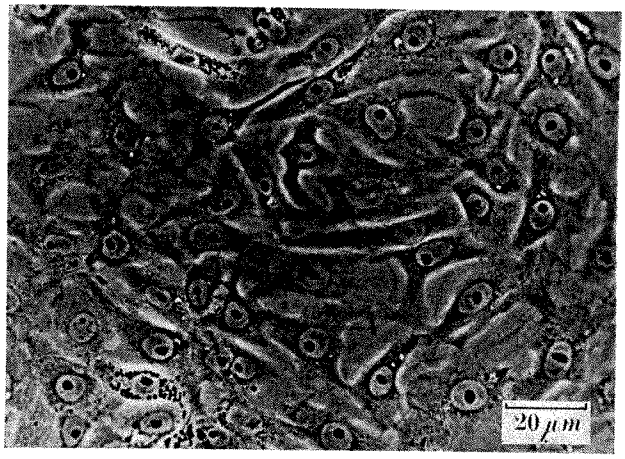


Fig. 1. Primary monolayer culture of trypsinized adult 1-*nu* kidney cells, 3 days after plating, showing only one nucleolus in each nucleus. For some experiments cultures were prepared as outgrowths from explants in fibrin clots. All cultures were maintained in modified L-15 medium[5,11].

Although partial blastulae cannot develop normally, it has been shown in previous work[1,5] that some of their cells may contain nuclei which can support normal development. The cells of a partial blastula may be dissociated and used to provide a source of donor nuclei for a second series of nuclear transfers. In this way it is possible to prepare a clone of embryos all of which contain nuclei derived by mitosis from the same original cultured cell. Nuclear transplantation sometimes results in chromosomal and developmental abnormalities[6-9]; so the most normal serial-transplant embryo within a clone provides a minimum estimate of genetic information contained in the originally transplanted nucleus.

Table 1. THE DEVELOPMENT OF EGGS RECEIVING SINGLE NUCLEI TRANSPLANTED FROM CULTURED CELLS

Source of cultured cells	Total number of nuclear transfers	Percentage cleaving to partial or complete blastulae (stage 9)* (ref. 13)
Stage 40 tadpoles	3,546	30·4†
Adult kidney	789	26·2
Adult lung	502	23·9
Adult heart	273	22·0
Adult testis	73	26·1
Adult skin	1,031	31·2

* At least half of each egg in this category cleaved completely to reach the late blastula stage.
† Data from ref. 5.

The results of serial nuclear transfers from cultured cells of various origins are summarized in Fig. 2. Two conclusions can be drawn from first as well as serial transfers. First, the development supported by nuclei from cultured cells of different origins is strikingly similar. Second, the developmental capacity of nuclei from cell cultures of adult organs closely resembles that of nuclei from tadpole cell cultures.

Before firm conclusions can be drawn concerning the gene content of a transplanted nucleus, it is necessary

to prove that the egg nucleus has not contributed to the development of the nuclear-transplant embryo. Although the reliability of ultraviolet irradiation for enucleating eggs of *Xenopus* has been demonstrated[10] it is desirable to prove that the nuclei of a transplant-embryo are the mitotic products of the transplanted nucleus and not of the recipient egg nucleus. For all the experiments described here, cell cultures have been grown from animals which are heterozygous for the anucleolate mutation[11] (1-*nu*) and therefore have only one nucleolus per diploid chromosome set (Fig. 1). All the frogs whose eggs were used as recipients for either first or serial nuclear transfers were shown to be 2-*nu* (Fig. 3) and therefore have two nucleoli per diploid chromosome set. When a 1-*nu* diploid nucleus is injected into an egg laid by a 2-*nu* frog, participation of the egg nucleus could give rise to a 1-*nu* haploid or 2-*nu* diploid. Alternatively, the combination of the egg nucleus with the injected nucleus could give rise to 2-*nu* triploids or 3-*nu* tetraploids but the egg nucleus could not contribute to 1-*nu* diploid embryos. Care must be taken in interpreting the origin of embryos which are 2-*nu*, for the nuclear transfer procedure is known to raise the normal low incidence of tetraploids[12]; these would therefore be 2-*nu* even though the transplanted nucleus is the sole source of their nuclei. In previous work[5] we have excluded the possibilities that nuclear-transplant embryos might consist of a mosaic of haploid and diploid cells, or that ultraviolet irradiation could cause the recipient egg nucleus to change from a 2-*nu* to 1-*nu* constitution.

Altogether, 5,200 eggs have received nuclei serially transplanted from adult somatic cells. It has not been possible to test the origin of the nuclei in all the embryos

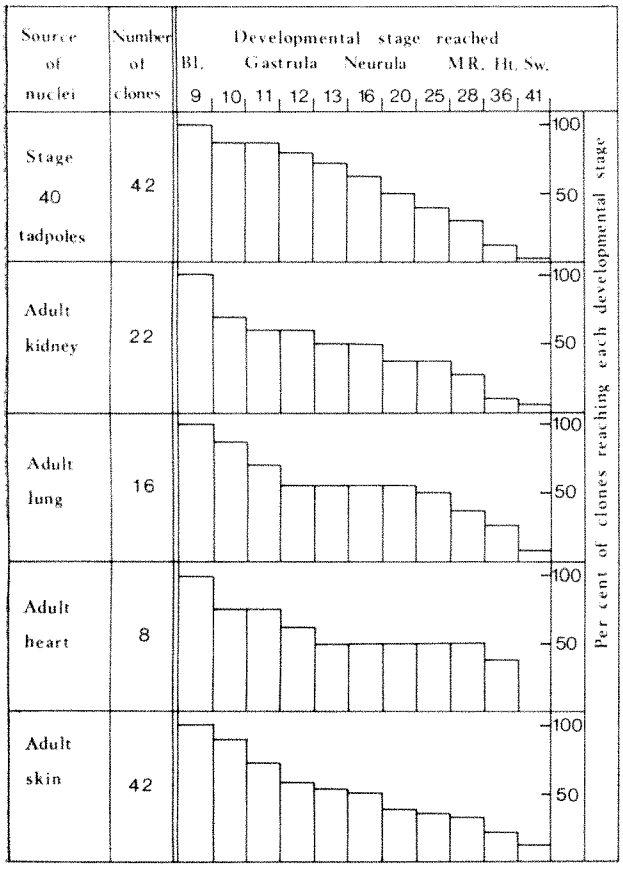


Fig. 2. The development of embryos in clones derived by serial transplantation of nuclei from cultured cells. The developmental stage reached by the most normal embryo is shown for each clone. Abbreviations: Bl, blastula; MR, muscular response; Ht, heart beat; Sw, spontaneous swimming.

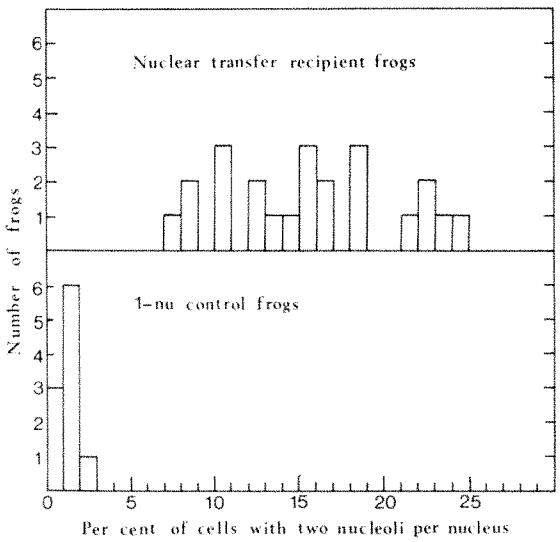


Fig. 3. The nucleolar number of frogs whose eggs were used as recipients for nuclear transfers. Skin explants from each frog were cultured in plasma clots. Five hundred cells in the monolayer outgrowth from each explant were scored to determine the percentage of cells with two nucleoli per nucleus. The histogram relates this value to the number of frogs tested. All the frogs used to provide recipient eggs were clearly different in nucleolar number from 1-*nu* controls, as expected of wild type frogs.

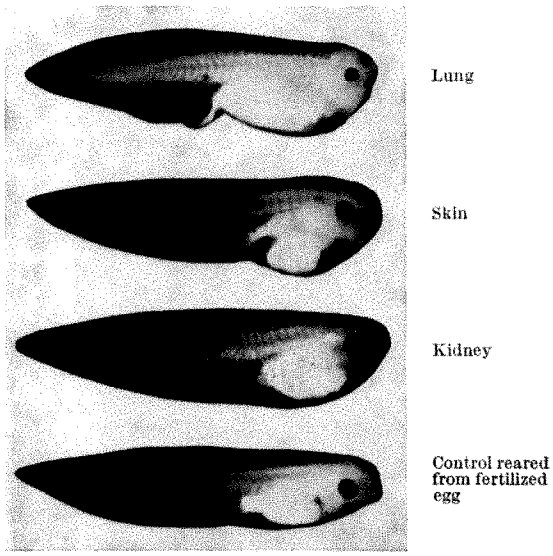


Fig. 4. Swimming tadpoles obtained by serial nuclear transfers from cultures of adult somatic cells. The tadpoles derived from lung, kidney and skin cells were all 1-*nu* diploids (±5), and their nuclei were therefore authenticated products of the transplanted nucleus (×15).

developing from these, and attention has therefore been focused on those embryos which developed as far as or beyond the stage with functional contracting myotomes. The nucleolar number has been determined for sixty-six embryos of this and later developmental stages contained in sixteen serial transfer clones. 71 per cent of these embryos were 1-*nu*, 11 per cent were 2-*nu* and 17 per cent were 0-*nu*. Most of the 0-*nu* embryos were contained in a single clone, and probably arose by loss of the nucleolus-bearing chromosome (or part of it) soon after nuclear transfer. Chromosome preparations were made from thirty-three of the 1-*nu* embryos (Table 2). Thirty-two of these were diploid or near diploid and only one was haploid. Similarly chromosome preparations from 2-*nu* embryos showed two to be tetraploid and therefore products of the transplanted nucleus. From these results it

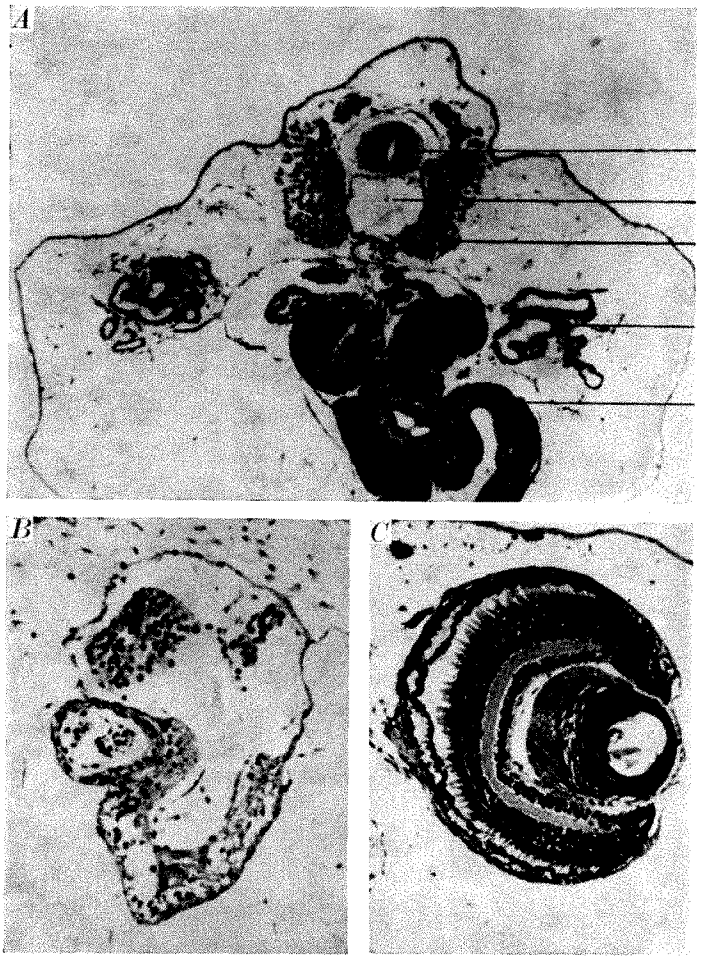


Fig. 5. Transverse sections through a 1-*nu* diploid swimming tadpole obtained by serial nuclear transfer from an adult skin cell. *A*, Dorsal region of mid-body (×150); *B*, heart containing nucleated erythrocytes (×420); *C*, eye (×300).

is clear that the egg nucleus has not participated in most of the nuclear-transplant embryos which reached advanced stages of development. In conclusion, 1-*nu* diploid tadpoles obtained in these experiments constitute a valid test of the developmental capacity of the transplanted nuclei.

Eighty-eight serial-transfer clones were prepared from the nuclei of cell cultures derived from adult organs; 32 per cent contained at least one tadpole which reached the stage of contracting myotomes (about stage 28 of Nieuwkoop and Faber, 1956). Some of these tadpoles were proved to be products of nuclei from adult kidney, lung and skin (Table 2). Furthermore, some tadpoles from each of these sources developed to more advanced stages (stage 41 and beyond[13]) as shown in Fig. 4. They performed frequent normal swimming movements, indicating functionally normal nerves, muscles, and neuromuscular connexions. An extensive and rapid circulation of red blood cells was maintained by a heart contracting regularly, indicating normal cardiac muscle function and erythrocyte differentiation. The nuclear-transplant tadpoles differ from stage 41 tadpoles reared from control fertilized eggs in only one obvious respect, the development of anal and cardiac oedema, a possible cause of their subsequent death. Examination of sections of these tadpoles revealed many different tissues and cell-types of apparently normal construction. Erythrocytes, skeletal and cardiac muscle, pronephros, notochord, nerve cord and complex sensory structures could be identified (Fig. 5). No cell-type could be recognized in tadpoles reared from fertilized eggs which could not be found in the nuclear-transplant tadpoles.

Table 2. VALIDITY OF NUCLEAR TRANSFER EMBRYOS REACHING THE MUSCULAR RESPONSE STAGE

Source of donor nuclei	Number of tadpoles examined	Number of tadpoles known to be derived from only the transplanted nucleus	
		1-*nu* diploids*	2-*nu* tetraploids†
Adult kidney	13	13	—
Adult lung	6	4	2
Adult skin	16	15	—

* 36±5 chromosomes in at least 4 metaphases.

† 72±10 chromosomes in at least 4 metaphases.

The most important conclusions are first that three different somatic tissues of an adult vertebrate contain nuclei which can support the development of tadpoles and must therefore have genes necessary for the formation of many cell-types; and second that no difference has been detected between the development supported by nuclei from adult kidney, lung and skin.

The results reveal very little about the proportion and identity of the cells, within the host tissue, whose nuclei were transplanted successfully. About 25 per cent of nuclear transfers from adult cells gave first-transfer embryos suitable for use as serial transfer donors; of these 32 per cent yielded clones which contained some tadpoles capable of muscular movements. Our two conclusions therefore do not necessarily apply to more than 8 per cent of cells tested. The use of cultures grown from explants in plasma clots largely avoids the specific selection of cells able to stick to the substrate, and the use of nuclei from primary cultures within a week of preparation prevents heavy selection of a minority cell type. Thus our conclusions should apply to typical cells of the host tissues concerned. We are now attempting to extend these experiments to cells which are demonstrably differentiated or determined, using cells derived from adult frog skin.

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Neuronal Populations in the Human Brain

THE deterioration of mental function in old age may be due to loss of neurones or decay of their function. Several studies support the former view[1,2] but sample sizes and the number of cases reviewed were small. Accordingly we studied the ventral cochlear nucleus (VCN) because it is well defined and small enough to make accurate counts feasible.

Brains from neurologically normal cases were obtained from autopsies at the Baltimore City Hospitals, the Johns Hopkins Hospital and from the Infectious Disease Hospital in Madras, India. The twenty-three cases ranged from the newborn to 90 years of age. The brain-stem was embedded as a single block in celloidin. Serial sections, 20 μM thick, were taken; every tenth section through the VCN was stained with cresyl violet. The VCN on each section was outlined with India ink, clearly separating it from surrounding structures. Under the 25× objective and with a magnification of 312×, all neuronal nucleoli in each section were counted with a reference grid[3]. Nucleoli or nucleolar fragments less than 1μ in diameter were not counted. Two counts done by different observers were averaged and the sum of the means multiplied by ten. A correction for split nucleoli was used[3].

The neuronal population estimates in the twenty-three cases, arranged by race, sex and age, are shown in Table 1. The figures under "basic count" columns are the totals of the uncorrected neuronal nucleolar counts for each of the two sides. The percentage difference between the two counts is shown in the next column. The "corrected counts" column on the right shows estimated totals adjusted by the nucleolar factor and period. The mean was 48,010 cells with an s.d. of 4,550. The 95 per cent tolerance interval on the central 95 per cent of cases was 37,210–58,810 cells. The percentage difference between the two sides was 0·01–12 per cent with a mean of 4·2 per cent.

Covariance analysis of the regression of count on age suggests no heterogeneity and accordingly the pooled results can be represented by the regression line:

$$y = 47{,}808 + 19{\cdot}77x$$

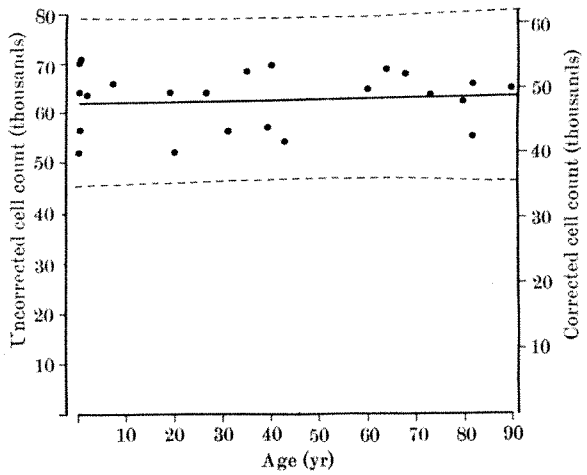


Fig. 1. The relationship between the mean neurone count and age. On the left scale are the uncorrected values and on the right scale are the corrected values. The continuous line is the least squares linear regression line and the interrupted lines are the 95 per cent tolerance limits on the central 95 per cent of the distribution.

where y is the mean of the cell counts in the two nuclei and x is the age in years. The regression line has a positive gradient, but a one-tailed test against the alternative hypothesis that the regression slope is positive gives $t = 0{\cdot}650$, $P = 0{\cdot}2614$. Thus the positive regression slope is not significantly different from zero.

A plot of the mean count against age is shown in Fig. 1 with the fitted regression line through them. Also shown are the 95 per cent tolerance limits on the central 95 per cent of values[4]. Sample estimates of components of variance are:

Within subjects (from comparison of the two sides): $4{\cdot}2 \times 10^6$ counts^2; among subjects: $17{\cdot}7 \times 10^6$ counts^2. Thus more than 80 per cent of the total variance arises from differences among individuals.

In the method used here there are four sources of error. First, outlining structure: the VCN was distinguished by its characteristic medium-sized, plump, ovoid cells, which contrast with the spindle-shaped cells of the dorsal cochlear nucleus; there is little difficulty in separating the VCN from the adjacent pontobulbar body. Second,

Table 1. COUNTS OF NEURONE POPULATION IN THE VENTRAL COCHLEAR NUCLEUS

Case	Age in years	Basic count Side 1	Basic count Side 2	Difference	Corrected count Side 1	Corrected count Side 2	Corrected average
				White male			
1	0·3	6,736	7,461	10 per cent	51,380	56,910	54,145
2	43	5,149	5,710	10 per cent	39,275	43,554	41,415
3	64	6,896	6,913	0·2 per cent	52,601	52,730	52,666
4	68	6,683	7,040	5 per cent	50,970	53,699	52,338
				Negro male			
5	0	6,992	7,042	0·7 per cent	53,333	53,714	53,524
6	0	5,171	5,231	0·01 per cent	39,443	39,901	39,672
7	35	6,784	6,961	3 per cent	51,747	53,097	52,422
8	60	6,380	6,591	3 per cent	48,665	50,274	49,470
9	80	6,262	6,283	0·02 per cent	47,765	47,925	47,845
				Indian male			
10	1·5	6,281	6,455	3 per cent	47,910	49,237	48,573
11	7	6,598	6,601	0·05 per cent	50,328	50,351	50,339
12	19	6,391	6,451	1 per cent	48,749	49,206	48,978
13	40	6,752	7,237	7 per cent	51,502	55,202	53,352
				White female			
14	0	6,323	6,461	2 per cent	48,230	49,283	48,756
15	0·1	5,446	5,874	8 per cent	41,541	44,805	43,173
16	27	6,264	6,603	6 per cent	47,780	50,366	49,073
17	31	5,307	5,998	12 per cent	40,480	45,751	43,116
18	73	6,308	6,500	3 per cent	48,116	49,580	48,848
19	82	5,448	5,688	4 per cent	41,556	43,387	42,471
20	82	6,246	7,005	11 per cent	47.643	53,432	50,538
				Negro female			
21	40	5,689	5,777	1 per cent	43,394	44,065	43,730
22	90	6,370	6,724	5 per cent	48,589	51,289	49,939
				Indian female			
23	20	5,123	5,308	3 per cent	39,977	40,488	39,782

Ventral cochlear nucleus: basic counts are the actual neuronal nucleolar counts on each side. Corrected counts are the product of the basic count, the period (10) and the NCF (0·76266).

sampling error: a systematic (periodic) 10 per cent sample gives an accuracy of within 5 per cent of the true count[5]. Third, counting errors occur when sections are poorly stained so that nucleoli are not clearly seen; when sections are too thick, resulting in difficulty in keeping track of the cells counted in a small field; when there is excessive crush cell artefact with nucleoli poorly seen in the densely stained nucleus; when directions are reversed during counting, a portion of a row being missed or recounted; when small neurones are not counted; or when some glial cells are taken for neurones. One author (B. W. K.) took periodic counts checking the accuracy of the two technicians. All counts not clearly wrong were averaged and taken as the number for the nucleus on that slide. Fourth, split cell error, which was corrected approximately[5] by the factor $t/(t+2a)$, where t is the section thickness in microns and a is the thickness of a sector of a nucleolus which presents a cross-section 1 μM in diameter. This formula assumes that the nucleolus is of the same consistency as the remainder of the tissue. There is evidence, however, that it is tougher and may be pushed by the microtome knife into one or the other sections[6]. If this is so, the true neuronal population would lie between the count calculated with, and that without, the nucleolar correction factor. We therefore give the basic count (the sum of the counts on each of the slides), and the corrected count (the product of the total count, period and nucleolar correction factor).

In our group of twenty-three cases, ten had audiometric evaluations. These cases, arranged in order of increasing numbers of neurones in the VCN, showed no clear relationship to the simple hearing tests done. With increasing age the human brain loses both volume and weight. A few studies have suggested that there is a loss of neurones[1,2]. Brody[2] studied blocks of cortex from several areas, in all only about 0·0005 per cent of the total neuronal population of the cerebral cortex. Ellis's[1] estimate of Purkinje cells in the cerebellar cortex was based on a small sample and again inconclusive. Our work suggests that there is no neuronal loss with age in a particular nucleus of the brain stem; but loss may occur elsewhere. Other structures of the brain need to be studied to determine whether the effect of age is uniform. No suggestions have been offered as to why the female brain is lighter than the male. Covariance analysis of the regression of counts in thirteen males and ten females on age shows that the regression slopes are not dissimilar but women have slightly lower mean values. While the difference just attains significance at the 5 per cent level, too much must not be made of this result which may be an artefact of the multiplicity of comparisons. In our series were eleven Caucasians, seven Negroes and five Indians. No significant differences of slope or intercept were found.

Hall[7] found a mean VCN neuronal population, in nine nuclei, of 68,700 (s.d. = 8,000). This mean figure is slightly but not significantly higher than our uncorrected mean count (62,939). His standard deviation was about twice that obtained by us, perhaps because of the smaller sample used in his counts.

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Integumental Pigments of the Amphipod, *Jassa*

THE integumental pigments of the isopod Crustacea are ommochromes[1,2], but by contrast the effectively similar colour of the amphipod *Gammarus* is due to carotenoids[3], probably in the form of carotenoproteins. The implication of these findings was that this constitutes a general distinction between these rather closely related orders.

It now seems, however, that *Jassa marmorata* Holmes, also in the suborder Gammaridea, and at least one member of another amphipod suborder have ommochromes as integumental pigments. The possibility was first recognized when the patchy distribution of the dark pigment of *Jassa* was first noticed, that of *Gammarus* being very uniformly distributed over the body. Under the microscope *Jassa* was seen to have a granular pigment, ranging in colour from old gold to dark slate, segregated in the astroid chromatophores characteristic of the Crustacea. The carotenoids of *Gammarus* are very diffusely distributed, not in this special type of cell.

After extracting with methanol the limited amount of carotenoids present in *Jassa* (chiefly, if not entirely, in the viscera, which are not easily removed from such small animals), the integumental pigments were extracted with the usual ommochrome solvents, in particular methanol–HCl and formic acid. Their properties proved very similar to those of isopods[2] and, as in the latter, both ommatin and ommin sub-classes were found to be present.

Fig. 1 shows the absorption spectra, for the range 200–700 nm, of the ommatins extracted by (A) a mixture of 19 parts of methanol and 1 part of concentrated HCl (v/v) and (B) the same mixture diluted with one quarter of its volume of water. The four peaks characteristic of ommochromes[4] are evident. In 5 N HCl the peaks were at 222, 241, 348 and 475 nm, the bathochromic shift of the one in the visible range also being characteristic of ommatins. Also, like xanthommatin, this pigment formed a crimson precipitate on reduction and raising the *p*H to 3–4. The precipitate dissolved in isoamyl alcohol, its absorption spectrum then having peaks at 235, 284, 389 and 486 nm. The oxidized form dialysed through visking tubing, like known ommatins.

After exhaustive extraction of the ommatin, in its yellow oxidized form, mixture A extracted ommin, in its purple reduced form, having absorption peaks at 251, 351 and 514 nm. In 90 per cent formic acid solution the peaks were at 267, 393 and 523 nm. Both ommatin and ommin formed purple to violet "halochromes" in high concentrations of sulphuric acid, in the same conditions as in isopods[2].

Chromatographically, also, using as developer the v/v mixture, formic acid : methanol : concentrated HCl : water (15 : 3 : 1 : 6), the ommatin and ommin behaved like those of isopods. In all significant respects, therefore,

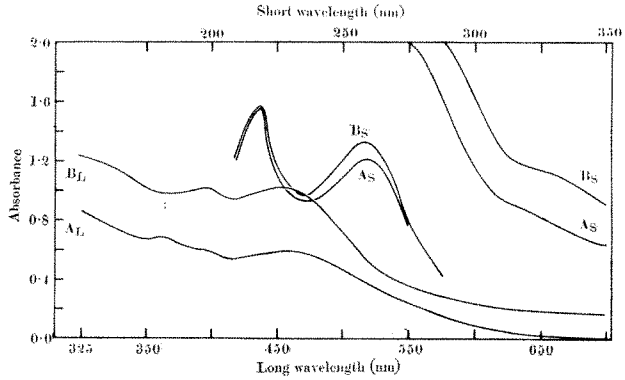


Fig. 1. Absorption peaks of ommatins. S, Short wavelength; L, long wavelength. See text for further explanation.

the ommochromes of *Jassa* are closely related to those of isopods.

As in the isopods, there was some indication that more than one ommatin was present. Thus the material extracted by methanol : HCl : water had its *A*, *B*, *C*, *D* absorption peaks respectively at 219, 257, 393 and 449 nm (Fig. 1), whereas that extracted by undiluted methanol : HCl gave peaks at 218, 259, 360 and 455 nm, with an indication of a second *C* peak at the 393 nm position. On chromatograms there was some indication of two ommatin spots, but overlapping each other as in the isopod material. If there is a second ommatin, similar to the "oniscoid" ommatin[2], it must be a very minor component, quantitatively, for the *D* peak of all extracts from fresh material was in the 450–456 nm range. A lower value, in the range 440 to 450 nm, with a lower peak, was obtained only from moribund material and from material stored for more than 2 days in methanol before extraction, and so may be artefactual.

The absorption spectrum in the various states and solvents, and most other properties examined, imply that the principal ommatin in living *Jassa* is xanthommatin, the most common of biological ommatins. The precipitated, reduced form, however, proved insoluble in phosphate buffer at *p*H 7·0 and the halochrome resembled that of the oniscoid ommatin in its lack of brilliance[2].

An unidentified species of "skeleton-shrimp" belonging to another suborder of amphipods, the Caprellidae, also was found to have integumental ommatin and ommin, arranged in much the same patterns as in *Jassa* and with very similar chemical properties. This group of pigments therefore may be relatively common in Amphipods. They were also found in the same general forms in a tanaid (probably *Tanais cavolini* Milne Edwards), although this is not surprising because the Tanaidacea are generally regarded as closely related to the isopods, but it strengthens the possibility that integumental ommochromes may be characteristic of the Peracarida in general. They have been found in the integument also of decapods and stomatopods[5] and so may be common to the Malacostraca in general. They occur in the eyes of the "Entomostraca" and, altogether, may be as common in Crustacea as in the Insecta.

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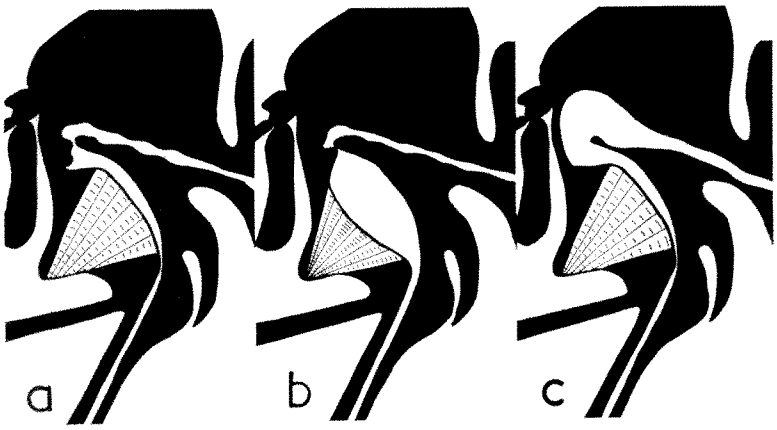


Fig. 1. Diagrammatic sections of tsetse fly head, showing the action of the cibarial pump.

Function of Resilin in Tsetse Fly Feeding Mechanism

Resilin is an elastic structural protein which was discovered by Weis-Fogh in the wing-hinges of locusts[1]. The substance can be identified with certainty only by the use of several tests, by means of which it has been demonstrated in specific areas of cuticle in insects and crustacea[2].

In tsetse flies and other Diptera, food is sucked into the gut by the action of cibarial dilator muscles[3,4]. When the muscles contract, they draw the anterior wall of the cibarial pump away from the more rigid posterior wall, opening the lumen, which fills with food drawn up through the proboscis. Suck-back from the oesophagus is prevented by the dorsal plate, which swings across the oesophageal opening. When the dilator muscles relax, the anterior wall reverts to its resting position and at the same time the dorsal plate swings back, allowing the passage of food into the oesophagus (Fig. 1).

In *Glossina austeni*, the cibarial pump has been operated *in vitro* by depressing the anterior wall with an electromagnetically activated probe[5]. In such experiments the return action of both anterior wall and dorsal plate immediately follows withdrawal of the probe and is independent of muscular activity. The transfer of food from the pump into the oesophagus is therefore a passive process, relying on energy stored in some elastic part of the dilated pump.

The pigmentation of the anterior cibarial wall in tsetse flies has been illustrated by Jobling[3]. Around the dark brown median apodeme there is a more lightly sclerotized area, the rest of the wall being fairly transparent. When the pump is operating, it is the transparent parts which are bent, the peripheral regions of the anterior wall being doubled over[5]. There is little bending at the hinge between the anterior and posterior walls. In the electron microscope the transparent, flexible parts are seen to be unstained and almost completely electron lucent—a characteristic of pure resilin[2]—whereas the posterior wall at the same locus has a dense cuticular appearance (Fig. 2).

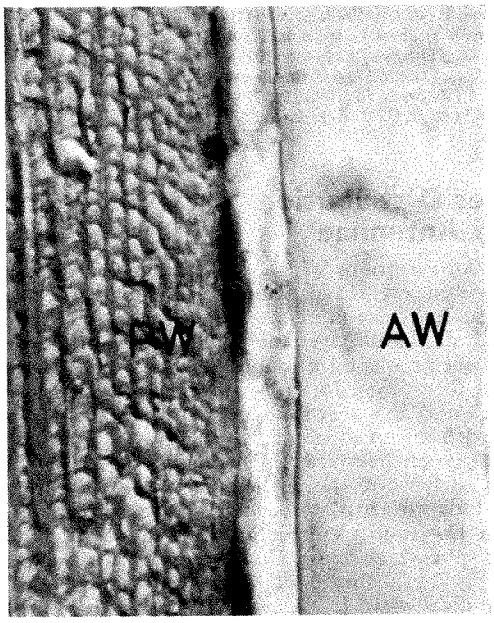


Fig. 2. Electronmicrograph of a section of the anterior (AW) and posterior (PW) cibarial walls of *Glossina austeni*.

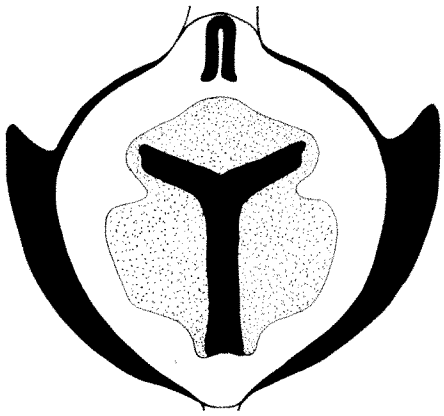


Fig. 3. Diagram to show the topography of resilin (unshaded areas) in the anterior cibarial wall.

The transparent material of the anterior wall fluoresces blue in ultraviolet at *p*H 12 and is stained by aqueous methylene blue solutions at *p*H 7. These are two further characteristics of resilin[2]. Electronmicrographs of the hinge region show that here both anterior and posterior walls are composed of continuous, curved lamellae of cuticle.

The topography of resilin in the anterior cibarial wall of the tsetse fly is illustrated in Fig. 3. Potential energy is stored in this material when it is flexed by the contraction of the dilator muscles. As the muscles relax, the resilin recoils to its undistorted shape, the stored energy being utilized to return the anterior wall and swing back the dorsal plate, so pushing food out of the pump and into the oesophagus.

The structure and function of the cibarial pump in other Diptera (my unpublished observations) and in *Rhodnius prolixus* (ref. 6 and H. C. Bennett-Clark, personal communication) indicate that resilin is a significant structural component of the sucking pump in many different insects.

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Species Distribution of New Structures on Fly Antennae

Antennal receptors which mediate various stimuli are concentrated on three segments in higher Diptera[1-3]. The scanning electron microscope (Cambridge Stereoscan) has now disclosed previously undetected structures on the pedicel or second antennal segment of a number of common flies (Figs. 1*a*, *b*). (Specimens were gold coated at 200 Å and the microscope was operated at from 3 to 10 kV with an aperture of 200μ.)

In the green bottle fly, *Phaenicia sericata*, a patch of median surface of the pedicel is usually glabrous and convoluted, with a plate occupying each depression (Fig. 1*c*). In the newly emerged fly, each plate contains a bulbous seta which tapers distally. The plate is bordered posteriorly by a cluster of fine hairs oriented anteriorly into the air stream (Figs. 1*c*, *d*). The number of plaques varies from six to sixteen per antenna, variations occurring between specimens and sometimes between pedicels of the same individual. There are no sexual differences in this species.

Altogether sixteen species of flies belonging to five families, chiefly muscoids, were examined for these plaques. They were found to be maximally developed Calliphoridae (*Calliphora vomitoria*, *Calliphora vicina*, *Cynomyopsis cadaverina*, *Phormia regina*, *Pollenia rudis*, *Phaenicia sericata*, *Phaenicia pallescens*, and *Phaenicia eximia*) and in Sarcophagidae (*Sarcophaga bullata*). They were less numerous or absent in the Muscidae (present in ♂ and ♀ *Musca domestica* in a longitudinal row of two or three plaques; ♂ *Fannia canicularis* had one and ♀♀ had two; missing in both sexes of *Stomoxys calcitrans*; present in both sexes of *Muscina stabulans* in a longitudinal row of four; and present in ♀ *Scatophaga stercoraria* in a transverse row of two). They were absent from a male tachinid parasitoid, *Nemorilla maculosa*, and from both sexes of the non-muscoid *Drosophila melanogaster*.

Intraspecific variation occurs in the number and arrangement of these plaques, but such sibling species as *P. sericata* and *pallescens* are separable. Generic and familial differences are even more distinct: calliphorids have their plaques in a cluster, whereas they are more linear in the sarcophagid and in the muscids which we examined (Fig. 1*e*). The posterior tuft of fine hairs was present in the sarcophagid and in all calliphorids but absent in muscids. A fuller description of species patterns will be published elsewhere.

The tuft of fine hairs is present in old flies but the bulbous setae are lost with age. The latter are solid and tend to fragment before detachment from the socket. A plug often remains in the socket after detachment (Fig. 1*f*). Other antennal setae do not seem to be so fragile and transient. Its rate of loss varies with species. There was a 50 per cent loss among ten-week-old *P. sericata* caged at 23° C, whereas, in *P. pallescens*, the loss was 30 to 80 per cent by the fourth day. In both species loss of these setae was complete or nearly so, after seven weeks. There was no obvious sexual or spatial pattern in the way these setae were lost, and differences could be found between the antennae of the same fly. Collisions of the flies with the interior of the cage may have produced some loss but there were no significant differences after flies were immobilized or had their wings clipped.

The state of these hairs may give a rough indication of a fly's age and this was used to determine the stage in which some of the common flies in the United States survive severe winters. We buried pupae of several species under 4 inches of dry or wet compacted soil and examined the antennae of teneral adults. Antennae of flies emerging from wet soil had a considerable accumulation of particles and could be mistaken for those of old flies. There was, however, only a 0 to 10 per cent loss of bulbous setae in *P. sericata*, and a 0 to 50 per cent loss in *P. pallescens*. The antennae of both species of fly emerging from dry soil retained all bulbous setae and only a few soil particles. The ptilinum undoubtedly protects the delicate antennal structures as it clears an emergence path.

Wild flies were trapped in early spring to determine the stage in which they had overwintered. Cluster flies, *P. rudis*, known to overwinter as adults, were trapped on April 9. Their antennae had no bulbous setae, were covered with debris and had many broken setae. Early spring blow flies, *C. cadaverina* and *C. vomitoria*, trapped during the first two weeks of April had antennae in the same condition. Additional evidence which suggests these flies had hibernated as adults was: (1) most had frayed wings; (2) a number of females had well developed ovaries and when isolated from males in the laboratory laid eggs which produced larvae; and (3) none showed pupal fat balls which are characteristic of recently emerged flies. Because early spring temperatures were sufficiently low to minimize flight activity until the second week of April, it is unlikely that exhaustion of pupal fat balls,

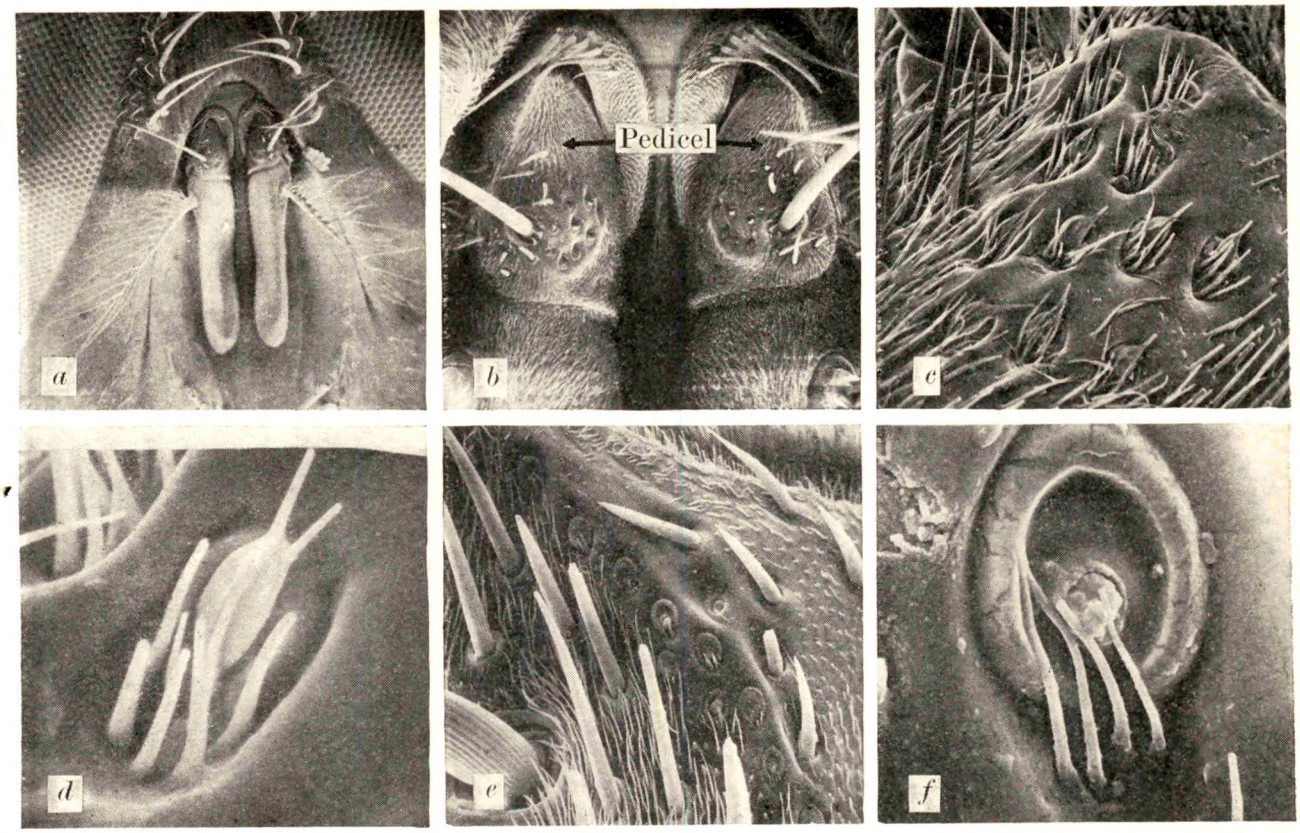


Fig. 1. *a*, Antennae of *P. sericata* (♀, ×32); *b*, same, showing area of pedicel which contains newly described structures (×131); *c*, same, showing pockets of bulbous setae and hairs directed anteriorly (×600); *d*, same, ×2,790 (forked hair is unusual); *e*, more linear arrangement of plaques in *S. bullata* (♀, ×336); *f*, same, showing socket after loss of bulbous seta (×3,330).

ovarian development and insemination, and fraying of the wings could have all taken place in these flies by early April. (Virgin or nulliparous flies may also overwinter. A female *P. regina* collected indoors on January 24, 1970, in Du Page County, Illinois, by Mrs Noreen Ash, had antennae and wings of a hibernating fly with immature ovaries.) The first spring specimens of *P. sericata* taken two weeks later correlated well with teneral adults on the basis of the above criteria. This species must therefore hibernate in a pre-adult stage.

Use of a cuticular structure for age approximations has the advantage that it may be applied to museum specimens. A few of our specimens were more than 20 years old and some retained a high percentage of bulbous setae. Histological and behavioural studies are under way to elucidate the function of these antennal structures.

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Experiments in Binocular Synchronization

As applied to eye guidance, Helmholtz's classical empirical theory of space perception[1] contains two basic assumptions: first, that direction and location in space are learned by temporal association between visual and tactile experience, and second, that the two eyes are exactly conjugate in their response to visual stimuli. The second assumption is logically necessary because the theory of temporal association permits no time discrepancy between the two eyes in forming visual and tactual associations. If such binocular differences did occur, each eye would "learn" separate directions. We have set out to test this second assumption.

A real-time computer system[2–4] (Fig. 1) was arranged to control and to measure the time intervals between the points of maximum velocity in binocular saccadic eye movements while reading. The movements of each eye were converted to electrical analogues by a binocular transducer fitted to a pair of goggles. The subject read samples of easy and difficult material presented by a motor-driven Teleprompter. To begin with, the computer system was used to control and code recordings of the binocular eye movements on a precision magnetic tape recorder. When the observations were completed, these recordings were reprocessed by computer programming to measure the time differences between the peak velocities of paired binocular saccadic fixations: the primary eye movement signals were differentiated to produce an electrical analogue of their velocity, and the input velocity signals sampled and converted to digital form at 1,000 samples/s. The time measures were accurate to the precision of the sampling rate. Millisecond timing could be achieved because the peak velocity of the eye movement signals could be detected with that precision.

A similar procedure was used in the second experiment to measure time differences in the velocity peaks of vertical eye movements, except that in this experiment the subject watched an oscilloscope target which shifted vertically in position in steps of 2·5 and 5·0 degrees. For this experiment, the photoresistor, binocular, vertical eye-movement transducer was used (Fig. 1). In the first

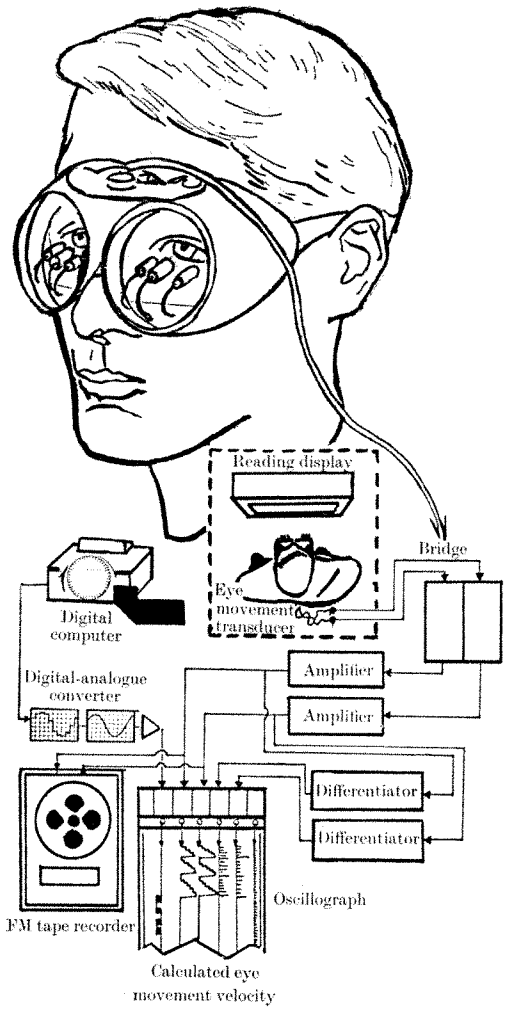


Fig. 1. Vertical photoresistor binocular eye movement transducer and real-time computer system used to measure time differences between the two eyes in visual tracking.

observations, the computer was used to shift the target spot at intervals of 0·35 and 0·70 s and to code the recording of the vertical eye movements. Later, the recorded movements were reprocessed and their velocity derivative obtained and recorded at a rate that would make possible accurate visual measurement of the time differences between the peak velocities of paired binocular saccades.

In the reading experiment, three subjects were tested in a repeated measures experimental design that varied reading difficulty and orientation of the reading display. Three orientations were used: normal, rotated 15 degrees to the right, and rotated 15 degrees to the left. The pattern of the recorded binocular movements are shown in Fig. 2 (top two eye movement traces) with a record of the velocity component of the movements. The computer measured the time differences between the velocity peaks of paired right-eye and left-eye saccades, as illustrated in these velocity records.

The distribution of eye time leads for the three subjects was plotted for the least difficult reading passages (Fig. 3). All three curves have three modal frequencies. Subject 1, unlike the other two subjects, gave a modal right-eye lead at about 5 ms. This subject showed a relatively low

Table 1. TIME LAG IN MILLISECONDS OF THE LEFT AND RIGHT EYES RESPECTIVELY IN RESPONDING TO CHANGES IN TARGET POSITIONS

Subject	Left eye	Right eye	Difference
1	164	161	3 (RE lead)
2	149	171	22
3	208	214	6
4	201	208	7
5	237	248	11

modal lead at 16 ms, and a very high frequency left-eye lead at 1 ms. In contrast subject 2 showed a high frequency left-eye lead at 15 ms and a low left lead mode at 1 ms. Subject 3 showed a somewhat different distribution with the highest modal frequency at 0·0 and nearly equal modal frequencies at 14 and 9 ms. These subject differences in modal

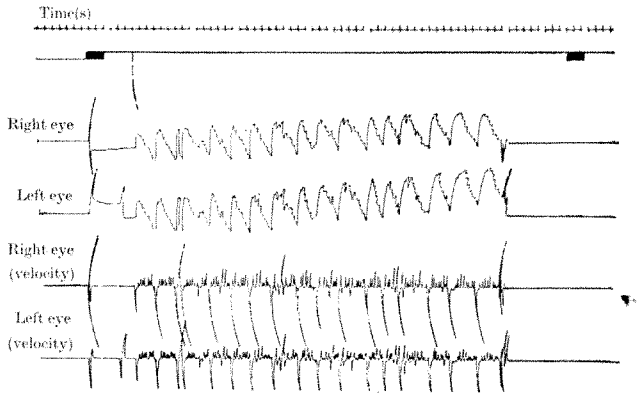


Fig. 2. Records of horizontal binocular eye movements in reading and traces of their velocity. High-precision measurement of binocular differences is achieved by detecting differences between paired velocity measures of the two eyes.

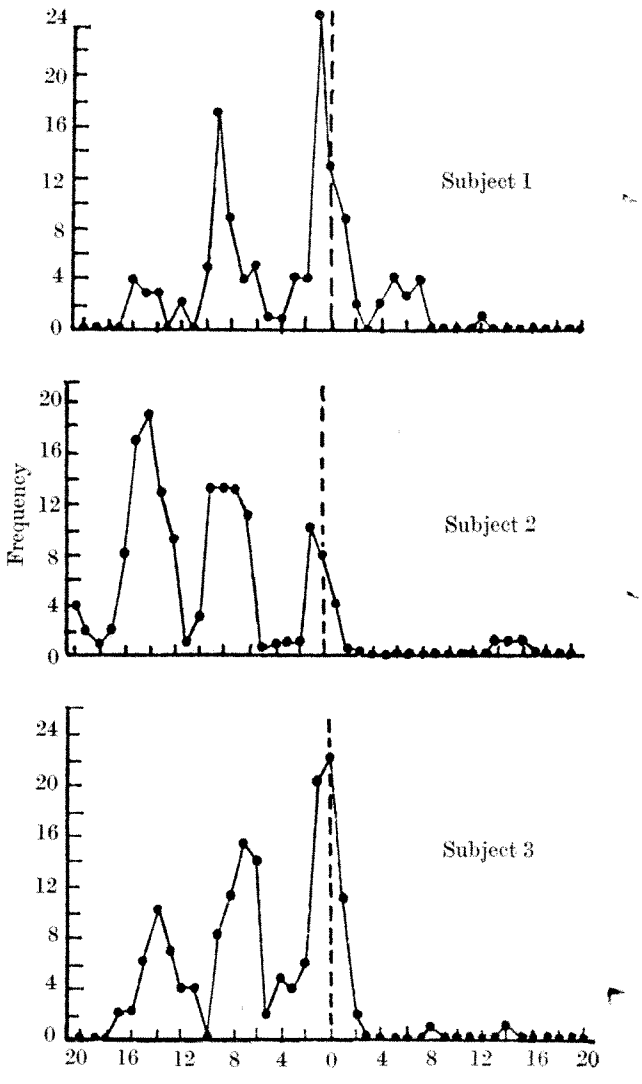


Fig. 3. Distribution of the frequency of right and left eye leads in reading among three subjects.

frequencies at different times were duplicated almost exactly in the other two conditions of difficulty of the reading material and were not changed by its orientation. Statistical tests showed that the differences in frequency of occurrence of the left-eye leads was significant.

The pattern of the vertical eye movements, their velocity characteristics and the difference in position between the right and left eyes are illustrated in Fig. 4. The experimental measurements consisted of time differences between the peak velocities of paired right-eye and left-eye movements, as shown in the lower two traces of the record. Results on five subjects showed that the mean time difference in ms between the two eyes favoured the left eye in tracking the vertical shifts in target position, favoured the left eye in four subjects, and the right eye in one (see Table 1). These differences between eyes were statistically reliable. Over a series of eight practice trials, the mean time between target movement and eye response was reduced significantly from a mean of 220 ms to about 200 ms. With practice, the mean time difference between the right and left eye was significantly increased from approximately 1 ms to about 9 ms.

The results of these experiments on horizontal and vertical binocular movements do not confirm the classical theory of Helmholtz that the two eyes are conjugate in binocular coordination. The finding that the left eye led the right eye in most of the measures of reading substantiates the observations of Hyde[5], who found that "outward" movements of the eye were slower than "inward" movements. The difference was 58 degrees/s (Hyde herself attributed this difference to lack of precision in her technique, and concluded that the eyes are completely conjugate).

The experiment illustrates how real-time computer systems can be used to obtain high-precision measurements in optometry and visual science. These methods, which may be extended to all fields of physiology and psychology, are based on the analogue–digital–analogue computer both as a control element in a behavioural–physiological system and as a high precision clocking and measuring device.

These findings raise questions about the validity of the claims of informational engineers[6] that the dynamics of saccadic movements in vision can be modelled adequately in terms of binary sampling processes related to sensory and neural input, which have an optimal sampling constant or response latency of 0·2 s. The results suggest that binocular visual coordination depends on several levels of continuous eye movement–retinal feedback timing and that these differ significantly for the two eyes and for each pattern of vertical and horizontal eye motion.

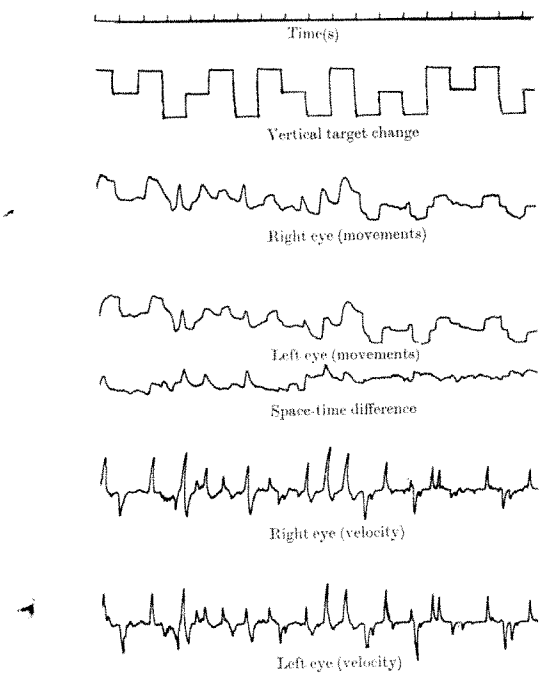


Fig. 4. Records of binocular vertical eye movements in relation to the step-wise vertical shifts in the observed target, with traces of the velocity of the eye movements.

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Effect of Blurring on Perception of a Simple Geometric Pattern

FIG. 1 is a modification of the Poggendorff illusion which has been drawn so that it is clearly visible at large distances. When viewed from a distance the central bar is perceived as being more horizontal and wider than it is drawn. This distortion increases as distance from the figure increases so that distortions of 15° can be found in most subjects. (Similar but much less striking distance and size effects have been noted in standard illusion figures[1–5].) The distortion is greater in subjects with poor visual acuity; and it can be increased by artificially increasing the blurring by optical means. The effect does not depend on accommodation, and can also be observed by placing a hair on a fine grating.



Fig. 1. A variant of the Poggendorff illusion.

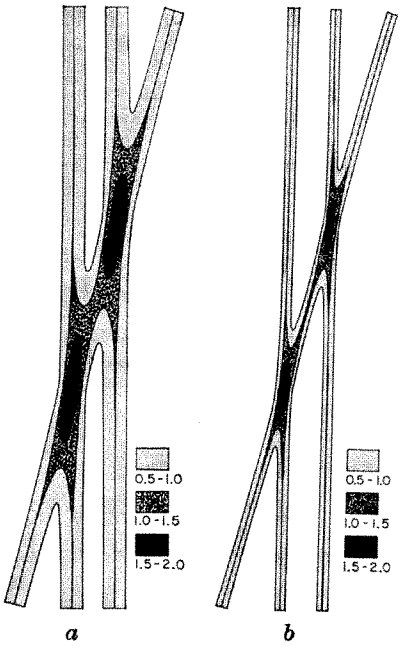


Fig. 2. *a*, The contour map of intensity which results when a line pattern, formed by two parallel lines crossed by a third at a 15° angle, is convolved (blurred) with a Gaussian of unit height. The distance between the parallel lines is twice the half-width of the Gaussian; *b*, the same as *a*, but with the distance between the parallel lines four times the half-width of the Gaussian.

The illusion seems to be caused by the faulty interpretation by the visual system of an image blurred by any combination of optical, motor or neural processes[6]. Here, however, I consider the effects of blurring in foveal vision, for which the blur function has been experimentally determined and may be approximated by a Gaussian whose half-width depends on the pupil size (the half-width is about 1·2 min for a 3·8 mm pupil)[7,8]. The retinal intensity pattern for foveal viewing of a luminous line pattern formed by two parallel lines crossed by a third at a 15° angle, can be computed, and is illustrated for distances between the parallel lines of twice the half-width of the Gaussian (Fig. 2*a*) and four times the half-width (Fig. 2*b*). Away from the points of intersection in Fig. 2, the extent of the blurring is about equal to the visual acuity and we perceive a straight sharp edge (perhaps as a result of neural inhibitive processes in the visual system)[9]. In the vertex of the acute angle, however, there is a plateau of rather slowly changing illumination several times larger than that at the limit of visual acuity. Although it is not easy, it is possible, looking carefully at Fig. 1, to see this blurred region in the vertex of the angle (see ref. 1, Fig. 29). Ordinarily, however, we do not notice these fine details and at large distances we perceive a tilted bar whose boundaries appear to be a "best fit" to a contour of constant intensity (Fig. 2).

In the past, many investigators have proposed that the inability to resolve the vertices of acute angles may lead to their perceptual enlargement, and consequently to many of the geometric illusions (Poggendorff, Zöllner, Hering, Orbison)[1-5,10-13]. Among the many demonstrations which support this hypothesis are the disappearance of the Poggendorff illusion when the ends of the oblique lines meeting the verticals are bent slightly outward near the point of contact[14]; the necessity of acute angles for the Hering illusion[4]; the enhancement of illusions by artificial increases of blurring[10,15]; persistence of illusions in haptic presentation[13]. Although these demonstrations provide evidence that distortions induced by acute angles are important in causing the illusions, many have persuasively argued that other factors must also be involved[1,15-18]. Certainly this is so. Even for such simple patterns as the geometric illusions, it is necessary to consider how information reaching the higher visual system simultaneously through parallel channels, or sequentially because of eye movements: is integrated to yield a single percept. The state of attention of the subject to fine details as well as expectations concerning what the stimulation pattern is will certainly affect this integration.

I have here presented a visual distortion which seems to be caused by the erroneous interpretation of a blurred image. Although only optical blurring has been discussed, "neural blurring" of the visual image at higher levels of the visual system will enhance the optical blurring which is already present at the retinal level. The partial persistence of the geometric illusions in binocular presentation[19] would therefore be expected. Study of the size and distance effect observed here should be useful in determining the rules by which people extract information from visual patterns, and may indicate that the comparatively small regions distorted by blurring in large figures have a large effect on the final percept.

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Relation of Age and Mongolism to Lateral Preferences in Severely Subnormal Subjects

HILDRETH[1] estimated the incidence of left-handedness in very young children to be about twice as high as in the adult population. This is consistent both with an effect of social training and also with maturation in lateral dominance, but in either case one might expect a subnormal population to show, at least up to a certain age, a greater incidence of left-handedness. Burt[2] and Gordon[3], relying partly on the observations of teachers, both found this; Clausen[4], however, using three objective tests, found no differences between retardate and normal groups aged

8 to 10 years. Severe subnormality, however, has scarcely been investigated; the children examined by Burt and Gordon, and the 46 per cent of Clausen's retardate subjects with IQs above 60, would almost certainly not be so graded. An attempt[5] to differentiate by diagnosis ranked the incidence of left-handedness in groups of subnormal males in the (diminishing) order: "familial", "brain-damaged", "mongols". It therefore seemed worthwhile to investigate lateral preferences in normal and severely subnormal groups, examining separately the effects of age and mongolism.

The greater the number of items used to assess laterality, the more may it appear a matter of degree and we used a wide range of items to make sure of detecting residual left-sided preference. Performance items were used because questionnaires, although they have obvious advantages, are not practicable with the severely subnormal. Also there is evidence[6] of considerable unreliability between questionnaire responses and performance on the same items.

The severely subnormal subjects were thirty-two patients at South Ockendon Hospital divided into four groups of eight (Table 1). The subjects selected were all attending the school, without obvious physical handicap and cooperative. Within these limitations it was not possible to balance the number of males and females, even with a hospital population of more than 1,100, but, because most investigators find a higher prevalence of sinistrality among males (Oldfield[7]), it was felt that the proportion of males must be kept equal between the main groups. Accordingly, there were eleven males in groups 1 and 2 combined, and in groups 3 and 4 combined; the proportion of males in the two normal groups (5 and 6) was made to match this. There were 32 normal subjects (Table 1). The 16 children were selected randomly from a class of 30, with the constraint that 11 must be boys.

The inventory was largely selected from that used by Oldfield, excluding unfamiliar items, dangerous activities, and tasks which could not be initiated by verbal instruction alone. One item added was that of blowing the nose. Thus the items were: writing, drawing, use of scissors, comb, toothbrush, knife without fork, dessert spoon, hammer, screwdriver, broom (upper hand), striking a match, dealing cards, threading a needle, throwing a ball, blowing the nose, kicking a ball and looking through a tube.

The order of presentation was randomized and every item was presented using both hands. If the subject showed uncertainty the score was divided, but this was rare. Three of the subnormal subjects failed to complete some of the items but their results are included because all three completed over half of the inventory. Laterality quotients (LQs) were obtained for each subject, from the commonly used formula:

$$\frac{\Sigma R - \Sigma L}{\Sigma R + \Sigma L} \times 100$$

where R, L are right hand and left hand preference scores respectively. Total right-handedness is then $+100$, and total left-handedness -100.

The frequency distributions of the LQs are shown in Figs. 1 and 2. By inspection the normal group seems the more positively right-handed. Both groups, however, have a clear bimodal distribution, making analysis of variance an inappropriate means of comparison. We decided to use the Kolmogorov–Smirnov test[8] to examine the significance of the largest difference between the cumulative distributions. This showed that the LQs of the normal subjects are higher than those of the subnormal ($P < 0{\cdot}05$, one tailed test); within the normal group there is no significant difference between older and younger subjects, but, within the subnormal group, the LQs of the older subjects are higher ($P < 0{\cdot}05$, one tailed test). Within the subnormal group there is no significant difference between the LQs of mongols and non-mongols. The mean LQ score of the former is 47·1 and of the latter is 25·7; there are, however, only ten males amongst the mongols and twelve amongst the non-mongols, and we also found that males tend to have stronger left-sided preferences than females. Combining groups 1, 2, 3 and 4, the mean male LQ is 29·8 and the mean female LQ is 50·9; combining groups 5 and 6, the figures are, for males and females respectively, 52·0 and 70·0.

No further independent comparisons are possible within the data, but the implication that the greatest

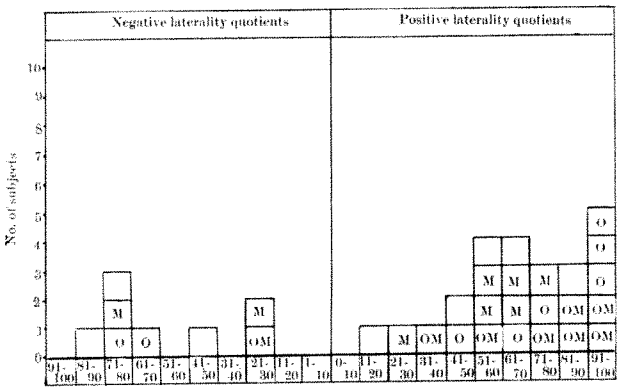


Fig. 1. Distribution of laterality quotients for 32 severely subnormal subjects (groups 1, 2, 3 and 4). Each cell represents one subject. M, indicates membership of a mongol group; O, indicates membership of an older age group.

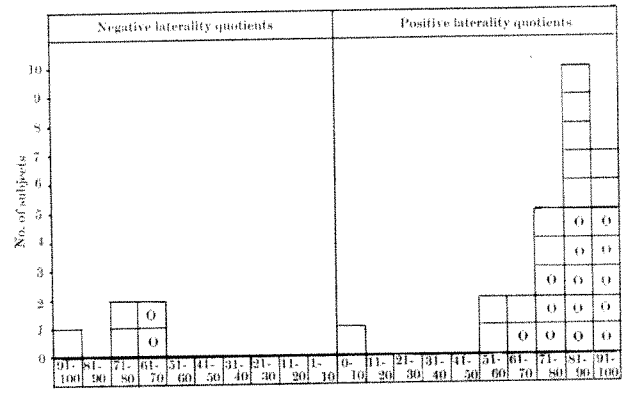


Fig. 2. Distribution of laterality quotients for 32 normal subjects (groups 5 and 6). Each cell represents one subject. O, indicates membership of the older age group.

Table 1. DESCRIPTION AND AGE, SEX AND INTELLECTUAL STATUS OF THE FOUR SUB-GROUPS IN THE SEVERELY SUBNORMAL SAMPLE AND THE TWO SUB-GROUPS IN THE NORMAL SAMPLE

Group No.	Description	N	Males	Females	Age range	Mental age range*	Mean IQ
1	Subnormal mongol	8	4	4	12–7 to 18–10	2–9 to 4–0	< 32·0
2	Subnormal non-mongol	8	7	1	10–9 to 17–10	> 2–0 to 6–1†	< 35·4
3	Subnormal mongol	8	6	2	19–4 to 24–0	3–6 to 5–10	< 30·5
4	Subnormal non-mongol	8	5	3	19–5 to 21–6	2–7 to 7–2	< 35·5
5	Normal	16	11	5	14–4 to 15–8	Comprehensive school pupils (mostly selected for grammar school initially)	
6	Normal	16	11	5	18–1 to 24–0	University candidates, first year students and technicians	

$N = 64$. * Based on assessment by Stanford-Binet. † One MA based on Seguin Form Board.

departure from right-handedness arises from the LQs of the younger subnormals is upheld by consideration of the mean LQs. These are for group 1 and 2: 20·2, for groups 3 and 4: 52·7, for group 5: 45·5, and for group 6: 69·6.

The test used does not discriminate between demonstrating increased left-handedness in the sense of showing the presence of a greater number of complete left-handers and of showing a more even spreading of the LQs from the extreme positive end, but inspection of the Figures suggests the latter is nearer to the truth. Because subnormals attending a hospital school probably receive at least as much training as normals in many of the activities investigated, a possible explanation of the results is that lateralization of hand preference in subnormals develops more slowly but not less completely than in normal subjects. Larger samples, however, might show that the difference remains within older subjects, though to a lesser extent.

Further results will be described elsewhere, in particular those relating to the effects of perinatal injury, to performance on individual items of the inventory and to the correlation between performance and questionnaire versions.

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Some Parameters of High Output CO_2 Laser Experimental Surgery

FOUR years ago, investigations on carbon dioxide laser surgery were initiated in our laboratory. This laser was relatively inexpensive by comparison with ion lasers, the only other high output continuous wave (CW) lasers with surgical potential. The CO_2 laser could be coupled with beam guides used as manipulators, was flexible, had a high efficiency and provided the continuous wave (CW) power needed for an "optical knife". One disadvantage was, of course, the output of an invisible beam far out in the infrared at 10,600 nm and the consequent expensive infrared optics required. The safety programme for investigative laser surgery has been reported before by our laboratory[1]. Specific hazards for the high output CO_2 laser arise from the reflexions from target areas and from surgical instruments. Our initial surgical experiments with 25 W output (Perkin Elmer CO_2 laser) were done on metastatic melanoma of man. Non-specific thermal coagulation necrosis developed after laser surgery. There was no local dissemination of tissue fragments from the CO_2 laser.

Recently, an American Optical 50–300 W CO_2 laser furnished with a flexible beam manipulator and with an endoscope and gastroscope has become available for investigative laser surgical research[2]. This led to renewed interest in the possibility of using CO_2 lasers, especially for so-called bloodless surgery in highly vascularized areas. Sterile coverings of the manipulator head can be used to ensure sterility during the operation. Animal experiments with this laser at 30 W output included the production of partial and complete heart block of dogs by Naprstek and Rockwell[4]. In these experiments the laser was used for the initial skin incision, the thoracotomy and entry into the endocardium. No other types of instruments were used for haemostasis. Precise lesions could be produced in the conductive bundle as observed by the monitoring instruments. In these investigations, the precision and relative bloodlessness of the laser were evident.

Detailed experiments were done by Rockwell and Hishimoto on the CO_2 laser parameters of the liver surgery of rats, and by Siler, Naprstek and Hoefer on surgery of the liver of dogs. These experiments[7] quantified the laser reaction in tissue as regards heat retransmission, depth of incision and focused power density; they also collected data on histology, histochemistry and liver function. These data will be reported later. Such research in liver surgery is necessary for there has been increased frequency of liver damage from the sudden pressure of seat belts in deceleration in automobile accidents. In these preliminary studies the CO_2 laser was found to be superior to the current instrument of preference, the high frequency electro-surgical knife: haemostasis from capillary bleeding was controlled well. Bleeding from moderate and large vessels, however, was not controlled. Experiments are now under way with special thrombin and gelfoam packs combined with laser "welding" techniques to close bleeding points. At present, for extensive liver surgery in the dog, 125 W output is preferred. Similar results in liver surgery with the CO_2 laser were found by Mullins, Jennings and McClusky[5] and by Goodale, Okada, Gonzales, Borner, Edlich and Wangensteen[6]. Goodale has also used the CO_2 laser in the treatment of a stress ulcer. These investigators have used this laser also in treatment of experimental gastric haemorrhage in dogs. Stellar (personal communication) has used the CO_2 laser for neurosurgery in animals and for excision of third degree burns in the pig. Hobe'ika in our laboratory has used the CO_2 laser with the endoscope for surgery of the larynx in dogs. Miller, Winston and Wilson in our laboratory are also using the high output CO_2 laser for cutting and welding bones in the rabbit and dog.

Recently, another type of CO_2 laser was used. This was a pulsed high frequency laser (249 pulses/s) developed by Douglas Linn (Linn, D., personal communication) of Lasers Systems, Inc. Initial experiments were done on liver, kidney and bones of the dog. With outputs of 80 W, the cutting of tissues was excellent but plumes of blood were observed and active haemorrhage in tissue developed. To retain the rapid and precise surgery of these high output instruments and to reduce bleeding, some of the output parameters of these lasers are being varied.

The American Optical high output CO_2 laser system at 35 W has been used to excise two malignancies in man. This was done primarily because the area of the cancer was highly vascularized in these patients. One patient had a 2·0 cm basal cell epithelioma of the posterior aspect of the neck recurrent after previous electrosurgery and X-ray radiation. This was in an area where extensive actinic changes with telangiectasia had developed. This area bled freely. The CO_2 laser was used with local anaesthesia to excise an oval piece of tissue. No other instruments were used. There was only minimal haemorrhage. The wound was closed with interrupted black silk sutures reinforced with Steri-Strips. Detailed biopsy studies showed adequate excision of the epithelioma in all planes with only a narrow rim of coagulation necrosis which did not extend into the invasive tumour cords. When the sutures were removed at 7 days, some broadening of the scar was seen but the

lesion had healed. The area was soft and has remained free of cancer during the brief observation period of 8 months.

The second patient had 2·5 cm lobulated angiosarcoma of one finger. Under local anaesthesia and tourniquet, three separate sections were removed with the scalpel, the CO_2 laser and the high frequency electrosurgical unit respectively. Biopsy sections from the scalpel and the laser were adequate for detailed histological studies, whereas the sections from the high frequency electrosurgery instrument showed complete charring of the entire tissue mass. With the release of the tourniquet, extensive bleeding occurred only in the scalpel control area and three blood vessels had to be ligated. There was a small recurrent nodule 3 weeks after surgery only in the area of the scalpel. This was treated effectively with local X-ray irradiation to the nodule only. Follow up studies after only 9 months showed smooth, soft scarring, but no evidence as yet of any local recurrences.

With planned safety programmes, the high output CO_2 laser can be used in investigative laser studies. Increased flexibility is necessary for continued, surgical research with this laser. The high output CO_2 laser then is another significant development for current investigative surgery.

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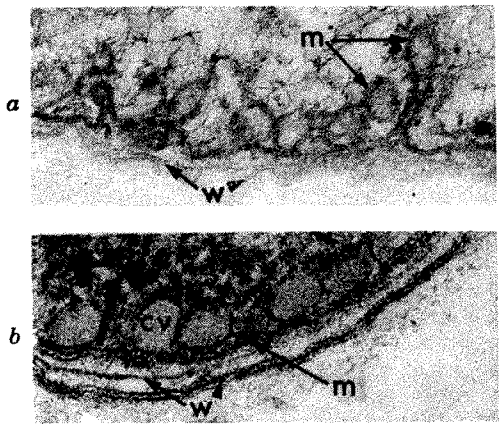


Fig. 1. Electron micrographs of small areas of the cortical region in (*a*) a purple bacterium (*Rhodospirillum rubrum*) and (*b*) a green bacterium (*Pelodictyon clathratiforme*), to show the relations between the cell wall (w), unit membrane (m) and chlorobium vesicles (cv). *a*, ×112,000; *b*, ×160,000.

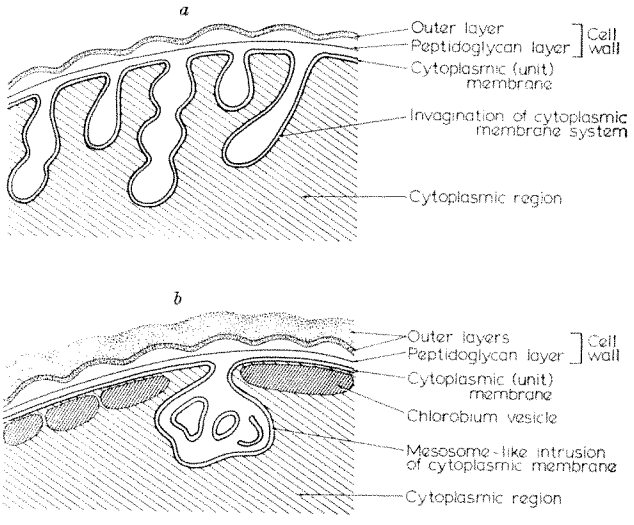


Fig. 2. Schematic interpretations of the structure of the cortical region of the cell in: *a*, a purple bacterium with vesicular membranous intrusions, of the type shown in Fig. 1*a*; and *b*, a green bacterium.

Chlorobium Vesicles: the Photosynthetic Organelles of Green Bacteria

Among photosynthetic organisms, green and purple bacteria are distinguished by their failure to evolve oxygen, a reflexion of their inability to use water as a photosynthetic electron donor. Although the mechanism of photosynthesis is grossly similar in these two groups of bacteria, its machinery is not. The photopigment systems of green and purple bacteria are group-specific; and their intracellular locations differ[1,2].

Electron micrographs of parts of the cortical area of each type of cell are shown in Fig. 1, and diagrammatic interpretations of the topological relationships between the cortical cell structures in Fig. 2. The cell membrane of purple bacteria is topologically very complex; numerous intrusions, with the form of vesicles, lamellae or tubules, penetrate deeply into the cytoplasmic region. The photopigments are incorporated into this complex unit membrane system[2,3]. In green bacteria, infolding of the cell membrane is less extensive. It occurs at only a few points to form structures similar to the mesosomes found in many groups of non-photosynthetic bacteria. Underlying the cell membrane (but not connected with it) is a cortical layer of oblong vesicles known as chlorobium vesicles, with which the photopigment system is associated[3–6]. Each vesicle is approximately 35 by 120 nm, and is bounded by a non-unit membrane only 2–3 nm thick.

Certain acyl lipids, which include mono- and digalactosyl diglycerides, are specifically associated with the eukaryotic photosynthetic apparatus[7]. These two galactolipids are present in the cells of blue-green algae[8], but have not been detected in the cells or photosynthetic membrane systems of purple bacteria, which contain predominantly phospholipids[8,9]. The cells of green bacteria, however, contain large amounts of glycolipids in addition to phospholipids[10,11]. The glycolipids of *Chloropseudomonas ethylica* have been characterized chemically[11]. "Glycolipid I" is a monogalactosyl diglyceride, and therefore belongs to one of the classes of glycolipids associated with the photosynthetic apparatus of oxygen-evolving photosynthetic organisms. "Glycolipid II", which contains galactose, rhamnose and a third unidentified sugar, has not been found in any other biological group. These analyses were performed on whole cells, and therefore do not reveal the specific intracellular locations of glycolipids I and II.

After glutaraldehyde fixation of crude, cell-free extracts (essential to stabilize the chlorobium vesicles), we have succeeded in fractionating the particulate components of green bacteria by a combination of differential centrifugation and sucrose gradient electrophoresis[12,13]. The preparations of vesicles and membrane fragments so obtained

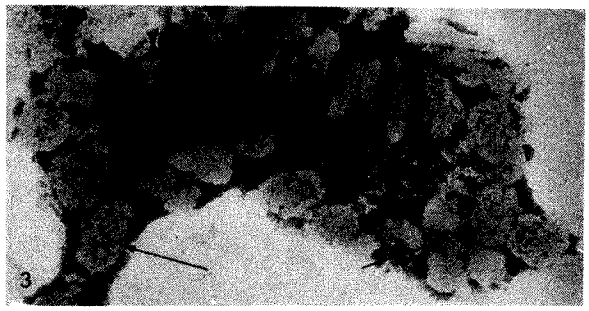


Fig. 3. Electron micrograph of the purified vesicle fraction of *C. thiosulfatophilum* 6130, negatively stained with phosphotungstate. ×80,000.

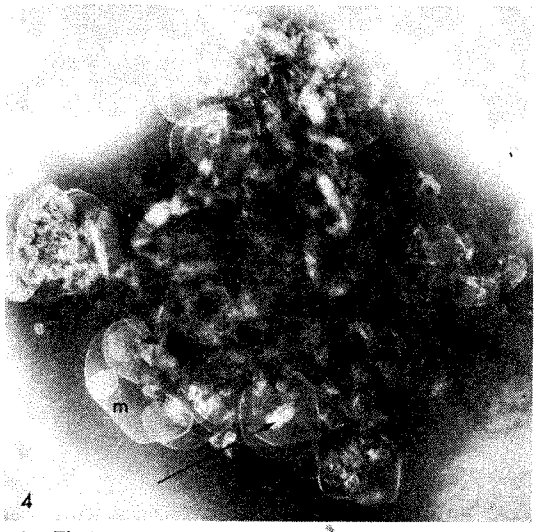


Fig. 4. Electron micrograph of the purified membrane fraction of *C. thiosulfatophilum* 6130, negatively stained with phosphotungstate. ×48,000. The preparation consists predominantly of flattened membranes, probably derived from mesomal intrusions.

seem from their appearance in the electron microscope (Figs. 3 and 4) to be relatively pure. Table 1 presents analytical data on these fractions, isolated from a strain of *Chlorobium thiosulfatophilum*. The purified vesicle fraction is extremely rich in chlorophyll; its specific chlorophyll content is 9 times that of the initial crude cell-free extract. Although the membrane fraction contains some chlorophyll, its specific chlorophyll content is less than 10 per cent of that of the vesicle fraction, and can be reasonably attributed to contamination with vesicles or comminuted fragments derived from them. Particulate succinic and malic dehydrogenase activities are found almost exclusively in the membrane fraction; these two enzymatic activities are characteristically associated with the cytoplasmic membrane system in other bacteria.

Table 1 also shows the phosphate and carbohydrate contents of the lipids extracted from vesicles and membranes. Both glycolipids and phospholipids are present in each fraction; but glycolipid predominates in the vesicles, and phospholipid in the membranes. The glycolipids of the vesicle and membrane fractions were characterized by the methods of Constantopoulos and Bloch[11]. Fig. 5 shows that one specific glycolipid is associated with each fraction. The glycolipid in the vesicle fraction corresponds to "glycolipid I" of Constantopoulos and Bloch[11], and the glycolipid in the membrane fraction to "glycolipid II". We have found the same glycolipids, with the same localization, in vesicle and membrane fractions of another strain of *C. thiosulfatophilum*, and of a strain of *C. limicola*. "Glycolipid I" and "glycolipid II" are therefore probably characteristic cell constituents of all green bacteria.

Table 1. PROPERTIES OF VESICLE AND MEMBRANE FRACTIONS ISOLATED FROM A CRUDE CELL-FREE EXTRACT OF *Chlorobium thiosulfatophilum* STRAIN 6130

	Specific content, per mg of protein*			Lipid composition‖	
	Bacteriochlorophyll *c*† (μg)	Succinate dehydrogenase‡ (units)	Malate dehydrogenase§ (units)	Phosphate (μg/mg total lipid)	Carbohydrate (μg/mg total lipid)
Vesicle fraction	845	0·010	< 0·001	8·5	42·0
Membrane fraction	83	0·229	0·015	43·8	9·3

* Determined by Lowry–Folin method[14].

† Determined after methanol extraction by absorbancy at 667·5 mm (ref. 15).

‡ Assay method and unit[16]. About 80 per cent of the initial activity of this enzyme survives glutaraldehyde treatment of crude extracts.

§ Assay method and unit[17]. About 30 per cent of the initial activity of this enzyme survives glutaraldehyde treatment of crude extracts.

‖ Total lipids extracted from lyophilized preparations with chloroform–methanol (1 : 1) for several hours. Analytical methods have been described in refs. 12 and 13.

The identification of "glycolipid I" as a monogalactosyl diglyceride[11], coupled with the present findings, shows that chlorobium vesicles contain one of the two classes of galactolipids associated with the photosynthetic apparatus of oxygen-producing photosynthetic organisms. This is the only galactolipid present in the photosynthetic apparatus of green bacteria. "Glycolipid II", which has not been detected in other photosynthetic organisms, is a constituent of the unit membrane system of green bacteria, and therefore has the same intracellular location as the glycolipids which occur in many non-photosynthetic bacteria[18,19].

This work confirms the hypothesis that chlorobium vesicles are functionally specialized organelles, which house the machinery of photosynthesis in green bacteria. The absence of unit membranes is the most remarkable property of these organelles. In other groups of photosynthetic procaryotes and in the chloroplasts of eucaryotes, the lipid-soluble photopigments and the associated machinery of photosynthetic electron transport are invariably built into unit membrane systems ("thylacoids"). Although the chlorobium vesicle is to some extent a functional analogue of the chloroplast, the differences between the two are so great that they cannot be construed as homologous. The green bacteria seem to be an isolated and highly specialized group of photosynthetic organisms, remote from the major line of cellular evolution among procaryotes which led to the emergence of organisms capable of using water as a photosynthetic electron donor (exemplified by the contemporary blue-green algae), and eventually to the implantation of this mode of photosynthesis among eukaryotes.

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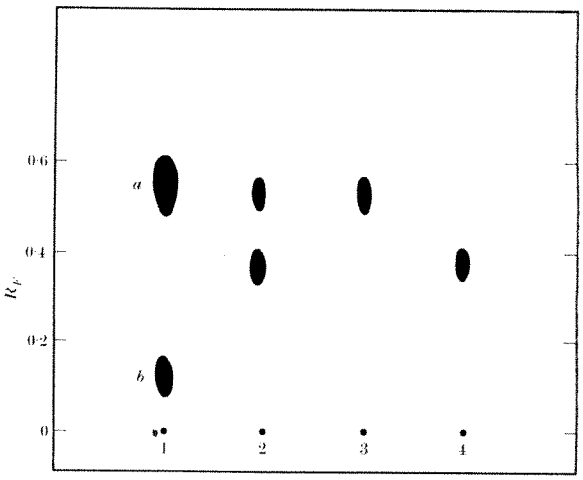


Fig. 5. Thin-layer chromatogram of the glycolipids of *C. thiosulfatophilum* 6130, compared with those of the unicellular green alga *Chlorella pyrenoidosa*. Adsorbent: 'Adsorbosil-I'; solvent system; chloroform–methanol–acetic acid–ethyl ether (35 : 15 : 5 : 45): spray: diphenylamine reagent. (1) Lipids extracted from *Chlorella pyrenoidosa* (*a*: monogalactosyl diglyceride; *b*, digalactosyl diglyceride). (2) Lipids (65 μg) prepared from a crude extract of *C. thiosulfatophilum*. (3) Lipids (75 μg) from a purified vesicle fraction. (4) Lipids (35 μg) from a purified membrane fraction.

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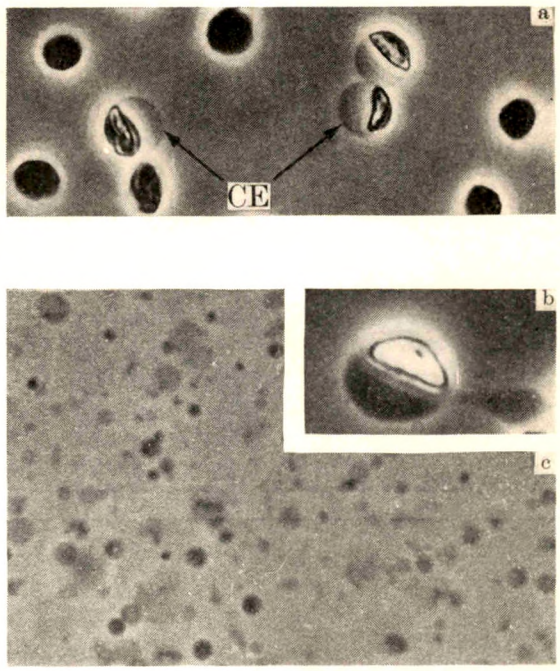


Fig. 1. Phase contrast micrographs of suspensions containing chloroplasts (*a* and *b*) and chloroplast envelope membranes (*c*). In *a* the chloroplast envelopes (CE) of the swollen chloroplasts are seen as greyish halos: the other chloroplasts are devoid of envelopes. The pinching off of a portion of the envelope is shown in *b*. Photography as previously described[17] except that Kodak 'Tri-X Pan' (400 ASA) film was used. (*a*, ×800; *b*, ×1,500; *c*, ×1,430.)

Isolation of Chloroplast Envelope Membranes

LITTLE is known of the nature of the chloroplast envelope: investigations have been concerned chiefly with its role in granal membrane development (for review see ref. 1) and in the transport of metabolites between the chloroplast and the cytoplasm[2–13]. The lack of knowledge of the physical and chemical properties of the plastid envelope limits our understanding of its function in photosynthesizing chloroplasts and of its precise role in plastid development. As relatively few methods are available for the investigation of the envelope *in vivo*, it seemed valuable to devise a method for the isolation of envelope membranes from other chloroplast components and then to study the properties of the isolated envelopes. In this paper we give details of a protocol for the isolation of a membrane fraction containing substantially pure chloroplast envelope membranes.

Vicia faba L. (var. Dwarf White Fan) plants were grown in controlled environment conditions as described previously[14]. The plants (17–21 days old) were placed in the dark for 66–72 h before harvesting, and chloroplasts were isolated from 30–40 g leaf tissue as previously described[14]. The grinding medium was 53 mM Na_2HPO_4 – KH_2PO_4 (*p*H 7·3) containing 0·5 M D(−)-sorbitol, 10 mM $MgCl_2$ and 10 mM EDTA (disodium salt). The chloroplast suspension, which contained 50 per cent–70 per cent intact plastids as judged by phase contrast microscopy, was the starting material for the chloroplast envelope isolation. These suspensions (0·3–0·4 mg chlorophyll/ml.) showed succinic dehydrogenase activity, indicating the presence of mitochondria, and the bacterial count was about 25,000 colonies/ml. Because both mitochondria and bacteria would be a potential source of membrane contamination, the chloroplasts were centrifuged again at 500*g* for 15 min through a 4 cm layer of isolation medium in which the density was increased by substituting 0·4 M sucrose for 0·5 M sorbitol. This centrifugation reduced both the mitochondrial and the bacterial contamination by 60 per cent. The supernatant was carefully removed by surface suction.

Each washed chloroplast pellet was resuspended in 10 ml. grinding medium from which the sorbitol had been omitted, and incubated at 0° C for 10 min. The suspensions were homogenized in a Ten-Broek ground-glass homogenizer by raising and lowering the plunger rapidly three times. The homogenized suspension was diluted with 20 ml. of incubation medium, and centrifuged at 3,000*g* for 10 min. Floating material was removed and the supernatant decanted and recentrifuged at 20,000*g* for 30 min. The pellet from this spin contained the chloroplast envelope membranes.

At each stage during the isolation procedure the suspensions were observed by phase contrast microscopy. Initially the intact chloroplasts were opaque and shining in appearance, but after incubation the suspensions contained many chloroplasts with greyish halos (Fig. 1*a*). In each of these chloroplasts the lamellae system was excentric within the greyish balloon-like structure. This image was interpreted as the side view of an intact chloroplast in which the envelope had become greatly distended. Occasionally the greyish structure was pinched off (Fig. 1*b*) free of lamellae and resembled a red blood cell ghost. These ghosts were assumed to be derived from the chloroplast envelopes, and increased in number after homogenization. The later stages in the procedure were designed for the collection of the ghosts free from lamellae membranes and the final envelope fraction contained numerous ghosts of varying sizes (Fig. 1*c*). When examined in the electron microscope, the pellet of the membrane fraction (20,000*g*) showed a gradation (Fig. 2). Near the top (*A*) the pellet consisted of an almost pure membrane fraction of double-membrane bound vesicles. These vesicles ranged in diameter from 0·3 μm to 1·0 μm

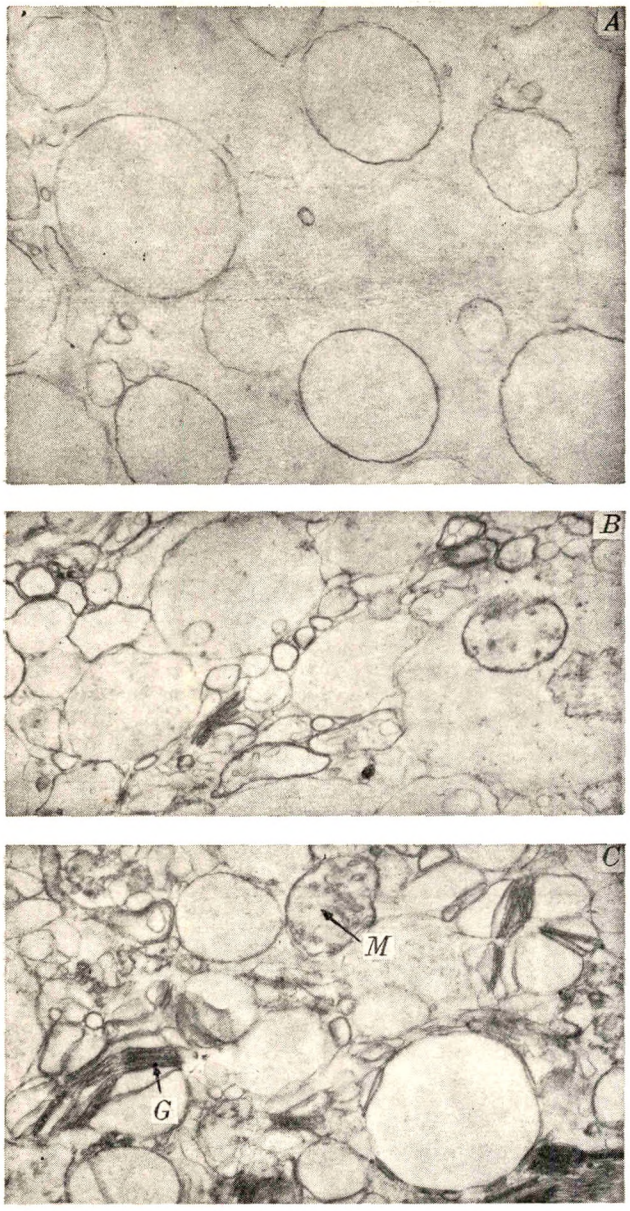


Fig. 2. Electronmicrographs of thin sections from the top (*A*), middle (*B*) and bottom (*C*) of a pellet of the chloroplast envelope fraction. The pellet was fixed with 2·5 per cent glutaraldehyde and post-fixed in 1 per cent osmium. Fixation and postfixation were each for 30 min at room temperature and the fixatives were phosphate buffered at *p*H 7·0. The pellets were dehydrated in an acetone series and embedded in 'Araldite'. The sections on uncoated grids were stained with lead citrate[20]. *M*, Mitochondria; *G*, granal membrane stacks. (*A*, ×31,875; *B*, ×11,250; *C*, 15,000.)

and have the appearance to be expected of contracted isolated chloroplast envelopes. Towards the middle, the pellet became much more mixed and contained vesicles bounded by double and single membranes, recognizable mitochondria (*M*), and granal membrane stacks (*G*). Some of these vesicles probably come from membranes detached from the chloroplast lamellae during incubation and homogenization. At the bottom of the pellet (*C*) an occasional entire chloroplast stripped of its outer envelope was found.

The lamellae membranes contain chlorophyll which may be assumed to be absent from the chloroplast envelope[15]. To assess the degree of contamination of the membrane fraction with lamellae membranes, chlorophyll and protein composition was determined for several preparations (Table 1). The protein : chlorophyll ratio for lamellae from *Vicia faba* chloroplasts is 1 : 1 (ref. 16 and R. O. M., unpublished results) and values greater than this indicate the presence of other protein-containing membranes which do not contain chlorophyll. Despite the fact that small changes in the chlorophyll values give relatively large changes in the protein : chlorophyll ratio (see experiments 2 and 3) the calculated per cent contamination by lamellae membranes is consistent between experiments. It is clear that only 8 per cent – 16 per cent of the membrane protein in the chloroplast envelope fraction is lamellar in origin. Similar calculations for mitochondrial protein, based on estimates of succinate dehydrogenase activity, give values of a maximum of 1 per cent–3 per cent mitochondrial protein in the final membrane preparation.

Table 1. CHLOROPHYLL AND PROTEIN COMPOSITION OF SEVERAL PREPARATIONS

Experiment	Age (day)	Protein (A) (μg/ml.)	Chlorophyll (B) (μg/ml.)	Ratio A : B	Lamellar protein
1 (4)	18	47·7	7·8	6·2	16 per cent
2 (2)	19	38·5	4·7	8·2	12 per cent
3 (4)	19	42·5	3·4	12·5	8 per cent
4 (6)	21	21·8	2·9	7·7	13 per cent

Composition of a fraction containing chloroplast envelopes prepared as described in the text. The number of replicas for each experiment are shown in parentheses. Protein determined on an 80 per cent acetone precipitate according to Lowry *et al.*[18]; chlorophyll determined by the method of Arnon[19]. Lamellar protein is expressed as a per cent of the total protein and assumes protein : chlorophyll ratio of 1 : 1 in the lamellae membranes (ref. 16 and R. O. M., unpublished).

Table 2. COMPOSITION OF FRACTIONS CONTAINING CHLOROPLAST ENVELOPE MEMBRANES

Experiment	μg/100 g leaves Protein	μg/100 g leaves Chlorophyll	Non-lamellar protein	Per cent intact chloroplasts in starting material
1	450	46	404	40–50
2*	293	86	207	15–25
1	130	25	105	5–10

The percentage of intact chloroplasts is a visual estimate of preparations viewed by phase contrast microscopy.
* Incubation at 10° C. Postincubation centrifugation 1,000*g* for 15 min.

The difference between the total protein and the lamellae protein in this membrane fraction gives a good quantitative estimate of the chloroplast envelope material present. The greater the percentage of intact plastids in the original chloroplast suspension, the greater should be the proportion of envelope protein in the final membrane fraction. The data in Table 2 shows that there is a direct relationship between the percentage intact plastids in the original chloroplast suspension and the non-lamellar protein in these membrane fractions isolated from it.

The appearance of the membrane fraction by phase contrast microscopy and in the electron microscope and the demonstration that it is enriched with membrane protein not associated with chlorophyll suggests that it is suitable for further investigation of the chemical and enzymological properties of the chloroplast envelope membrane.

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Light Compensation Points and Photorespiration

SOME results of an investigation into light compensation points of photosynthesizing leaves are relevant to arguments about the occurrence or absence of photorespiration in illuminated leaves of species which do not release carbon dioxide into carbon dioxide-free air, and which have a carbon dioxide compensation point (Γ) close to zero[1–3].

At the light compensation point equivalent amounts of carbon dioxide are evolved and fixed photosynthetically within leaves, and because increases in temperature cause greater increases in the rates of processes which produce carbon dioxide than in rates of photosynthesis, light compensation points increase with temperature (Fig. 1). The points shown are the means of values obtained with four leaves from each species; temperatures stated are measured leaf temperatures. Light was supplied by water-cooled incandescent lamps suspended at variable heights above a constant temperature leaf chamber; intensities were measured with a barrier cell held in a very flat frame and calibrated with a megatron light meter; the cell could be moved to immediately above the leaf surface inside the chamber. The light compensation point was taken as the intensity at which the concentration of carbon dioxide in the closed circuit was maintained at 310 p.p.m. for 30 min.

With one notable exception the light compensation point was apparently most closely related to leaf thickness and the amount of non-chlorophyllous tissue in the leaves; this is best illustrated by the sun and shade leaves of *Impatiens parviflora* (Balsam) and of copper beech. In the latter, however, the sun leaves were reddish-brown and the shade leaves a dull green. There was a similar relation between the light compensation points of sun and shade leaves in *Rhododendron*.

The somewhat fibrous leaf of sugar cane (a zero-Γ plant) was clearly exceptional but the other zero-Γ plant, maize, did not have this exceptionally low light compensation point and differed very much from sugar cane in its temperature response. Both plants were grown in a glasshouse; it was not possible to distinguish between sun and shade leaves in these plants. On many days during the growth of these plants light intensities reached 80,000 lux. Day temperatures were usually hotter than 25° C and often reached 37° C.

It could be argued that in sugar cane leaves the rate of evolution of carbon dioxide in the light was as low as in shade leaves, but this is not so for maize with a light compensation point of 2,300 lux at 35° C—its optimum temperature for photosynthetic performance. As reported earlier[4], maize does not establish zero-Γ at temperatures hotter than 30° C and the minimum light intensity to reach zero-Γ at 30° C is about 5,000 lux. In spite of its low light compensation point the same is true of sugar cane; its light requirement for establishing zero-Γ at 30° C was higher than that of maize. The dark respiration rate of sugar cane was 0·8 mg of CO_2 dm^{-2} h^{-1} at 25° C and 1·6 mg of CO_2 dm^{-2} h^{-1} at 35° C; for maize the values were 1·6 and 3·4 mg of CO_2 dm^{-2} h^{-1} respectively. Anatomical differences between the two species relate to the distribution of chlorenchyma, but it is difficult to see a connexion between these and the very different light compensation points.

These results support the view that in illuminated maize leaves (and perhaps sugar cane too) carbon dioxide is evolved but not released into carbon dioxide-free air, because of an effective carboxylation mechanism which is probably not restricted to cells containing distinct chloroplasts[5,6]. This does not preclude the possibility that at intensities above the light compensation point the evolution of carbon dioxide is progressively inhibited, but there is no good evidence for this view.

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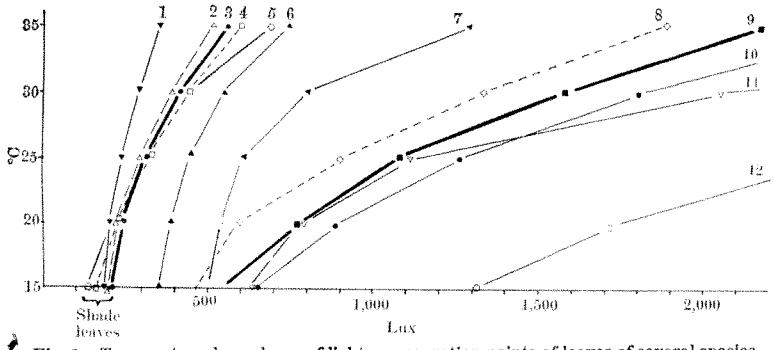


Fig. 1. Temperature dependence of light compensation points of leaves of several species. The percentages indicate the proportion of light transmitted by the leaves when placed directly on the barrier cell. Leaf thicknesses are shown for those species of which sun and shade leaves were used. 1, 27 per cent, shade balsam, 80μ; 2, 30 per cent, lettuce, 120μ; 3, 13 per cent, sugar cane, 375μ; 4, 20 per cent shade beech, 80μ; 5, 8 per cent, shade *Rhododendron*, 220μ; 6, 17 per cent, partial sun balsam, 120μ; 7, 13 per cent, sun balsam, 180μ; 8, 4 per cent, sun beech, 150μ; 9, 8 per cent, maize, 270μ; 10, 11 per cent, sugar beet, 260μ; 11, 10 per cent, wheat, 230μ; 12, 4 per cent, sun *Rhododendron*, 480μ.

CO_2 Assimilation by Chloroplasts illuminated on Filter Paper

CHLOROPLAST suspensions from *Spinacea oleracea* or *Pisum sativum* assimilate CO_2 in the light at rates comparable with those observed with intact leaves[1–3]. With most preparations the major photosynthetic products are phosphoglyceric acid (PGA) and dihydroxyacetone phosphate (DHAP)[2,4,5]. Although the synthesis of these compounds is consistent with the operation of the photosynthetic carbon reduction (PCR) cycle[6] in the isolated plastids it is still not clear why these particular intermediates accumulate. It has been shown[5] that the outer membranes of the chloroplasts are permeable to both PGA and DHAP. Thus a large proportion of the carbon fixed into these compounds may diffuse into the external medium. The amount of carbon lost from the chloroplasts will presumably depend on the ratio of the volume of external medium to that of the aqueous phase of the chloroplasts. For chloroplast suspensions containing 100 μg chlorophyll per ml. this

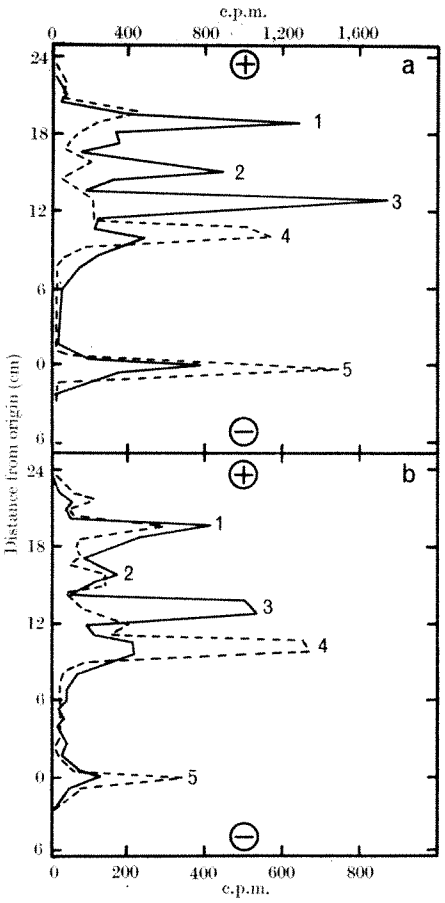


Fig. 1. Distribution of radioactivity into photosynthetic products after 20 min illumination of (*a*) spinach chloroplasts and (*b*) pea chloroplasts in suspension (———) or on filter paper disks (- - - -). Ethanol extracts were resolved using high voltage paper electrophoresis in pyridine–acetic acid–water. The principal peaks of radioactivity correspond to (1) phosphoglyceric acid, (2) sugar diphosphates, (3) dihydroxyacetone phosphate, (4) hexose monophosphates, (5) glycine plus serine plus alanine.

ratio would be over 100 : 1, contrasting markedly with the fluid environment of the chloroplasts *in vivo*, where the ratio of the volume of non-chloroplastic cytoplasm to that of the chloroplasts is usually less than one. It seemed possible, therefore, that more of the PGA and DHAP formed might be metabolized further if the volume of medium in the experimental sample was reduced to a minimum by layering the chloroplasts on filter paper.

Chloroplasts prepared by conventional techniques[7] from leaves of spinach (*Spinacea oleracea*) or peas (*Pisum sativum*) were resuspended in a medium containing 0·33 M sorbitol, 0·001 M $MnCl_2$, 0·001 M $MgCl_2$, 0·002 M versene, in 0·05 M HEPES (N-2-hydroxyethyl-N′-2 ethane sulphonic acid) buffer (*p*H 7·5). Chloroplasts in suspension, equivalent to 100 μg chlorophyll, were spread on disks of Whatman number 42 filter paper (diameter 2 cm) placed on a coarse glass sinter connected to a filter pump. Suction was maintained until all visible moisture had been removed. The disks were illuminated in 'Perspex' chambers of 5 or 10 ml. volume at 60,000 lx and 25° C. Radioactive $^{14}CO_2$ (0·5 per cent by volume) was generated in the chambers by injecting $Na_2{}^{14}CO_3$ into central wells containing lactic acid. The chloroplasts were inactivated and extracted by plunging the paper disks into hot 80 per cent ethanol. Alternatively chloroplasts equivalent to 100 μg chlorophyll were illuminated in 300 μl. medium containing 3·3 mM $Na_2{}^{14}CO_3$ in glass centrifuge tubes sealed with Subaseals[8] at 60,000 lx and 25° C.

In the first type of experiment the chloroplasts were assimilating gaseous CO_2, whereas in the second, carbon was available as bicarbonate ions. This made it difficult to compare the effective CO_2 concentrations of the two systems. The amount of carbon fixed, however, was not increased if the amount of carbon introduced into the experimental system was increased, so it was concluded that the chloroplasts were saturated with CO_2.

The time course of $^{14}CO_2$ assimilation by chloroplasts illuminated on filter paper supports was basically similar to that reported for chloroplasts illuminated in suspensions[1]. A lag period of about 3 min was followed by a period of rapid CO_2 fixation which lasted about 10 min. The rate of fixation then decreased significantly. The maximum rate of fixation extrapolated from time course experiments was equivalent to about 15 μmoles CO_2 assimilated per mg chlorophyll per hour. This compared with rates of about 20 μmoles CO_2 per mg chlorophyll per hour for chloroplasts illuminated in suspensions.

Photosynthetic products were separated by two-dimensional paper chromatography in phenol/water (100 : 40) followed by *n*-butanol–acetic acid–water (75 : 20 : 50)[9] or by one-dimensional high voltage paper electrophoresis in pyridine–acetic acid–water (4 : 6 : 490) buffer (*p*H 4·5)[10] at 4 kV for 30 min. Positions of radioactive products, revealed by autoradiography, were compared with those of authentic compounds detected using spray reagents. The largest radioactive areas on the electrograms were eluted and the identity of the substances established by co-chromatography and co-electrophoresis with authentic compounds.

As expected, PGA and DHAP were the major radioactive products recovered in extracts of chloroplasts which had been illuminated in suspensions (Fig. 1). In contrast, the amounts of these early intermediates of the PCR cycle produced by chloroplasts which had been illuminated on solid supports were significantly lower. More than 85 per cent of the radioactivity incorporated into ethanol soluble compounds was recovered in hexose monophosphates and amino-acids. About 10 per cent of the total carbon fixed was incorporated into a glucan (starch). These chloroplast preparations did not form sucrose. It has been suggested[11] that an enzyme concerned in the conversion of triose phosphate to hexose monophosphate controls the biosynthesis of sucrose in isolated chloroplasts. It might therefore be expected that the accumulation of monophosphates by chloroplasts illuminated on filter paper disks would favour sucrose formation. Because sucrose was not formed, however, it seems that the factors controlling its biosynthesis are more complex.

We thank Mr J. E. Brown for technical assistance.

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Book Reviews

TYING THINGS TOGETHER

Systems Psychology

Edited by Kenyon B. de Greene. (McGraw-Hill Series in Management.) Pp. xviii + 593. (McGraw-Hill: New York and Maidenhead, July 1970.) 178*s*.

THIS is an expensive book but it is a large one—about 400,000 words long. There are fifteen authors, all American, who are mostly psychologists but a few engineers and administrators are included. They deal with topics such as systems analysis techniques, accidents and safety, control systems, man–computer interrelationships, psychophysiological stress and "esthetics, architecture and city and regional design". The writing is discursive, usually factual, sometimes tedious, sometimes emotional and is rather deceptively informal. Deceptive in that there is an enormous expertise and a quality of structured thinking which may not be obvious to the reader who has not tried to do this kind of work. The authors are clearly men of extensive experience in the design of large scale systems, and many have a background of laboratory experimental work with which they tend to be disillusioned. For example, Chapanis (p. 76), "the fact of the matter is that most laboratory experiments in psychology have only very limited relevance for the solution of practical problems".

Systems psychology emerged from experiences on the post-war defence systems such as SAGE. This book is an advance on earlier ones in that it includes extensive work on space systems such as Apollo, industrial problems of computer based information systems and some tentative and entirely speculative excursions into current social problems. Typically the systems approach is defined as "a pragmatic approach to tying things together" justified by a rather nice quotation from John Muir, "When we try to pick out anything by itself we find it hitched to everything else in the universe". There are various dimensions and hierarchies of systems: behavioural—biological—equipment, particles—atoms—molecules—colloids—nuclei—cells—tissues—organs—organisms—man–machine systems—groupings, parts—units—components—subsystems—systems—macrosystems, elements—tasks—duties—jobs, automatic—man–machine—socio-technical—social. The systems psychologist is the specialist in human behaviour within this structure; he is the advocate for the human operator particularly when there is arbitration about the relative roles of men and machines. He functions most effectively as a member of design and development teams especially for technologically advanced systems. His tools are chiefly those of systematic observation, recording and analysis of real behaviour: task analysis, skills analysis and error analysis. He occasionally resorts to the study of isolated variables in the laboratory situation but only as a source of supporting evidence for his systematic observation. For the conduct of experiments he more often uses a simulator where the operator's task and environment are as similar as possible to "reality". He believes in training but seems to need the superficial security of hardware dominated training which relies on computers, simulators, teaching machines, mock-ups and visual aids: the teacher has become either a "management developer" or a "lesson planner". His more formal concepts are mathematically based: information theory, decision theory and control theory. He tries to steer a difficult course between the need to be comprehensive in his consideration of relevant variables and his preference for hard evidence. His justification must be that no more than a fair share of real system failures result from his design mistakes.

The book brings us up to date on current practice. There are interesting but dangerous new trends in the recognition of social problems and the substitution of humanistic values for cost effectiveness—dangerous in that the systems psychologists have at present no more idea of how to tackle these problems than anybody else has. The sweeping nature of systems concepts has an unfortunate tendency to induce grandiose thinking. In common with every other scientist or technologist, the systems psychologist can only deal with problems in relation to which he has both relevant concepts and relevant evidence. The interesting question is whether a young psychologist can operate more effectively as a laboratory experimentalist or a systems designer. My vote goes to the latter. Not because laboratory work is necessarily invalid but rather because the advancement of knowledge by good laboratory experiments is just too difficult except for a very tiny minority of research workers. Systems design is also difficult but those who are not sufficiently competent are more easily found out.

The book lacks an author index. References are placed at the end of each short chapter and so its value as a guide to the literature is not high. Diagrams and tables of data are excellent but there are not enough of them.

W. T. SINGLETON

RECORDS OF IRON-AGE HORSEMEN

Frozen Tombs of Siberia

The Pazyryk Burials of Iron Age Horsemen. By Sergei I. Rudenko. Translated from the Russian and with a preface by M. W. Thompson. Pp. xxxvi + 340 + 180 plates. (Dent: London, September 1970.) 252*s*.

PROFESSOR Sergei Ivanovich Rudenko was born in 1885. He graduated in Leningrad and was until his recent death a senior scientific member of the Institute of Archaeology of the Russian Academy of Sciences. Primarily an anthropologist, he readily appreciated that in Siberia the primitive present was very close to the primitive past. In 1947–9 he excavated four large barrows at Pazyryk. Frozen barrows had been dug in the Altai by Radloff in 1865, and by Gryaznov in 1925–9. One of Gryaznov's barrows was Pazyryk 1. No further work was carried out until 1947–9 when the other four large barrows at Pazyryk were excavated by Rudenko. It is the work at Pazyryk in 1929 and 1947–9 that formed the subject of Rudenko's *Kultura Naseleniya Gornogo Altaya v Skifskoe Vremya* published in Moscow/Leningrad in 1953. This present book is an English translation by Dr M. W. Thompson, and has the benefit of revisions by the author. The results of excavations since 1949 in other barrows in the Altai, where the finds were far less spectacular than those in the five Pazyryk barrows, were published by Rudenko in 1960 in his *The Culture of the Population of the Central Altai in Scythian Times* (Moscow/Leningrad), together with some revised judgments on his earlier work.

The dead in the Pazyryk barrows were buried in wooden chambers in shafts below the mounds. The unusual preservation of materials and human bodies from the ancient and prehistoric past of man is one of the great fascinations of the archaeological record. No student of the past can fail to be excited by the bodies surviving in the tree-coffins of Denmark or by discoveries such as those of Tollund and Grauballe man in the Danish peat bogs. Great dryness or wetness can preserve human beings, leather, wood and textiles as we know from Europe and America, but in a limited area of Asia—Mongolia and Siberia—constant freezing has the same spectacular results.

In the five great Pazyryk barrows the peculiarity of construction, the climate and the altitude caused the contents to freeze up after burial. Subsequently, robbers broke into these tombs, just as robbers have broken into allegedly rich tombs in Egypt and elsewhere; they took away much of the contents, but they never got to the accoutred horses outside the burial chambers. The skins of these horses survived intact and show us their colours and breeding. The condition of the human bodies was so good that it is possible to study the tattooing and to make one wonder again whether classical writers, describing the ancient Britons, were describing painting in woad or Pazyryk-like tattooing.

The artefacts of bone, wood and horn are remarkable as one might expect from the graves of important persons. There were found many bridles and saddles as well as an elaborate carriage. Perhaps the most exciting finds were pieces of Chinese silk, a Chinese mirror and a Persian carpet and a complete apparatus for inhaling the fumes of Indian hemp.

In his preface Thompson writes "the refrigerated objects . . . not only throw a flood of light on aspects of the life of the people who used them which have normally left no trace: but also throw some light on the civilized areas with which these people were in contact: the Persian and Chinese textiles at Pazyryk are older than any surviving examples in Persia or China".

These were the tombs of semi-nomadic people, pastoral ancestors of the Kazakhs and other peoples who lived from the Caspian to the Altai mountains: people who practised little cultivation but kept herds of horses, cattle, sheep and goats. Carbon-14 dating tells us that these barrows date from between 500 and 200 BC: possibly 400 BC is the most likely date.

This is a remarkable book and we are fortunate in having it available in English in such a lavishly illustrated form. Dr Thompson has not only done the translation but contributed a long and learned introduction that is itself a most valuable essay. GLYN DANIEL

EVOLUTION AND GENETICS

Evolution and Genetics in Honor of Theodosius Dobzhansky

Edited by M. K. Hecht and W. C. Steere. Pp. xv + 594. (North-Holland: Amsterdam; Appleton-Century-Crofts: New York, 1970.) 152*s*.

Evolutionary Biology

Vol. 3. Edited by Theodosius Dobzhansky, Max K. Hecht and William C. Steere. Pp. viii + 309. (North-Holland: Amsterdam; Appleton-Century-Crofts: New York, 1969.) 147*s*.

THE inevitable *festschrift* for Dobzhansky has appeared; it is a handsome book with fifteen articles on evolution and genetics and an introductory chapter about Dobzhansky himself. With what trepidation must honoured academics first open the pages of their *festschrift*, scan the titles, and start to read. Will the papers be irrelevant to their life's work; will they be rehashes of previous tired reviews; or will the papers be there simply because they will not be critically refereed ? (One has seen them, unfortunately.) The famous must have some tense moments.

Dobzhansky's admirers have, on the whole, done him well. Naturally, most of the articles are reviews. Two are historical or philosophical. The essay by Simpson, on "Uniformitarianism", is a delight to read. Rensch's article on "Panpsychistic Identism" is almost completely opaque, but there are signs that the translation is very unhelpful. The reviews are often long, and can treat the subject to a satisfying depth. In particular, the paper by Carson, Hardy, Spieth and Stone (100 pages long) is fascinating. There are four other reviews on aspects of *Drosophila*. Beardmore discusses the relationship between the variability of the environment and the variability of gene pools. Spiess gives a long review of the influence of the genotype on mating ability in *Drosophila*. Brncic discusses Chilean *Drosophila*, and it is interesting to compare the relatively staid evolution of these drosophilids with the florid evolutionary behaviour of the Hawaiian forms. Ayala deals with Volterra's equations in relation to competition experiments with *Drosophila*, and he attempts to discern the effects of competition on the genotype.

Stebbins discusses the progress in plant evolutionary studies since his book was published in 1950. The article by Li on human genetic adaptation, though a useful summary, is somewhat too slight to be very satisfying, but it is amusing reading, particularly for the way he can dispose of opponents with a deft phrase. Two papers, by Florkin and Schoffeniels on adapted molecules, and by White on parthenogenetic animals, complete the reviews. The former paper is extremely slight.

Creed, Dowdeswell, Ford and McWhirter have nearly brought up to date their studies of the peculiar "boundary phenomenon" in *Maniola jurtina*. Although some interesting thoughts spring from their paper, it is frustrating not to have a clue as to what selective forces are working on the species. Eiche and Gustafsson report on a massive experiment to determine the hardiness of Scots pine transplanted to more or less climatically unfriendly sites in Sweden. The writers, and the reader, are troubled at times by the interaction of climatic and inbreeding effects. The Rizkis report some peculiar patterns of enzyme activity in *Drosophila* species hybrids. Finally, Wallace, in a short paper, astonished the non-drosophiliac reviewer with the very small distances *Drosophila* feels it necessary to move if conditions are right.

All in all we have here a solid, reasonably digestible mass of the kinds of evolutionary study that Dobzhansky and the rest of us will appreciate.

The other book is the third volume of a series edited by Dobzhansky, Hecht and Steere. So far, this series has been a useful one, with a good range of topics. Here there are articles on aspects of evolution in oats, social bees and parthenogenetic weevils. The article on defences against visually hunting predators is interesting but it is rather out of place. Other articles cover sexual selection in *Drosophila* and cancer cells as temperature sensitive mutants, and, finally, there is a long and welcome article by Hopson and Crompton in which they argue for the monophyletic origin of mammals. J. D. CURREY

THE *DROSOPHILA* OVARY

Ovarian Development in *Drosophila melanogaster*

By Robert C. King. Pp. x + 227. (Academic: New York and London, July 1970.) 154*s*.

Drosophila melanogaster is probably the commonest experimental animal, and the literature on it increases by more than two papers a day. Most of this output is concerned with genetics, of course, and there is still a surprising shortage of publications on *Drosophila* physiology, even reproductive physiology. Professor King's book is therefore very welcome because it fills one such gap by dealing with the important subject of ovogenesis. In essence, it is a summing up of the researches carried out or stimulated by his group during the past fifteen years, and anybody familiar with this work will expect this monograph to be well illustrated with clear line drawings and by relevant photomicrographs; their expectations will be fulfilled. This book will therefore be useful as a convenient standard reference; others will find it a useful review of muscoid ovogenesis, as typified by *Drosophila*. The skeleton of the subject is necessarily descriptive morphology, covering everything so far published

from the origin of the pole cells to the mature, functioning organ. This anatomical detail is extensively illustrated. But the text is also an essay in developmental genetics, for the advantage of *Drosophila* is that mutants are available which interfere with normal processes and thus provide comparative material for experimental study.

Not all the general problems of morphogenesis are subsumed in the ovary, but a large number are: from the origins of polarity in the undifferentiated larval rudiment, through the many problems of stem cells, the exact regulation of division of their products to form 16-cell cysts and the differentiation of all but one of these cells into polytene nurse cells, to cell interactions and to the hormonal initiation of egg production and the regulation of egg output. All of these are discussed, but only a few mutants affecting some morphogenetic events have so far been found and can be examined. This book should stimulate the search for more. Using female sterile mutants (*fused*, *female sterile* and *narrow*) it has been possible to show, for instance, that each gene affects the restriction of cystocyte division, leads to the development of "tumorous" cysts, and acts autonomously. King produces a molecular model to explain this, which he recognizes is too speculative but which may be a useful working hypothesis. Similarly, he uses crossover suppressor mutations to examine the cytology of the oocyte nucleus, and shows that synaptonemal complexes are not then formed. This leads to an extensive discussion of recombination, and to further models. The effects of other mutants influencing vitellogenesis, development of follicle cells and of nurse cells are also indicated, and the book concludes with a short section on interactions of the ovary with other organs. Unfortunately these interesting analyses throw little light on the major problem of relating the detailed events of ovogenesis to embryonic determination in this mosaic egg.

In some respects too much material is compressed into too little space, and the book is far too expensive for its size. But it is a mine of information, covering about 500 references, and it should stimulate further work, as intended. There is a comprehensive index.

JAMES H. SANG

ORGANELLE DEVELOPMENT

Control of Organelle Development

Edited by P. L. Miller. (Symposia of the Society for Experimental Biology, No. 24.) Pp. viii+524. Published for the Company of Biologists on behalf of the Society for Experimental Biology. (Cambridge University: London, October 1970.) 140*s*.

ALTHOUGH the non-chromosomal transmission of mutations involving chloroplast and mitochondrial functions had been studied for many years, it is only in the past decade, following the discovery that these organelles contained DNA and were capable of incorporating labelled amino-acids into their proteins independently of the cytoplasmic ribosomal systems, that a combined assault by biochemists, cytologists and geneticists on the problem of organelle development has been sustained.

It is difficult to point to the major contributions made by individual authors in this volume because many contributors have attacked the problems across a broad front. Overall, however, a coherent picture of the influences involved in the assembly of chloroplasts and mitochondria emerges. Their DNA, RNA and protein synthesizing systems are shown to resemble the corresponding systems in bacteria and reinforce the idea, favoured by a number of contributors, that these organelles have evolved from endosymbiotic prokaryotes. It is made equally clear that the degree of autonomy of chloroplasts and mitochondria is limited and that nuclear genes provide considerable information for the structure of the organelles and control of their assembly. Even the nucleus enjoys only a limited autonomy for its activities are shown to be influenced by the status of the cytoplasm.

Three contributors describe their work on recombination of chloroplast and mitochondrial genes. Of particular interest in this respect is the suggestion by Slonimski and his co-workers that, in yeast, the tendency for mitochondrial genes to undergo recombination depends not only on their separation, but also on their distance from a polarity locus which may vary from strain to strain. This implies a kind of mitochondrial sexuality, possibly independent of the cell mating type.

On the whole, the contributions maintain the high standard set by previous symposia organized by the Society for Experimental Biology and the book provides a more comprehensive approach to the field of organelle development than has been previously achieved.

PETER A. WHITTAKER

PLANT VARIETIES

Genetic Resources in Plants

Their Exploration and Conservation. Edited by O. H. Frankel and E. Bennett. (IBP Handbook No. 11.) Pp. xxi+554. (Blackwell Scientific: Oxford and Edinburgh.) 100*s*

THIS book, published in the IBP Handbook Series as a guide for biologists participating in international programmes, includes the papers presented during an FAO/IBP technical conference held in Rome in 1967. It is divided into six sections. The first deals with the evolution of cultivated plants and includes descriptions of the pattern of genetic variability and of adaptation in cultivated plant populations. This is an extremely useful section emphasizing important concepts and principles. This subject leads on to the tactics of plant exploration and to the ecological considerations involved. The third section describes the methods of exploring variability in individual crops and forest tree species and is rather inadequate in that no crop is dealt with fully. Section four includes the evaluation and utilization of genetic resources, and chapter 37 by Krull and Borlaug stresses an important point that effective utilization of variability in breeding improvement depends on breeding to a well defined objective. The final sections, on documentation and conservation, contain information which is new. The methods suggested for the conservation of genetic resources include the management and provision of an inventory of wild communities or primitive cultivars, the maintenance of plant introduction collections at breeding stations, world seed banks and the development of composites or mass reservoirs of variability. Because there is no practical way in which plant material can be maintained with complete genetic integrity, any of these methods must be a compromise and the problems of maintenance of variability are clearly discussed by Allard in chapters 8 and 41. Although the conservation of variability is important it would be quite impossible to conserve all the variability that now exists and the plant breeder could never utilize all this variability. Effective utilization of variability by the plant breeder must be emphasized; so often genetic advance is not limited by variability but by the selection pressures to which the organism is subjected.

This book with its 554 pages and more than forty contributors is not recommended for consumption in one sitting but rather as a book to be dipped into like an encyclopaedia. As such, it provides a most useful source of information not available elsewhere; the editors and contributors have succeeded in stimulating thought and possibly action on this world wide problem of the conservation of genetic resources in plants. A. M. EVANS

ELECTROCHEMICAL PROCESSES

Electrochemistry

By J. Koryta, J. Dvorak, and V. Bohackova. Pp. xv+350. (Methuen: London, September 1970.) 105*s*.

TEXTBOOKS and monographs on electrochemistry are fairly common but here at long last is one that can be recommended. The material for the book originated in lectures given at the Charles University in Prague. The four principal chapters deal with equilibria in electrolyte solution, transport phenomena, equilibria in heterogeneous electrochemical systems and the kinetics of electrochemical processes.

Although first year undergraduates might find the going rather tough, the level is suitable for a third year course or for postgraduates starting on electrochemical research. For instance, there are useful sections on such topics as the acidity function, convective diffusion and experimental methods in electrochemical kinetics; there is also a valuable discussion of the double layer, and the authors quite rightly devote as much space to diffusion as to conductivity in electrolyte solutions. There is necessarily a large number of equations but the mathematics is well explained and there is a useful appendix on the solution of the differential equations found in electrochemistry. The authors' descriptions are concise and they certainly believe in using the deep end: the Debye–Hückel theory starts on page 17 and the pace continues to be as fast. But the topics are ordered in a logical rather than in a traditional or historical fashion.

The standard of the English is good and there are plenty of helpful diagrams. One can criticize one or two small points; for instance, there is only just over a page on the actual measurement of activity coefficients and the examples of important electrode processes are too briefly described. I would have preferred more references than the twenty or so key review articles given at the end of each chapter. But all in all this is a book to be recommended for teachers and students. Professor Koryta and his co-authors will now have to get busy re-writing the book in SI units, no doubt a more difficult task than translating it into English.

W. J. ALBERY

RADIATION CHEMISTRY

The Radiation-induced Decomposition of Inorganic Molecular Ions

By Everett R. Johnson. Pp. ix+144. (Gordon and Breach: New York and London, August 1970.) 145*s*; $17.40.

I DID not immediately comprehend from the title that this was a book about the chemical effects of high energy radiation on inorganic solids. At a first glance and even on reflexion it is easy to confuse the stable molecular ions of this book with the transient molecular ions which have little or no connexion with the subject matter of the book. Indeed, the investigation of such transient molecular ions, both positive and negative, constitutes one of the most active areas of chemical physics at the present time. Workers in this field would certainly feel cheated if they bought this book, and in any future edition it would certainly be advisable to select a less ambiguous title.

Title apart, the book is most useful and presents in a most readable manner the chemical changes induced by ionizing radiations in inorganic solids such as nitrates, chlorates, perchlorates, bromates, azides and permanganates. An introductory chapter gives certain background material in solid state chemistry, and this is followed by a general account of the factors affecting the radiation decomposition of inorganic solids. Thereafter, the solids are discussed individually according to the nature of the anion. The account is both descriptive and critical and the book can be recommended to graduate students who are freshly entering the field. The subject, which has not progressed far beyond the descriptive stage, is still fraught with inconsistencies and remains removed from the main stream of chemistry. Nevertheless, the author has made a determined and praiseworthy attempt to systematize the available data. The major limitations are inherent in the subject itself.

GLYN O. PHILLIPS

WORK OF A MASTER

Gesammelte Abhandlungen

By David Hilbert. Second edition: Band 1: Zahlentheorie. Pp. xvi+539. Band 2: Algebra, Invariantentheorie, Geometrie. Pp. viii+453. Band 3: Analysis, Grundlagen der Mathematik, Physik, Verschiedenes, Lebensgeschichte. Pp. vii+435. (Springer-Verlag: Berlin and New York, 1970.) DM98; $27 the set.

THE firm of Springer has given us, in the same year as Constance Reid's excellent life of Hilbert, a reprint of the master's collected papers, first published in 1932–5. It would be an exaggeration to say that these papers cover the whole field of mathematical development in the half-century 1885–1935; for instance, Hilbert seems to have had little interest in the Lebesgue theory. But nobody else of the time could match his power of making deep and fundamental penetrations on so wide a front.

Arrangement is not chronological, but by topics. The first volume deals largely with his series of great memoirs, establishing the principles of class field theory. It ends, however, with a dessert dish in the proof of Waring's conjecture; the first note in 1909 used a 25-ple integral, but the second note in the same year made a 5-ple integral suffice. In the second volume, we find the three pages in which Hilbert disposed of Gordan's theorem on the existence of a finite basis for invariants, a proof which provoked Gordan to remark "This is not mathematics, this is theology". The whole contents of this volume are set in perspective by two surveys, one by van der Waerden on the algebraic part, and one by Schmidt on the geometric work, principally the axiomatic formalization of geometry.

Volume three is a rich miscellany. The papers on analysis deal chiefly with the rehabilitation of Dirichlet's principle, and some axiomatics. Then there is the famous paper given to the international congress in Paris in 1900, the list of unsolved problems which governed the direction of much of twentieth-century mathematics. The memoirs on integral equations, later collected into a book, are not included, but there is a most valuable survey of this topic, up to and including Hilbert's powerful contributions, by Hellinger.

The volumes have been printed with the publisher's usual care and skill, and any library with mathematical pretensions, however meagre, must find room for them.

T. A. A. BROADBENT

REVISED KUHN

Atomic Spectra

By H. G. Kuhn. Second edition. Pp. xvii+472. (Longman: London, January 1970.) 105*s*.

THE publication of a second edition of *Atomic Spectra* indicates that the book has found its place in the physics literature in that it covers the subject more thoroughly than the more general textbooks while being less mathematical than the more specialized texts such as that by Condon and Shortley.

For some years now the principal trend in high resolution atomic spectra has been the use of radio frequency

methods where very small level differences can be more easily and accurately measured; some 15 pages have therefore been added to the first edition covering such topics as atomic beam resonance and optical pumping. As might be expected from the author's interests, the sections on width and shape of spectral lines have also been increased with a discussion on Voigt profiles following recent work. Recent developments in the field of auto-ionization have also been included. Basically the book remains as in the 1962 edition—an excellent summary of the theoretical interpretation of atomic spectra, with hyperfine structure and isotope shift being most thoroughly treated. The omission of even a passing mention of applied spectroscopy, either in the spectrochemical aspects or its use in astrophysics, for example, solar corona and ultraviolet spectra (see the work on the solar eclipse described in *Nature*, **226**, 229 and 1097; 1970), emphasizes the academic outlook. The book will continue to prove most useful for the more advanced undergraduate and the research worker both in the title subject itself and related fields where interpretation of spectra is essential.

L. Bovey

AIR POLLUTION MEASUREMENT

The Analysis of Air Pollutants

By W. Leithe. Translated by R. Kondor. Pp. x+304. (Ann Arbor–Humphrey: Ann Arbor and London, July 1970.) $18.75.

This book is a translation of the original German edition of 1968. In this English edition is included the author's addenda (11 pp.) giving additions and bibliographic references which apply in particular to British and American conditions.

The book is divided into six parts. The first is a discussion on the problems of clean air. History and legislation are considered as well as sources and control of pollution, meteorological effects on dispersion and the hygiene of air pollutants. In the space available these aspects can be but briefly summarized. Regulations operative in German-speaking countries predominate and proposed values of maximum ground level concentrations, are the MIK-values of the Union of German Engineers. The addenda relating British legislation is disappointing. The work of the Alkali Inspectorate is noted and the Clean Air Act (1956) is briefly—and incorrectly—summarized in six lines. More recent legislation is not mentioned.

The next two parts deal with special techniques for air sampling and for preparation of gaseous mixtures of known composition. These are most useful sections, the techniques being most clearly described. The fourth part considers special analytical methods. A selection of apparatus specifically designed for estimation of pollutants, from the simple Dräger tubes to sophisticated automatic continuous devices of British, American and German manufacture, is described. The generous treatment of gas chromatographic methods of pollution measurement is particularly interesting.

The last two parts (which account for more than 50 per cent of the book) contain a selection of methods for the estimation of all types of pollutants. The working procedures are described with typical Germanic thoroughness and attention to detail. All methods are supported by an extensive bibliography. (The bibliography contains 327 entries and an additional 88 references are listed in the addenda.) It is disappointing that the Warren Spring method for the estimation of smoke—as used in the British National Survey—is omitted but, in any selection, one expects to find certain personal favourites excluded.

Overall, the work should prove to be a vade mecum for the analyst engaged in any aspect of pollution measurement.

Frederick J. Allan

Short Notices

World Nuclear Directory.

Fourth edition. Pp. lxxix+764. (Harrap: London, November 1970.) 300*s*.

The latest edition of this comprehensive reference book remains basically an international list by countries of bodies concerned with the study and exploitation of nuclear phenomena. The entries under the name of a particular country are divided into subgroups such as national agencies, government departments, universities and industrial concerns; each entry gives a short account of the nuclear activities of the body or institution with its address and the names of its chief personnel (in most cases). One of the principal additions is a list of reactors throughout the world, with their commonly used abbreviations or names and their state (under construction, proposed or dismantled, for example). A list of world nuclear periodicals and their editors or editorial boards is also included. The book can be slightly criticized on the grounds that the subgroups run on from one to the next without distinction. It is possible that a reader unfamiliar with the layout and not using the index might look in the wrong subgroup because each is listed alphabetically.

X-rays.

By Brian Bowers. (A Science Museum Booklet.) Pp. 36. (HMSO: London, 1970.) 4*s* 6*d*.

This booklet is primarily a history of the development of X-rays and an account of their everyday use in such diverse applications as medicine, materials testing and the study of painting. As a Science Museum booklet, it begins predictably enough with a brief survey of the museum's own collection of X-ray equipment and early radiographs. The booklet commends itself to sixth formers and to students about to visit the museum, and indeed to anybody, specialist or not, interested in an overall picture of the growth of the subject. One unusual feature of the publication is a description of popular attitudes to X-rays soon after their discovery at the end of the last century; a New York newspaper, for example, announced that X-rays were being used "to reflect anatomic diagrams directly into the brains of the students, making a much more enduring impression than the ordinary methods of learning anatomic details".

Non-Solar X- and Gamma-ray Astronomy.

Edited by L. Gratton. (International Astronomical Union Symposium No. 37, Rome, Italy, May 8–10, 1969.) Pp. x+425. (Reidel: Dordrecht, 1970.) Hfl. 70.

Over the past eight years high energy astronomy has become an important source of information about the universe. The expected launch in 1971 of a satellite devoted exclusively to high energy astronomy should, if successful, provide more information each day than has so far been gleaned by all the balloon and sounding rocket experiments. This, then, is a good time to take stock of the situation in high energy astronomy. The symposium held in Rome in 1969 achieved just such a stocktaking, and the publication of its proceedings provides a landmark in the development of the topic. Observations are, in general, not yet sufficiently definitive for detailed theories of the sources to be constructed, but reviews of the theoretical position as of 1969 are very complete, so that anybody working in the field of high energy astronomy would need little more than this volume and those papers published since mid 1969 in order to place his own work in perspective. When so much has already been achieved with limited techniques the potential of the X-ray Explorer satellite is awe-inspiring; this may well be the last opportunity for any normal individual to have any hope of achieving a widespread knowledge of the whole field.

Correspondence

Citation Idiosyncrasies

SIR,—In continuing the discussion on the use of the Science Citation Index for historical purposes, Mr Cawkell is undoubtedly right in arguing that it is necessary to work on probabilities rather than certainties. You, sir, are equally correct in your editorial in pointing out that comparatively little is known as to why authors cite particular papers when writing an account of their own work.

In my review of the Science Citation Index for 1964 (*Nature*, **208**, 717; 1965) I made two particular points. First, that the value of the Index could only be truly assessed when volumes covering a number of years were available. That is now the situation, and it is reasonable to say that it has proved to be a useful information retrieval tool. As a generalization it can be said that, if the use of any major information retrieval system results in the retrieval of some 60 per cent of the relevant papers (for example, ref. 1), then the Science Citation Index will also be able to produce references to some 60 per cent of the relevant papers; the value of the index is that it is likely to retrieve a different sub-set and therefore the use of the two systems might well result in an increase of, say, 85 per cent in the overall recall ratio.

My second point was that an improvement in the citing habits of authors would do more than anything else to improve the usefulness of a citation index, and suggested that the time was coming when editors of scientific journals should take the same active steps to obtain a higher quality of citing as they have successfully been doing in relation to titles and abstracts. While the ultimate value of citation indexes was still uncertain, this was perhaps an optimistic hope, but the adoption of a simple code showing the purpose of a citation, for example, that it referred to a standard experimental technique, would undoubtedly increase the usefulness of the Science Citation Index. It would not, of course, result in the retrieval of a single additional relevant paper, but it would eliminate some of the noise which, *pace* Mr Cawkell's remarks, is as troublesome in the Science Citation Index as in any other information retrieval system.

Yours faithfully,

C. W. CLEVERDON

Library,
Cranfield Institute of Technology,
Cranfield, Bedford.

[1] Lancaster, F. W., *Evaluation of the Medlars Demand Search Service* (National Library of Medicine, Washington, 1968).

SIR,—The recent correspondence in *Nature* on the importance or otherwise of having one's papers cited extensively and the alleged structure of scientific development which can be perceived in a citation index has led me to wonder whether reference giving has not become an absurd convention which scientists unthinkingly adhere to. I claim no special virtue of freedom from infection in this respect.

To the outsider, the scientific referencing system must seem burdensome, pompous and irrelevant. What is the purpose of giving a reference? We answer that it allows the reader to enter a new field by giving him background, that it establishes the truth of our assertions and it enables us to indicate the extent of our own original contribution. Our rationalized view of reference-giving seems to me about as relevant to what really happens in science as the philosopher of science's concern with induction, deduction, hypothesis theory and law. The process of giving a reference is frequently wayward, dishonest or self-centred. We are fairly well constrained to tell the truth so all our pent-up human instinct to self aggrandisement comes bubbling to the surface in those first few paragraphs where we tell it "like it is". In that fertile ground we can tell you how we had the idea first—"The recent interesting proposal of Doe *et al.*[1] comes as a gratifying confirmation of my earlier ideas[2]", where it turns out that reference (1) is a full mathematical analysis with several experimental examples whereas (2) is the abstract of a speculative paper read to the Bruddesford Philosophical Society in which we managed to cover all possible developments in half an hour of arm-waving. Or we can tell you how extraordinarily well read we are, by quoting fifteen peripherally relevant articles in fifteen languages. Most of these articles will have been accumulated by accretion. Someone else referred to them and we added them to our list because they looked nice. This illustrates a fundamental law of reference giving—*it is quite unnecessary to have read or even seen the reference yourself before quoting it.*

Or we can show how up-to-date we are with all our references being not more than two years old. Or we can show how well connected we are by giving references to a large number of preprints. Or we can discreetly kick someone in the teeth by omitting to refer to his relevant paper. This last procedure guarantees us a large manilla envelope in the mail a few weeks later with his reprint inscribed with compliments. Or we can show our contempt for modern science by referring only to textbooks printed before 1900. Or we can spike criticism by a glowing reference to a potential critic. Or (the most common of all) we can take out our box of well-worn cards, shuffle them, xerox them and stick them on the end of the paper. "A mere orgy of dittography" as Evelyn Waugh's headmaster once criticized one of his essays. But we must be careful to mention every reference in the text; one of the more common sub-editorial comments on galley proofs is "not mentioned in text" against references.

With such an idiosyncratic background to references why bother at all? Practising insiders know how a paper fits into the mainstream of science and references are wasted on them. The intelligent outsider, if he can ever wade through our prose, is presumably sufficiently intelligent to write to us if he wants to know more. Perhaps most persuasive, however, is the time that would be saved by secretaries, sub-editors and printers in not having to reproduce that page of staccato, italicized, underlined, abbreviated, semi-colonized non-prose. And not having to worry whether the abbreviation for "Royal" if *roy.* or *Roy.* or even *r.*

As you must be aware, sir, authors of articles in *Nature* deem it desirable to have 25 ± 10 references in their papers. By reducing references to mention of previously unnoticed papers, useful equations and proofs, papers which one takes issue with or papers of very recent vintage which need to be brought urgently to public attention this figure might be reduced to 5 ± 3. It does not seem to me that science would be impeded; some money might even be saved.

I repeat—*mea culpa, sed inter alios.*

Yours faithfully,

DAVID DAVIES

47 Cross Street,
Belmont,
Massachusetts 02178.

SIR,—Alphabetical ordering of names in multi-author publication results in ambiguity about who did what; non-alphabetical ordering enhances the visibility of the individual by suggesting the order of importance of his contribution. There are no strict rules regarding assignment of publication credit among collaborators; many practices are followed[1,2]. We show that the policy by which multiple authors of papers published in *The Journal of Physiology* are listed in alphabetical order by surname without indication of the relative contributions of individuals has exerted selective control over publication of research in the journal.

The percentages of papers with different sizes of author-set published in vols 155–175 (1961–1964) of *J. Physiol.* were calculated in cases where the surname of at least one author began with A–E (520 cases) or P–Z (503 cases). Alphabetical value had no significant influence ($\chi^2 = 0{\cdot}05$, $P > 0{\cdot}05$) on whether an author published singly or with collaborators, even though in cases of multiple authorship P–Z surnames received first listing in only 7·6 per cent of cases compared with 80 per cent for A–E surnames. The possibility next considered was that P–Z authors published collaboratively in *J. Physiol.* when their authorship position approximated their research contribution but elsewhere when they wished to overcome the ambiguity in assignment of credit imposed by alphabetical ordering. The bibliographies of the 1,023 papers considered above were searched to establish the practice each set of authors had followed when publishing in journals which had no fixed policy on author-sequencing. Alphabetical ordering was maintained at a rate (78 per cent of cases) well above the value (48 per cent) obtained from a count of all references cited in *Annual Review of Physiology*, **26** (1964).

We found, however, that researchers with P–Z surnames avoid publication in *J. Physiol.* relative to those with A–E surnames. Comparison of the relative occurrence of A–E and P–Z surnames as *J. Physiol.* authors and as authors in other journals cited in bibliographies in *J. Physiol.* papers (the first 1,023 cases in vols 155–156) showed that P–Z surnames were significantly underrepresented (by 12·1 per cent) as authors of *J. Physiol.* papers ($\chi^2 = 19{\cdot}2$, $P < 0{\cdot}001$). Evidence that *J. Physiol.* authors with P–Z surnames maintain alphabetical sequencing in other publications suggests they either are junior collaborators in most research they report or prepared to publish in a manner which at face value renders ambiguous their role relative to co-authors. In latter cases there are probably many factors unconnected with publication of specific research by which this ambiguity is lessened[2].

Yours faithfully,

R. OVER
S. SMALLMAN

Department of Psychology,
University of Queensland,
Brisbane, Australia.

[1] Spiegel, D., and Keith-Spiegel, P., *Amer. Psychol.*, **25**, 738 (1970).
[2] Zuckerman, H. A., *Amer. J. Sociol.*, **74**, 276 (1968).

Misconception on MIRVs

SIR,—May I comment on the statement in "Pugwash Worth its Weight in SALT" (*Nature*, **227**, 1185; 1970) that: "Although the main strategic implications of MIRVs are as a first strike weapon, they were apparently developed by the United States as a penetration aid against the sophisticated Soviet air defence system which, it is claimed, is capable of being upgraded into an ABM system". This view, or more simply that the US developed its MIRV as a response to the Soviet ABM, is nearly universally repeated by persons on all sides of the ABM debate, in and out of the US Senate. It appears to be wrong. This is documented by two pieces of Congressional testimony.

Dr John Foster, Director, Defense Research and Engineering of the US Department of Defense, gave this evidence in 1968:

"Senator Mansfield: Is it not true that the US response to the discovery that the Soviets had made an initial deployment of an ABM system around Moscow and possibly elsewhere was to develop the MIRV system for Minuteman and Polaris?

"Dr Foster: Not entirely. The MIRV concept was originally generated to increase our targeting capability rather than to penetrate ABM defenses. In 1961–62 planning for targeting the Minuteman force it was found that the total number of aim points exceeded the number of Minuteman missiles. By splitting up the payload of a single missile (deleted) each (deleted) could be programmed (deleted) allowing us to cover these targets with (deleted) fewer missiles. (Deleted.) MIRV was originally born to implement the payload split-up (deleted). It was found that the previously generated MIRV concept could equally well be used against ABM (deleted)."

Mr Laird, the Secretary of Defense, gave this evidence in 1969:

Reason for US MIRV development

"Senator Pell . . .

"Now, in connexion with the system around Moscow, the so-called Galosh, the existence of which we discovered, I think, in the early 1960s, haven't we responded to that with our MIRV program? Wasn't it the Titan MIRV at that time?

"Secretary Laird: Well, I don't believe that necessarily is the case, Senator Pell. I would like to agree with you but having worked on these programs through the years——

"Senator Pell: Much more closely than I have because you were on the Armed Services Committee.

"Secretary Laird: As much as I would like to agree with you, I can't.

"Senator Pell: You feel it is a separate response, a normal outgrowth of regular research and development.

"Secretary Laird: Yes; it was a separate response and it was something we had under development and under consideration for several years even prior to the time that we knew of the deployment of the Galosh. I would not want to mislead anyone, as much as I would like to agree with you.

"Senator Pell: But our basic reason for developing MIRV was to increase our own deterrent power, not related, in your view, then to Galosh?

"Secretary Laird: It was not related to Galosh because we started appropriating funds for the development of this program prior to the time we knew Galosh was in being. That is why I cannot respond that way to that question.

"I happen to have been involved in the appropriation process, and we were funding the research and development on the MIRV, and we knew about these possibilities and had funded work on it prior to the time that the Soviet deployment of Galosh around Moscow was discovered."

The development of MIRVs is a most important point to get straight. MIRV represents a quantum, and a critical, jump in strategic weaponry. Various US administration spokesmen, past and present, have been guilty of repeating on public occasions the common misconception as to its origin, despite the above evidence to the contrary. The wrong view has no doubt been perpetuated because it presents the development (and the deployment) of MIRV as a response to Soviet action. Post ABM, ergo proctor ABM?

It may serve a useful purpose for US administrations, both the previous Democratic and the present Republican one, to maintain this error in misunderstanding, but that is no excuse for the rest of us to follow suit.

Yours faithfully,

MILTON LEITEMBERG

Stockholm International Peace
Research Institute,
Sveavägen 166,
S-113 46 Stockholm.

University Politics

SIR,—I read with considerable interest the article in *Nature* of October 24 (**228**, 314; 1970) on University Politics and I would like to correct one statement. This is to the effect that "At both Universities (Cape Town and Witwatersrand) members of staff and students alike . . . talk of their Universities as liberal, but it may be significant that this attitude is not usually accompanied by a willingness to embrace modern causes such as student representation". The fact is that at Witwatersrand, student representation is a very live issue and a great deal has already been achieved in bringing it about. Furthermore, the relationship between the Students' Representative Council and the University Administration is extremely cordial and strong.

Yours faithfully,

G. R. BOZZOLI

Vice-Chancellor and Principal,
University of the Witwatersrand,
Johannesburg.

Obituary

Professor T. K. Walker

THOMAS KENNEDY WALKER, professor of industrial biochemistry at the University of Manchester from 1954, when the chair was established, until his retirement in 1959, died on June 29, 1970, at the age of seventy-seven. Shortly after his death a most stimulating and remarkable account of the development of his department, written by himself, appeared in *Advances in Applied Microbiology* (**12**, 1; 1970). It describes his very extensive research work, his most happy relations with his students and his contacts with numerous biochemists at home, and especially abroad, and gives a strong impression of a man happy in his work and surroundings, with a growing, but quiet, satisfaction in the progress and reputation of his department. Walker's comments on the mental outlook required by those who would journey from the domain of organic chemistry to that of biochemistry should be read by all who have followed, or would follow, the same path. An article of his on the pollution of the environment will appear shortly.

After graduating at Manchester in 1915, Walker joined Weizmann's group to work on the technical production of acetone and *n*-butanol by an organism related to Fernbach's bacillus. This entailed a period of study at the Lister Institute. His life-long devotion to microbiology arose from this early work. His interest in large-scale operations was also enhanced by two years spent at the Royal Arsenal, Woolwich. These were followed by three years' research with Lapworth at Manchester on the interaction of diazonium salts with reactive methylene groups. From 1922 to 1925, he collaborated with Pyman at the Manchester College of Technology in an examination of the bitter bacteriostatic substance in the resin of hop-cones. Extensions of this work continued at intervals until 1958.

In 1925 Walker began a study of the behaviour of carbohydrates and aliphatic acids in cultures of *Aspergillus niger* and *Aspergillus oryzae*, during which acetic, pyruvic, dimethylpyruvic and α-ketoglutaric acids were detected in cultures of *A. niger* on various substrates, and the effect of fluoride, iodoacetate and cyanide was examined. He was frequently consulted about bacterial infections in yeast and beer and so arose a long series of investigations, many of considerable importance, on *Candida*, *Acetobacter* and *Lactobacillus* species. He became a recognized authority on *Acetobacter*, some species of which were shown to produce cellulose. From 1948 to 1958 he directed, for the Medical Research Council, extensive work on the production of edible fat by mould fungi, in which the cultural conditions for the most suitable moulds and the composition of the fat were closely studied.

Walker's manner was always quiet, friendly and unruffled. His sympathetic and encouraging attitude to the problems of young research students caused him to spend much time at their benches. He thus imparted that "feel" for the contents of a test tube or of an unpromising mixture which is the secret of successful laboratory manipulation and is still the distinguishing mark of the organic chemist. He had many diverse interests, including poetry and philosophy, and a religious faith that deepened with the lapse of years.

Announcements

University News

Professor Frank Press has been appointed Robert R. Shrock professor of earth and planetary sciences at the **Massachusetts Institute of Technology.**

Dr L. Mirsky has been appointed to a personal chair in the Department of Pure Mathematics, **University of Sheffield,** and the title of visiting professor in botany has been conferred on **Professor R. F. Jones,** State University of New York at Stony Brook, for the period January 1 to August 31, 1971.

Appointments

Professor Zdzislaw L. Wilhelmi, head of the Department of Nuclear Physics in the University of Warsaw, has been appointed director of the Division of Health, Safety and Waste Management in the Department of Technical Operations, **International Atomic Energy Agency.**

Miss Julia Henderson, associate commissioner for technical cooperation in the United Nations, has been appointed secretary-general of the **International Planned Parenthood Federation,** in succession to the late **Sir David Owen.**

International Meetings

February 18, 1971, **Safety in Chemical Manufacturing,** London (Institution of Chemical Engineers, 16 Belgrave Square, London SW1).

March 3–5, 1971, **Environment and Cancer,** Houston (Mrs Jane Brandenburger, M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas 77025, USA).

April 22, 1971, **Freshwater Biology and Electrical Power Generation,** Leatherhead (D. S. Wilson, Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey).

June 27, 1971, **Improving the Utilization of Energy,** with Special Reference to Complex Uses, Bucharest (The Secretary, British National Committee, World Energy Conference, 5 Bury Street, St James's, London SW1).

July 7–9, 1971, **Science and the Arts,** Edinburgh (Dr Eric G. Forbes, Institute for Advanced Studies in the Humanities, 12 Buccleuch Place, Edinburgh EH8 9LW).

July 7–9, 1971, **The Structure of Matter,** Christchurch (B. G. Wybourne, Department of Physics, University of Canterbury, Christchurch, New Zealand).

September 5–12, 1971, **Conference of Women Engineers and Scientists,** Turin (Dr A. E. Anour, Corso Vinzaglio 14, 10121 Torino, Italy).

September 6–9, 1971, **Physics and Chemistry of Asbestos Minerals,** Louvain (Mr B. Lincdn, Turner and Newall Asbestos Fibre Laboratory, c/o TAC Construction Materials Ltd, PO Box 22, Trafford Park, Manchester M17 1RU).

September 6–10, 1971, **Atomic Masses and Fundamental Constants,** Teddington (Dr B. W. Petley, Division of Quantum Metrology, National Physical Laboratory, Teddington, Middlesex).

September 6–11, 1971, **Metal-Insulator Transition and the Development of Narrow Energy Bands,** Assois (Dr A. D. Yoffe, Cavendish Laboratory, Cambridge).

September 7–9, 1971, **Computers for Analysis and Control in Medical and Biological Research,** Sheffield (Manager, Conference Department, IEE, Savoy Place, London WC2R OBL).

September 7–16, 1971, **Mycological Congress,** Exeter (John Webster, Department of Biological Sciences, University of Exeter, Devon).

September 8–10, 1971, **Oral Biology,** Zurich-Horgen (The Secretary, ICOB, Dental Institute, CH-8028 Zurich, PO Box 138, Switzerland).

September 8–11, 1971, **Drugs Affecting Lipid Metabolism,** Philadelphia (Dr William L. Holmes, Lankenau Hospital, Philadelphia, Pennsylvania 19151, USA).

September 13–16, 1971, **Cell Differentiation,** Nice (Dr Harris, Department of Medical Genetics, The Royal Infirmary, Manchester M13 9WL).

September 13–18, 1971, **Phenomena in Ionized Gases,** Oxford (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

September 14–17, 1971, **European Biophysics Congress,** Baden (Mrs E. Weidenhaus, Wiener Medizinische Akademie, Stadiongasse 6–8, A 1010 Vienna, Austria).

September 20–24, 1971, **The Urgency of Decision,** Lancaster (Conference Committee, Operational Research Society, 64 Cannon Street, London EC4N 6AD).

September 21–23, 1971, **Infrared Techniques,** Reading (Joint Conference Secretariat, IERE, 8–9 Bedford Square, London WC1B 3RG).

September 21–24, 1971, **Pneumatic Transport of Solids in Pipes,** London (H. S. Stephens, Pneumotransport I, BHRA, Cranfield, Bedford).

September 22–24, 1971, **Nuclear and Particle Physics,** Oxford (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

September 27–October 1, 1971, **Biomechanics,** Rome (Professor Sergio Cerquiglini, Istituto di Fisiologia Umana, Università degli Studi, Rome, Italy).

British Diary

Monday, December 28

Man has a Very Short Neck and no Tail (3 p.m.) Dr. John Napier, Royal Institution, 21 Albemarle Street, London W1. (First of Six Christmas Lectures for young people on "Monkeys Without Tails": A Giraffe's Eye View of Man. Further Lectures on December 30, January 1, 4, 6 and 8.)

Tuesday, December 29

Holography and Lasers—Uses for Laser Light (2.30 p.m.) Dr W. R. Bradford, Institution of Electrical Engineers, at Savoy Place, London WC2. (Christmas Holiday Lecture. To be repeated on December 30.)

Monday, January 4

Computational Techniques as an Aid in Physical Metallurgy (two day meeting). Iron and Steel Institute, at the University of Leeds.

Thames Estuary: Restoration and Preservation of its Quality (6.30 p.m.) Dr D. Train, Society of Chemical Industry, jointly with the Industrial Water and Effluents Group, in the Scientific Societies Lecture Theatre, 23 Savile Row, London W1.

The Preservation and Waterproofing of Joinery Timber Prior to Painting (7 p.m.) Mr R. R. Hill, Oil and Colour Chemists' Association, at Hull College of Technology.

The Selection and Training of Engineers Abroad (5.30 p.m.) Mr F. H. Woodrow, Institution of Civil Engineers, at Great George Street, London SW1.

Winter Meeting, British Society for the History of Science, at Chelsea College, University of London, Manresa Road, London SW3.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

BBC. Annual Report and Accounts for the year 1969/1970. Pp. 244+16 plates. (London: HMSO, 1970.) 33*s* net. [1611

Diamond Information L25: An Appraisal of Diamond as a Steel Grinding Abrasive. Dr Henry B. Dyer. Pp. 11. (London: De Beers Industrial Diamond Division, 7 Rolls Buildings, Fetter Lane, 1970.) *Gratis.* [1611

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 268, No. 1185 (12 November 1970): Scattering and Information. Pp. 177–200. 13*s*; $1.70. Vol. 268, No. 1185. (12 November 1970): Baroclinic Waves in a Rotating Fluid Subject to Internal Heating. By R. Hide and P. J. Mason. Pp. 201–232. 17*s*; $2.20. (London: Royal Society, 1970.) [1611

Water Resources Board. TN8: Crump Weir Design. Revised edition. Pp ii+104. TN12: Dry Weather Flows. Pp. 149. (Reading: Water Resources Board, Reading Bridge House, 1970.) [1611

The Training of Psychiatrists: Proceedings of the Conference on Postgraduate Psychiatric Education, 6–8 March 1969, held at The Institute of Psychiatry, De Crespigny Park, London, SE5. (*British Journal of Psychiatry*, Special Publication No. 5.) Edited by G. F. M. Russell and H. J. Walton. Pp. 156. (Ashford, Kent and Headley Brothers Ltd, 1970. Published by authority of the Royal Medico-Psychological Association, 1970.) 36*s*; $5.25. [1611

General Electric Company Ltd. Hirst Research Centre—Descriptive Brochure. Pp. 20. (Wembley, Middx.: GEC, Hirst Research Centre, 1970.) [1611

Institution of Gas Engineers. Communications. No. 833: First Report of the Education and Training Committee, 1969/1970. Pp. 14. 10*s*. No. 834: Large-Volume Gas Measurement. By A. Griffiths and J. Newcombe. Pp. 17. 20*s*. No. 835: Modern Development of Plastics Systems for Gas Distribution. By A. Walker and G. Clerehugh. Pp. 15. 10*s*. No. 836: Engineering Problems on High-Pressure Gas Plants. By G. M. Rimmer. Pp. 20. 20*s*. No. 837: Recent Advances in Mathematical Analysis for Gas Application. By V. E. Chancellor, Y. Hamam and C. Yalcindag. Pp. 28. 20*s*. No. 838: The Blow-Off of Burner Flames. By H. Edmondson and M. P. Heap. Pp. 10. 10*s*. No. 839: New Developments in Radiant Heating Systems. By K. F. Coles and L. P. Bagge. Pp. 16. 10*s*. (London: Institution of Gas Engineers, 1970.) [1611

The Gas Council. Research Communications. No. 169: Report on the Gas Council Research Scholarships, 1970. Pp. 10. 10*s*. No. 170: Multi-Stage Jet Boosters. By H. B. Basset and J. J. Templeman. Pp. 15. 10*s*. No. 171: Spark Ignition of Natural Gas—Theory and Practice. By J. F. Sayers, G. P. Tewari, J. R. Wilson and P. F. Jessen. Pp. 17. 20*s*. No. 172: Flash Tube Ignition of the Cooker Hotplate. By A. J. Miles. Pp. 19. 10*s*. No. 173: The Effects on Plastic Pipes of Gas Conditioning Agents and other Additives. By J. T. O'Shea, R. O'Sullivan, A. V. Smith and L. J. Wood. Pp. 12. 10*s*. No. 176: The Technology of Large Boiler Firing Using Interruptible Supplies of Gas. By R. A. Hancock. Pp. 11. 10*s*. No. 177: Installation and Ventilation. By E. W. Berry, R. D. Bruce and B. C. Carter. Pp. 13. 10*s*. No. 178: The Odorisation of Natural Gas. By A. B. Densham, P. A. Gibbons, M. D. Glidewell, R. Palmer and G. F. I. Roberts. Pp. 14. 10*s*. No. 179: Non-Aerated Burners for Natural Gas. By B. C. Dutton, J. A. Harris and B. J. Kavarana. Pp. 16. 10*s*. (London: The Gas Council, 1970.) [1611

Tropical Doctor: a Journal of Modern Medical Practice. Vol. 1, No. 1, (January 1971.) Pp. 1–48. Published quarterly. Annual Subscription: 60*s*; $8. Single copies 15*s*; $2. (London: Royal Society of Medicine, 1971.) [1711

Bulletin of the British Museum (Natural History). Historical Series. Vol. 4 No. 1: John Cranch, Zoologiste de l'Expédition du Congo (1816). By Théodore Monod. Pp. 1–75+3 plates. (London: British Museum (Natural History), 1970.) 56*s*. [1811

Agricultural Research Institute of Northern Island. Forty-third Annual Report 1969/1970. Pp. 37. (Hillsborough, Co. Down: Agricultural Research Institute of Northern Ireland, 1970.) [1811

BBC Records. Sounds of My Life: Peter Scott talks about his Eventful Life. (Mono LP 33⅓ r.p.m.) (London: BBC Enterprises, 1970.) 28*s* 9*d*. (Available from Record Retailers.) [1811

A Longitudinal Study of the Dietary of Elderly Women. By B. R. Stanton and A. N. Exton-Smith. Pp. 32. (London: King Edward's Hospital Fund for London, 1970.) 8*s* (40*p*). [1911

Council for Nature—Report 1969/1970. Pp. 17. (London: Council for Nature, Zoological Gardens, London NW1, 1970.) [1911

Dinosaurs. By W. E. Swinton. Fourth edition. (Publication No. 542.) Pp. xiii+44 (11 plates.) (London: British Museum (Natural History), 1969.) 5*s*. [1911

X-rays: Their Discovery and Applications. By Brian Bowers. (Science Museum Booklet.) Pp. 36. (London: HMSO, 1970.) 4*s* 6*d* (22½*p*) net. [1911

Other Countries

Publications of the World Meteorological Organization, 1951/1970. Pp. 108. (Geneva: World Meteorological Organization, 1970.) [911

Tales of Near and Far East including Interlude in Iran, The Sompuras—Architects of the Moon, a Prime Minister for Singapore and Sampan Girl. By Morris Redman Spivack. Pp. 22. (Gothenburg: M. R. Spivack, Poste Restante, 1970.) [911

National Council on Radiation Protection and Measurements. NCRP Report No. 37: Precautions in the Management of Patients Who Have Received Therapeutic Amounts on Radionuclides. Pp. vi+61. (Washington, DC: National Council on Radiation Protection and Measurements, 1970.) $1.50. [1011

République Démocratique du Congo. Publications de l'Institut National pour l'Étude Agronomique du Congo. Série Scientifique No. 112: Rôle des Animaux Édaphiques dans la Fertilité des Sols Forestiers. Par M. E. Maldague. Pp. 245. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1970.) [1011

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